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# Annexe – Travaux post-2001

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### 5.6 Impact of nitrogen deposition on species richness of calcareous grasslands in Europe - some preliminary results

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#### Abstract

- This paper seeks to determine whether N-deposition has a negative impact on the species richness of calcareous grasslands at a European wide scale.
- 100 calcareous grasslands across the Atlantic region of Europe were sampled in one season. Species composition and richness of vegetation communities were compared to some key environmental drivers (climate and N deposition) indirectly estimated via surrogates (latitude, longitude, N concentration in bryophyte tissue).
- There are marked differences in species composition across the calcareous grasslands of the Atlantic biogeographic zone within Europe. Contrasts in mean species richness between regions are also detectable at a European wide scale. These natural gradients may mask any footprint of N deposition on vegetation at a European-wide scale.
- For grasslands located along the western range of distribution, there are indications of a decline in species richness as N concentration in moss increases. This suggests that N deposition may be reducing biodiversity in calcareous grasslands at a wide scale, but that this impact can only be detected at the regional, rather than cross-European, level.
- Further research is needed to investigate the impact of N deposition on calcareous grasslands, particularly through the direct assessment of potential drivers as well as the characterisation of variations in species pools at the European scale.

#### 5.6.1 Introduction

The increase of atmospheric deposition of nitrogen (N) in recent decades, due to fertilizer application and fuel consumption, represent nowadays a major threat for biodiversity in ecosystems (Langan 1999, Phoenix *et al.*, 2006). In Europe, where this trend has been particularly documented, the

effects of N deposition have been studied through empirical or experimental approaches (Bobbink 1998, Stevens *et al.*, 2004). Among several effects, N deposition affects nutrient availability, which is a major driver of plant community composition and species richness (Tilman and Pacala, 1993). As a consequence, the impact of N deposition is of major concern for those species-rich ecosystems which are strictly associated with nutrient-poor soils such as Natura 2000 grasslands and heathlands. Recent comparative studies, based on either spatial gradients or on time series analyses, have shown clear evidences of an impact of N deposition on acidic grasslands, leading to a decrease of plant species richness and a loss of species associated with less fertile conditions (Dupré *et al.*, 2009, Stevens *et al.*, 2004). Such evidence is also found, at local or national level, for other species-rich habitats such as calcareous grasslands or heathlands (Maskell *et al.*, 2009), or from experimental results (Bobbink, 1991, Willems and van Nieuwstadt, 1996). Whether such results are generally applicable is of particular importance because calcareous grasslands are of major interest for the conservation of biodiversity in Europe as they support communities of exceptional diversity and many rare and endangered species of plants, insects and birds (WalliesDeVries *et al.*, 2002).

The lack of knowledge at a broader scale has motivated the BEGIN project (Biodiversity of European Grasslands – the Impact of Atmospheric Nitrogen Deposition) which seeks to determine whether N-deposition is impacting the species richness of grasslands on a European wide scale. Different approaches have been used to assess the loss of biodiversity associated with N deposition in acidic grasslands: historical analysis (Dupré *et al.*, 2009), experimental and comparative surveys (Stevens *et al.*, 2004, 2010). Another objective of BEGIN was to investigate whether a similar decrease in biodiversity is occurring in a contrasting grassland system. The most important grassland type across Europe in terms of biodiversity are the calcareous grasslands of the Mesobromion alliance (Koch, 1926). Compared to acid grasslands, these habitats have a much greater species richness and larger number of rare species, and are also presumed to be sensitive to N deposition through increasing nutrient availability. We thus hypothesised that N deposition may be significantly impacting these grasslands.

#### 5.6.2 Aims and objectives

- We aim to determine whether any significant variability in plant species richness in calcareous grasslands across Western Europe could be detected and related to any regional-scale evaluation of N atmospheric deposition.
- In 2008, we surveyed 100 calcareous grasslands belonging to the Mesobromion alliance on a transect across the Atlantic biogeographic zone of Europe. Site selection was performed through a composition criterion (required presence of five species among a predefined list of target species) and a management criterion, in order to avoid abandoned grasslands. For each site, five 1 m × 1 m replicates were recorded. In each square meter, the cover of all occurring plant species (vascular plants and bryophytes) was visually estimated. Each site is therefore characterised by a list of species with average abundance (calculated from the five quadrats) and an average richness per plot (n=5 replicates).
- In this preliminary study, we only used environmental surrogates to account for the major environmental drivers we identified. A major predictor of large scale species richness is latitude (Hillebrand, 2004). We used latitude and longitude as aggregate variables integrating distinct climate factors i.e. mainly temperature (North-South) and precipitation (West-East) gradients. Total nitrogen concentration in bryophytes was used as a surrogate to estimate total N deposition at a high resolution (Harmens *et al.*, 2008). Because species-specific differences are expected, we considered only the sites where the same moss species (i.e. Ctenidium molluscum) was collected and analysed. This accounted for about half of the sampled sites (51 sites for the 100 sites of this study).

• We performed a correspondence analysis (CA) and a hierarchical clustering of the [100 sites x 161 species] data table in order to provide an ordination and classification of plant communities. The table was obtained after removal of species occurring in less than 5 per cent of the records in the initial table [100 sites x 225 spp]. Similar analysis was performed on the sub-set of 51 sites where some environmental surrogates were available. Simple regressions were performed, after data normality was tested (Shapiro-Wilk test), to assess correlations between environmental surrogates and species richness (i.e. mean species number for the five replicates) and species composition (floristic gradients from the CA). All analyses were performed with R free software (2007).

#### 5.6.3 Results and discussion

The Correspondence Analysis shows that gradients in species composition are well correlated to the geographical distribution of the sites (Figure 5.8a,b). Latitude and longitude are highly significantly correlated with respectively axis one (n=100,  $r^2=0.52$ , p<0.001), and axis two ( $r^2=0.63$ , p<0.001) of the CA. A hierarchical clustering (Ward method) performed on the output of this CA (Figure 5.8c)

gives a very similar result to clusters defined on a national basis. Three main types of plant communities can be defined (Table 5.3), related to sites from France (FR), United Kingdom and Eire (UK+IRL), and Germany (GER). Sites from north central Europe (Belgium, Netherlands, Denmark) are distributed within these three clusters, while Norway sites form a specific sub-cluster.

Looking for pattern of variation of community species richness along these floristic/geographic gradients of the CA, we found no evidence of a correlation with species richness for any of the CA axes. However, when considering the different clusters from the CA, species richness appear significantly different between some regions (Figure 5.9 - one way ANOVA; F= 3.01, df=99, p<0.05). As we avoided abandoned sites, these differences could not be due to management contrasts but rather to differences in species pool size, depending on regional specificity (soil, climate, history).

We analysed the sub-set of 51 sites to test whether species composition gradients and species richness variations could be correlated to N deposition, estimated via the N surrogate (N per cent in the moss C. molluscum). The 51 sites were distributed in the three main clusters-regions: 27/27 sites of the South-West (SW) of Europe (CL2), 13/36 sites of the North-West (NW) of Europe (CL3) and 11/32 sites of the Est (E) of Europe (CL1). These sites were also regularly distributed along CA axes. We then performed a new Correspondence Analysis (CA2) on these 51 sites, to built floristic gradients on this specific data set. Patterns were similar to the first CA, the CA2 axes being even more correlated to latitude and longitude (n=51, r<sup>2</sup>=0.56, p<0.001 for axis 1; r<sup>2</sup>=0.82, p<0.001 for axis 2). N per cent in bryophyte tissue was correlated only with the axis three of this second correspondence analysis (r<sup>2</sup>=0.10, p<0.05), suggesting at least that N deposition could be correlated to gradient of species composition in this data sub-set. We did not find any correlation

	Cluster	Region	Countries	Some differential species				
	CL1	E	GER, (B), (NL)	Silene vulgaris, Inula conyza, Poa angustifolia				
	(CL1bis)	E	Norway	Viola canina, Deschampsia flexuosa, Alchemilla filicaulis				
	CL2	SW	FR, (B)	Teucrium montanum, Gaudinia fragilis, Seseli montanum, Thesium humifusum				
	CL3	NW	UK, IRL, DK, (B), (NL)	Carex humilis, Festuca arundinacea, Ranunculus repens				



Figure 5.8: Correspondence analysis of the [100 relevés x 161 species] data table for the analysis of composition gradients in calcareous grasslands. a) Eigenvalues; b) F1×F2 plane showing distributions of relevés in the national surveys, c) F1×F2 plane with clusters performed from hierarchical clustering (Ward method) on relevés coordinates.



Fig.5.9: Box plots showing species richness (mean SR.m-<sup>2</sup>; n=5 replicates) variations in the four clusters from the CA. see text for details. Boxes sharing the same letter are not statistically different (P < 0.05, Tukey's HSD comparing all clusters).

between N surrogate and species richness of plant communities at a broad scale (Figure 5.10a; n=51, p=0.18). However, when we performed regressions at the regional level (Figure 5.10 b,c,d), correlation was significant for SW region (Cluster 2, n=27,  $r^2=0.20$ , p<0.05), while no trend was detected for other

clusters. When significant, correlation shows a decline of species richness at the highest levels of N concentration (deposition). The processes responsible for this decline may be found in the effects of N enrichment, resulting in changes in vegetation structure and species interactions to the benefit of competitive tall grasses (Bobbink, 1991, Liancourt *et al.*, 2005).

From our data, composition gradients in calcareous grasslands are marked at the European scale, even though we removed the less frequent species in the data set (i.e. with occurrence less than 5 per cent) which should attenuate contrasts between countries. This species turn-over is shown in phytosociological works (Royer 1985, Willems, 1982). Our survey confirms that climate gradients are likely to be the most important drivers of species turn-over in calcareous grasslands in Europe, as climate variables such as temperature and rainfall are known to be correlated with latitute and longitude (Ozenda 1994, Duckwoth *et al.*, 2000). Similarly, our data suggest that regions in Europe could be characterised by species pools of different sizes. However, this has to be confirmed with species pool studies (e.g. Dupré, 2000), based on more complete phytosociological datasets.

Because of the strong climate-driven variation in species composition and richness, it is difficult to detect a separate signal of N deposition as a potential driver of calcareous grassland diversity on a cross-Europe scale. The use of N concentration in moss as a surrogate for N deposition can also introduce some potential artefacts. Besides differing among different species, this relationship might also depend on other factors such as N speciation, the ratio of wet/dry deposition in N deposition, and local climate (Harmens *et al.*, 2008). Despite these limitations, there are some indications of an N-deposition signal on species composition at a European wide scale and on species richness for calcareous grasslands located at the western range of their distribution.



Fig.5.10: Regression plots between nitrogen concentration in the moss Ctenidium molluscum (N per cent dry weight) and species richness (mean SR.m-<sup>2</sup>; n=5 replicates) in calcareous grasslands according to different geographic ranges: a) subset covering the whole geographic range i.e. Atlantic Europe; n=51; b) subset from NW Atlantic Europe i.e. Cluster 3; c) subset from

#### 5.6.4 Conclusions

- There are strong gradients of species composition in calcareous grasslands in western Europe. Contrasts in mean species richness between regions are also detectable at a European-wide scale. These gradients and contrasts appear to be driven primarily by climate.
- Because of these strong environmental responses, it is difficult to detect a clear influence of N deposition on species richness and composition at the European scale.
- However, when filtering the data at the regional to national scale, there are indications of an N-deposition signal on species richness for grasslands sampled in western regions (Atlantic coast). If real, regressions suggest a fairly strong decline in diversity with increasing N deposition for these sites
- These intimations of a N impact on calcareous grassland diversity strongly point to a need for targeted research, particularly through the direct assessment of potential drivers as well as the characterisation of natural variations in species pools at the European scale.
- The above have strong implications for conservation and pollution mitigation actions for management of calcareous grasslands (Calciura and Spinelli, 2008).

#### References

- Bobbink, R. (1991) Effects of nutrient enrichment in Dutch chalk grassland. *Journal of Applied Ecology*, 28, 28-41.
- Bobbink, R., Hornung, M. and Roelofs, J.G.M. (1998) The effects of air-borne pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, 86, 717-738.
- Calaciura, B. and Spinelli, O. (2008) Management of Natura 2000 habitats. 6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates (Festuco-Brometalia). Report European Commission.
- Duckworth, J. C., Bunce, R.G.H. and Malloch, A.J.C. (2000) Vegetation-environment relationships in Atlantic European calcareous grasslands. *Journal of Vegetation Science*, 11,15-22.
- Duprè, C. (2000) How to determine a regional species pool : a study in two Swedish regions. *Oikos*, 89, 128-136.
- Dupre, C., Stevens, C., Ranke, T., Bleeker, A., Peppler-Lisbach, C., Gowing, D., Dise, N., Dorland, E., Bobbink, R. and Diekmann, M. (2009) Changes in species richness and composition in European acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. *Global Change Biology*, 16, 344-357.
- Harmens, H., Norris, D., Cooper, D., Hall, J. and the participants of the moss survey (2008) Spatial trends in nitrogen concentrations in mosses across Europe in 2005/2006. Programme Coordination Centre for the ICP Vegetation, Centre for Ecology and Hydrology, Bangor, United Kingdom (available at: http://icpvegetation.ceh.ac.uk/publications/documents/ FinalNreport2005-6surveyamendmentSwitzerland.pdf).
- Langan, S.J. (1999) *The impact of nitrogen deposition on natural and semi-natural ecosystems*. Kluwer Academic Publishers, Netherlands.
- Liancourt, P., Corcket, E. and Michalet, R. (2005) Stress tolerance abilities and competitive responses in a watering and fertilization field experiment. *Journal of Vegetation Science*, 16, 713-722.
- Maskell, L.C., Smart, S.M., Bullock, J.M., Thompson, K. and Stevens, C.J. (2010) Nitrogen Deposition causes widespread species loss in British Habitats. *Global Change Biology*, 16, 671-679.
- Ozenda, P. (1994) Végétation du continent européen. Delachaux et Niestlé, Lausanne, Switzerland.
- Phoenix, G.K., Hicks, W.K., Cinderby, S., Kuylenstierna, J.C.I., Stock, W.D., Deneter, F.J., Giller, K.E., Austin, A.T., Lefroy, R.B., Gimeno, B.S., Ashmore, M.R. and Ineson, P. (2006)

Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. *Global Change Biology*, 12, 470–476.

- R Development Core Team (2007) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0 (available at: http://www.R-project.org).
- Royer, J. M. (1985) Liens entre chorologie et différenciation de quelques associations du Mesobromion erecti d'Europe occidentale et centrale. *Vegetatio*, 59, 85-96.
- Stevens, C.J., Dise, N.B., Mountford, J.O. and Gowing, D.J. (2004) Impact of Nitrogen Deposition on the Species Richness of Grasslands. *Science*, 303, 1876-1879.
- Stevens, C.J., Duprè, C., Dorland, E., Gaudnik, C., Gowing, D. J.G., Bleeker, A., Diekmann, M., Alard, D., Bobbink, R., Fowler, D., Corcket, E., Mountford, J.O., Vandvik, V., Aarrestad, P.A., Muller, S. and Dise, N.B. (2010) Nitrogen deposition threatens species richness of grasslands across Europe. *Environmental Pollution*, 158, 2940-2945.
- Tilman, D. and Pacala S. (1993) The maintenance of species richness in plant communities. In: Species diversity in ecological communities (eds. Ricklefs, R. E. and Schluter, D.). Chicago University Press, Chicago, USA.
- WallisDeVries, M.F., Poschlod P. and Willems, J.H. (2002) Challenges for the conservation of calcareous grasslands in northwestern Europe: integrating the requirement of flora and fauna. *Biological Conservation*, 104, 265-273.
- Willems, J. H. (1982) Phytosociological and geographical survey of Mesobromion communities in Western Europe. *Vegetatio*, 48, 227-240.
- Willems, J.H. and Van Nieuwstadt M.G.L. (1996) Long-term after effects of fertilization on above-ground phytomass and species diversity in calcareous grassland. Journal of Vegetation *Science*, 7, 177-184.

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Société botanique de France



## Can contrast between forest and adjacent open habitat explain the edge effects on plant diversity?

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**Abstract:** Forest edges are key features in human-dominated landscape. Located between forest and non-forest habitats, edges induce biotic and abiotic changes, which may have profound consequences on vegetation diversity. Recent studies suggest the importance of different edge types in the modulation of edge-related responses. However, edge effect on the spatial dynamic of vegetation, from forest to non-forest habitats, remains unclear. Our aim was to compare the species richness and diversity of vegetation communities between forest and open habitats with their respective edges, in high-contrast versus low-contrast situations. The degree of contrast was defined according to the disturbance regimen of non-forest habitats. We surveyed vascular vegetation along transects in forest and open habitats and in their respective edges, in three regions of France. We showed that edge effects occur on plant diversity, whatever the region, but asymmetrically. Edge effect tends to be greater on the open side than on the forest side of the border. Species richness and diversity were generally higher in open edge than in open habitat, whereas no significant difference was observed between forest edge and forest habitat, whatever the contrast situation encountered. This study shows that the edge effects detected along a forest–edge–exterior habitat gradient may depend in large part on the disturbance regimen in open habitats as well as the vegetation pool size. We highlighted the need to carefully consider the edge types, e.g. their contrast with adjoining non-forest habitat, in further studies to identify the relevant factors and mechanisms behind edge-related response patterns of biodiversity in human-dominated landscapes.

Keywords: forest edge; open edge; vegetation; Shannon diversity; richness; disturbance

#### Introduction

Human-driven changes in land-use patterns have increased the need to understand how landscape structure and configuration affect species distribution (Collinge 2009). Mosaic landscape results from diverse and opposite dynamics: agricultural intensification, resource extraction and timber harvesting creating open habitats (Harper et al. 2005), abandonment of grazing or management, tree plantations leading to the increase in forest habitats. In such landscapes, interfaces between open habitats and forests, also called forest edges, became one of the dominant features. Forest edges may induce gradual changes from the border in abiotic and biotic conditions, i.e. "edge effects", through the modification of organisms, matter and energy flows (Cadenasso et al. 2003).

Hence, forest edges have a major ecological role, notably for biodiversity and associated ecological features. They can act not only as biodiversity hotspots, amalgamating species from both forest and open habitats (Matlack and Litvaitis 1999; Duelli, Obrist, and Fluckiger 2002), but also as corridors for species circulating in the landscape. Conversely, forest edges may represent ecological traps for species that select them as reproduction sites despite the high mortality risk (e.g. Ries and Fagan 2003). Hence, the response pattern of species to the forest edge can be positive, negative or neutral (Ries and Sisk 2004).

The composition and structure of forest edges seem to be of high relevance for biodiversity aspects because distribution of species and plant communities is largely shaped by the edge type, which is determined by the type of vegetation in adjacent habitat, the age of the forest, and the time since disturbance (Didham and Lawton 1999; Mesquita, Delamônica, and Laurance 1999; Harper et al. 2005; Chabrerie et al. 2013; Pellissier et al. 2013). Even when several factors that influence the spatial extent of the edge effect into forest patches are standardized, variability still exists (Hamberg et al. 2008) due to the importance of the type of edge studied. Edges with high contrast between forest and adjacent open habitat and edges with low contrast may have different structural characteristics and as a consequence, different functional characteristics (Gehlhausen, Schwartz, and Augspurger 2000; Laurance, Didham, and Power 2001; Cadenasso et al. 2003; Strayer et al. 2003; Ries et al. 2004). For example, López-Barrera et al. (2006) showed that edge effects detected on oak seedlings along a forest-edge-open habitat gradient in the

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highlands of Chiapas, depend in large part on the fact that the edge had low or high contrast with adjacent habitat.

Understanding how the edge type may alter the structure and composition of the vegetation diversity is of high importance not only for understanding and identifying response patterns to edge effect (Ries et al. 2004; Harper et al. 2005) but also for predicting vegetation community composition in human-dominated landscapes. This knowledge could help to improve the management of biodiversity in forest edges where the interactions between forests and agriculture are growing, particularly through the valuation of ecosystem services, such as pollination and pest regulation. In this study, we investigated the response patterns to edge influence of vegetation communities, through their species richness and diversity, according to the contrast between forest and open habitats, defined here by the frequency of disturbance in open habitats, in three regions of France. A large part of studies on edge effects focus on the forest side (e.g. Alignier and Deconchat 2013) and ignored the adjacent habitat, although theoretical models predict that edge effects occur on both sides of the edge (Cadenasso et al. 2003; Ries and Sisk 2004; Ries et al. 2004).

This study compares the richness and diversity of vegetation communities between a core habitat and its associated edge. Habitats considered are forests and their adjacent open habitats (fields, herbaceous communities). Edges inside the forest (i.e. forest edge) are compared to forest interior (i.e. forest habitat) and edges outside the forest (i.e. open edge) are compared to open habitat. Because the frequency and intensity of disturbance of the open habitat can be high or moderate, we distinguish respectively high- and low-contrast edges. Different patterns are expected according to the contrast between forest and adjacent open habitats, so-called edge type (Figure 1). In a high-contrast situation between forest and adjacent open habitat, a gradual transition is expected. The edge could play a role as a barrier or a filter limiting the flow of organisms between habitats: for example, forest specialist species that grow preferentially



Figure 1. Expected theoretical patterns of plant species diversity in high- and low-contrast situations between forest and open habitats. In low-contrast situation, edge effect is assumed to be positive, i.e. more species are encountered in the edge than in the core habitat (in grey), whatever the side (forest or open). In high-contrast situation, it is assumed to be mixed (see the text for details).

in shade conditions do not come out of the forest interior and heliophilous species from open habitats do not come in (Strayer et al. 2003). Assuming that the forest habitat – less disturbed – is richer than the adjacent open habitat, we hypothesized that vegetation diversity decreases along the gradient forest–edge–open habitat. In low-contrast situations, plant species may disperse from one habitat to another, develop and concentrate along edges. As a result, the diversity of species may be substantially greater along edges than within any of the adjacent habitats (Matlack and Litvaitis 1999).

#### Material and methods

#### Study area

The study was conducted in three regions of France (Figure 2). The Aquitaine region, with almost one million hectares of maritime pines, has the largest artificial forest in Europe. The landscape is dominated by a mosaic of maritime pine plantations of different ages, clear-cuts, heathlands (dominated by Molinia caerulea (L.) Moensch, Erica cinerea L., Ulex europaeus L.), scattered deciduous forest patches and herbaceous firebreaks. As a whole, forest covers 75% of the total area and open areas (25%) are patches of different sizes disseminated in a forested landscape. The climate is thermo-Atlantic (mean annual temperature 12°C, mean annual rainfall 700 mm) and the elevation is low (c.50 m above sea level). The Midi-Pyrénées region can be classified as a temperate agro-forested landscape. Forests are fragmented with patch size between 0.5 and 35 ha and cover approximately 15% of the total area. The main tree species are Quercus robur L., Quercus pubescens Willd, Carpinus betulus L., Prunus avium (L.) L. and Sorbus torminalis (L.) Crantz. The region is hilly (250-400 m above sea level) and has a sub-Atlantic climate and slight Mediterranean influences (mean annual temperature 11°C; mean annual rainfall 750 mm). The Centre region (Loiret) is mostly dedicated to intensive crop production. Most forests (patch size between 0.5 ha and > 2200 ha) are oak-hornbeam coppice with standards used for wood and



Figure 2. Location of the three studied regions with type of open habitat adjoining forests (high/low disturbed habitat corresponding to high/low contrast situation).

wood fuel production and cover near 35% of the region. The region is slightly hilly (110–300 m above sea level) and has a sub-Atlantic climate with some continental influence (mean annual temperature 10.5°C, mean annual precipitation 750 mm).

#### Sampling design

Twenty forest edges pertaining to different forest patches, were sampled in each region in May-June 2011 or 2012. For the Centre region, forest patch size varied between 0.6 ha and 818 ha (mean 71 ha). For the Midi-Pyrénées region, forest patch size varied between 0.8 and 47 ha (mean 12 ha), except for one wood of 505 ha. In each region, we considered 10 forest edges adjoining habitats with frequent soil disturbance and/or vegetation harvest (once or more per year). These high-contrast situations concerned firebreaks in Aquitaine, and oilseed rape in Midi-Pyrénées and Centre. The other 10 forest edges were located beside habitats with low disturbance (perennial communities or one vegetation disturbance per year, i.e. mowing, representing low-contrast situations) such as dune in Aquitaine, meadow in Midi-Pyrénées and orchard in Centre (Figure 2). Vascular vegetation sampling was conducted in both habitats (forest and open) and their respective edges. For each forest edge, a transect was performed perpendicular to the border. The border was defined as the line formed by mature trees. Along each transect, we established 2  $\times$  50-m plots parallel to the border, one beside the border in the edge (forest edge) and one 50 m into the forest (forest habitat) (Figure 3). If edge effects can extend up to 1 km (Laurance 2000), they generally do not exceed 30 m in temperate forests



Figure 3. Scheme of the sampling design. Two  $100\text{-m}^2$  plots parallel to the border were placed in the forest edge and the forest habitat, at 0 m and 50 m from the border, respectively. Two sets of five 4 m<sup>2</sup> plots were placed within the open edge and the open habitat, at 0 m and 30 m at least from the border. The border was defined as the line formed by mature trees between forest and open habitats.

(e.g. Piessens et al. 2006; Alignier and Deconchat 2013). We also established two sets of five  $2 \times 2$  m plots placed parallel to the border, one beside the border (open edge) and one at least 30 m away from the border in the open habitat (Figure 3). Vegetation sampling areas were defined to detect the majority of species in plant communities in each type of habitat. For that purpose, we estimated species cover with respect to the minimum sampling area method using the following standards: 100 m<sup>2</sup> for the forest side and 16 m<sup>2</sup> for the open habitats side (Guinochet 1973). The plots were designed to consider a similar spatial extent (50 m length) to avoid the larger environmental heterogeneity found in a larger sampling plot. At each plot, we recorded abundance-dominance of all vascular plant species pertaining to all strata (trees, shrubs and herbs) according to the Braun-Blanquet scale (Braun-Blanquet 1956).

Vegetation surveys in each habitat and its edge gave species richness (*R*) and allowed calculation of Shannon species diversity (*H*) according to the proportional abundance of each species ( $p_i$ ) as:

$$H = -\sum_{i=1}^{R} p_i \ln\left(\mathbf{p}_i\right)$$

In open habitat and open edge, abundance values for each plant species were averaged over the five 4-m<sup>2</sup> plots.

#### Statistical analyses

First, we compared mean species richness and mean Shannon species diversity between habitats and their respective edges in each region using pairwise Student's t-tests. Then, similarity in species composition was quantified for each habitat and its edge, in each region. We used the Bray Curtis similarity index, which is related to the Sorensen index and which assesses similarity between two localities based on differences in abundance of species, and not merely on presence/absence. It ranges from 0 (no similarity) to 1 (same species in both localities all, occurring at the same abundance in both sites). Because this index can only be computed with integers, abundance data were rounded up before the calculations were made; this has no effect on the resulting index values. We compared similarities in species composition according to contrast (1) for the forest side and (2) for the open side, using analysis of variance (ANOVA) tests. Statistical analyses were carried out using R 2.15.1 (R Development Core Team 2010).

#### Results

A total of 534 vascular plant species were recorded through the three regions. In the Aquitaine region, 154 species were recorded with 102 species (on average 18.3  $\pm$  5.9) in high-contrast situations (n = 4 transects; firebreaks) and 60 (on average  $8.7 \pm 5.2$ ) in low-contrast

situations (n = 16; dunes). The assemblage was dominated in high-contrast situations by Pinus pinaster Aiton (present in 50% of surveyed plots), and in low-contrast situations by Carex arenaria L. (57.8% of surveyed plots), Cerastium diffusum Pers. (54.7% of surveyed plots) and Aira praecox L. (53.1% of surveyed plots). In the Midi-Pyrénées region, 365 species were recorded with 181 species (on average  $18 \pm 11.2$ ) in high-contrast situations (n = 10 transects; oilseed rape) and 318 (on average  $35.8 \pm 9.7$ ) in low-contrast situations (n = 17; meadows). The assemblage was dominated in high-contrast situations by Hedera helix L. (present in 60% of surveyed plots), Crataegus monogyna Jacq. (55% of surveyed plots) and Ligustrum vulgare L. (52% of surveyed plots) and in low-contrast situations by Dioscorea (L.) Caddick & Wilkin communis (present in 94% of surveyed plots). Rubia peregrina L. (present in 88% of surveyed plots) and *Hedera helix* (86% of transects). In the Centre region, 331 species were recorded with 221 species (on average 26.6  $\pm$  13.0) in high-contrast situations (n = 10 transects; oilseed rape) and 197 (on average 25.7  $\pm$  10.3) in low-contrast situations (n = 11; orchards). The vegetation assemblage was dominated by *Galium aparine* L. (present in 52% of surveyed plots) in high-contrast situations, and *Hedera helix* (79%), *Quercus robur* (68%) and *Rubus fructicosus s.l.* (66% of surveyed plots).

Whatever the contrast situation encountered, a clear edge effect in terms of species richness and Shannon species diversity (H) was observed between the open edge and open habitat, whereas no significant differences were observed between the forest edge and the forest habitat for both the Midi-Pyrénées and the Centre regions (Figure 4; Appendix 1). Higher species richness and higher H were encountered in open edges. No



Figure 4. Species richness (mean  $\pm$  SD) and Shannon species diversity (× 10; mean  $\pm$  SD) in the forest and open habitats and their respective edges in the three regions of France. Capital letters (lowercase, respectively) indicate significant differences for mean species richness (mean Shannon species diversity, respectively) at  $\alpha = 0.05$ . Note that forest habitat was compared uniquely to forest edge and open edge uniquely to open habitat.



Figure 5. Bray–Curtis similarity in vegetation community composition between habitats and their respective edges for (A) the forest side and (B) the open side. Different letters indicate significant differences at  $\alpha = 0.05$ .

significant differences were observed between habitats and their respective edges in Aquitaine (Figure 4; Appendix 1).

Similarities between vegetation communities belonging to the forest side averaged 35% and did not differ according to the contrast situation or the region (ANOVA, F = 2.95, p = 0.194; Figure 5A). For the open side, similarities were lower (average near 25%) than the forest side. Similarities between vegetation communities belonging to open habitat and open edge were significantly higher for the Aquitaine region (ANOVA, F = 15.16, p = 2.4 e-09; Figure 5B).

#### Discussion

Edge effects occur on plant communities in three regions of France, but in an asymmetrical way. Edge effect on plant diversity tends to be greater on the open side than on the forest side of the border. Species richness and diversity were generally higher in open edge than in open habitat whatever the contrast situation encountered. No significant difference was observed between the forest edge and the forest habitat. Expected response patterns of vegetation diversity were not all satisfied. With a view to managing vegetation diversity in the edge so as to promote ecological services in both adjacent habitats, studies dealing with edge effect according to edge types and patch contrast in human-dominated landscapes are still required.

Higher species richness was found in vegetation communities for the Centre and Midi-Pyrénées regions than for the Aquitaine region. Indeed, the vegetation pool size in the Aquitaine region, dominated by pine plantations, was only about half as important as in the other regions, dominated by broadleaved forests. Natural forests are usually more suitable as habitat for a wider range of native forest species than plantation forests (Bremer and Farley 2010). But there is growing evidence that plantation forests can provide valuable habitats, even for some threatened and endangered species, and may contribute to the conservation of biodiversity by various mechanisms (Carnus et al. 2006; Brockerhoff et al. 2008). Another explanation could be related to local environmental conditions, especially to available light. The development of the forest cover from a quasi-pure pine plantation to a multi-storeyed mixed forest contributes to attenuation in radiation beneath the canopy (Porté, Huard, and Dreyfus 2004). Pine plantations displayed understorey habitats with more light available than in broadleaved forest, so the contrast between forest habitat and forest edge was reduced. In addition, the open edge may be less influenced by adjacent forest in pine forest, leading to homogenization between habitats. This may particularly endanger the rare, often habitat-specific, species that represent a large part of species richness (Gaston 1994).

Addressing the edge-related response of vegetation communities, we observed some variability by comparison with expected patterns (Figure 1). In high-contrast situations, species richness tended to decrease according to the gradient forest-edge-open habitat, in spite of there being no significant difference between forest edge and forest habitat. This pattern, in accordance with our expectations, was confirmed for the Midi-Pyrénées and Centre regions, but not for the Aquitaine region. Although edge effects in temperate forests generally do not exceed 30 m (e.g. Piessens et al. 2006; Alignier and Deconchat 2013), the lack of significant difference between forest edge and forest habitat indicates either that edge effect was longer than 50 m or that the size of our forest patches was too small to guarantee the presence of a core habitat zone. In that sense, forest patches can be considered to be entirely under the influence of the edge (Laurance and Yensen 1991) or of multiple edge effects (Fletcher 2005). Nevertheless, this last assumption is more debatable in the large forests of the Aquitaine and Centre regions. In low-contrast situations, the patterns were less clear. Only species richness in the Centre region was substantially greater in edges than in adjacent habitats, as expected. This result tends to demonstrate that although a low-contrast situation allows more dispersal than a highcontrast situation, species do not necessarily concentrate in edges and may ignore them. However, to justify labelling a species as insensitive, one should be able to demonstrate a consistent lack of response at several edge types (Ries and Sisk 2010). Focusing on Shannon diversity, only patterns observed in high-contrast situations for the Midi-Pyrénées region and in low-contrast situations for the Centre region conformed to our expectations. We have to note that no edge effect was observed in any contrast situation for the Aquitaine region. A likely explanation for the absence of vegetation response to edge effect is that we assumed that the forest habitat was richer than the open adjacent habitat, but it appears evident that it was not the case for the Aquitaine region. The dune and some firebreak habitats were richer than the forest habitat. As noted previously, stands are composed of intensive and homogeneous plantations of maritime pines in the Aquitaine region that may reduce the habitat heterogeneity and so impoverish vegetation communities (Bremer and Farley 2010). Conversely, open habitats like permanent grasslands could be as rich or even richer than forest habitats (Łuczaj and Sadowska 1997). Hence, our dichotomy of edge type, based on disturbance frequency, needs to be nuanced, as the contrast perceived by the human observer does not necessarily represent relevant heterogeneity to vegetation communities. A way to fix this could be to improve the description of disturbance regimens in each habitat and to relate them to the evolutionary history of the different plant communities.

Similarity in vegetation communities between edge and habitat averaged 35% in the forest side over all regions. Plant communities were as rich in forest edges as in forest habitats, but plant assemblages were quite different. This change in species composition may reflect the functional response of species. Indeed, forest habitat specialists are generally shade-tolerant and are known to avoid edges (Ranney, Bruner, and Levenson 1981) whereas edges advantage light-tolerant species (Brothers and Spingarn 1992). Similarity in vegetation communities between edge and habitat is lower in the open side. This result would indicate that exchanges - through dispersal - between vegetation communities in the open side were more limited than in the forest side. Similarity in vegetation communities between habitats and their respective edges did not differ according to the contrast, but according to the region. The Aquitaine region showed a significantly higher similarity between open edge and open habitat than in other regions. However, similarity increased where species richness decreased. So, inter-regional variation in similarity pattern was probably linked to the vegetation pool size.

This study has found a great variability in the response patterns of vegetation communities to edge effect. These patterns did not all correspond to the expected response patterns, depending on the region. The range of edge-related response patterns observed suggests that the contrast between forest and open habitats is not uniform and that vegetation diversity depends mainly on the region – through pool size – and the disturbance regimen. Taken together, our results highlight the importance of (1) improving the description of disturbance regimen of both forest and nonforest habitats, and (2) additional tests of the effects of different edge types (Ries et al. 2004) or patch contrast (Harper et al. 2005) on vegetation communities. Considering species assemblages and their functional traits (e.g. specialists versus generalists) may provide more accurate response patterns to edge influence than by considering the whole species through diversity indices. In addition, variability observed between regions suggests that these factors should be addressed more explicitly in further studies. To develop a comprehensive theory of edge effects and effective management of vegetation diversity in edges, future studies should identify the relevant factors and mechanisms behind edge-related response patterns of biodiversity in human-dominated landscapes.

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#### Notes on contributors

Audrey Alignier's research is targeted at the understanding of the spatial distribution patterns of vegetation communities. Didier Alard is a plant ecologist interested in conservation ecology and biodiversity monitoring. Richard Chevalier's research is targeted on the identification of the main biotic and abiotic drivers shaping forest plant communities. Emmanuel Corcket is a plant ecologist working on relationships between biotic processes and biodiversity.

Didier Alard, Emmanuel Corcket and Richard Chevalier conceived and performed the vegetation surveys. Audrey Alignier conducted data analysis. Audrey Alignier, Didier Alard and Emmanuel Corcket wrote the manuscript.

#### References

- Alignier, A., and M. Deconchat. 2013. "Patterns of forest vegetation responses to edge effect as revealed by a continuous approach." *Annals of Forest Science* 70: 601–609.
- Braun-Blanquet, J. 1956. "Plant Sociology: The study of plant communities." In *The study of Plant Communities*, edited by H.J. Oosting. San Francisco: W.H. Freemannand.
- Bremer, L.L., and K.A. Farley. 2010. "Does plantation forestry restore biodiversity or create green deserts? A synthesis of the effects of land-use transitions on plant species richness." *Biodiversity and Conservation* 19: 3893–915.
- Brockerhoff, E.G., H. Jactel, J.A. Parrotta, C.P. Quine, and J. Sayer. 2008. "Plantation forests and biodiversity: oxymoron or opportunity?" *Biodiversity and Conservation* 17: 925–951.
- Brothers, T.S., and A. Spingarn. 1992. "Forest fragmentation and alien plant invasion of Central Indiana old-growth forests." *Conservation Biology* 6: 91–100.
- Cadenasso, M.L., S.T.A. Pickett, K.C. Weathers, S.S. Bell, T.L. Benning, M.M. Carreiro, and T.E. Dawson. 2003. "A framework for a theory of ecological boundaries." *BioScience* 53: 750–758.
- Carnus, J.-M., J. Parrotta, E.G. Brockerhoff, M. Arbez, H. Jactel, A. Kremer, D. Lamb, K. O'Hara, and B. Walters. 2006. "Planted forests and biodiversity." *Journal of Forestry* 104: 65–77.

- Chabrerie, O., A. Jamoneau, E. Gallet-Moron, and G. Decocq. 2013. "Maturation of forest edges is constrained by neighboring agricultural land management." *Journal of Vegetation Science* 24: 58–69.
- Collinge, S.K. 2009. *Ecology of fragmented landscapes*. Baltimore MD: The Johns Hopkins University Press.
- Didham, R.K., and J.H. Lawton. 1999. "Edge structure determines the magnitude of changes in microclimate and vegetation structure in tropical forest fragments." *Biotropica* 31: 17–30.
- Duelli, P., M.K. Obrist, and P.F. Fluckiger. 2002. "Forest edges are biodiversity hotspots–also for Neuroptera." Acta Zoologica Academiae Scientarum Hungaricae 48: 75–87.
- Fletcher, R.J. 2005. "Multiple edge effects and their implications in fragmented landscapes." *Journal of Animal Ecology* 74: 342–352.
- Gaston, K.J. 1994. Rarity. London: Chapman and Hall.
- Gehlhausen, S.M., M.W. Schwartz, and C.K. Augspurger. 2000. "Vegetation and microclimatic edge effects in two mixed-mesophytic forest fragments." *Plant Ecology* 147: 21–35.
- Guinochet, M. 1973. Phytosociologie. Paris: Masson et Cie.
- Hamberg, L., S. Lehvävirta, M.L. Minna, H. Rita, and D.J. Kotze. 2008. "The effects of habitat edges and trampling on understorey vegetation in urban forests in Helsinki, Finland." *Applied Vegetation Science* 11: 83–98.
- Harper, K.A., S.E. Macdonald, P.J. Burton, J.Q. Chen, K.D. Brosofske, S.C. Saunders, E.S. Euskirchen, D. Roberts, M.S. Jaiteh, and P.A. Esseen. 2005. "Edge influence on forest structure and composition in fragmented landscapes." *Conservation Biology* 19: 768–782.
- Laurance, W.F. 2000. "Do edge effects occur over large spatial scales?" *Trends in Ecology and Evolution* 15: 134–135.
- Laurance, W.F., R.K. Didham, and M.E. Power. 2001. "Ecological boundaries: a search for synthesis." *Trends in Ecology* and Evolution 16: 70–71.
- Laurance, W.F., and E. Yensen. 1991. "Predicting the impacts of edge effects in fragmented habitats." *Biological Conser*vation 55: 77–92.
- López-Barrera, F., R.H. Manson, M. González-Espinosa, and A.C. Newton. 2006. "Effects of the type of montane forest edge on oak seedling establishment along forest–edge– exterior gradients." *Forest Ecology and Management* 225: 234–244.

- Łuczaj, Ł., and B. Sadowska. 1997. "Edge effect in different groups of organisms: vascular plant, bryophyte and fungi species richness across a forest-grassland border." Folia Geobotanica and Phytotaxonomica 32: 343–353.
- Matlack, G.R., and J.A. Litvaitis. 1999. Forest edges: Maintaining biodiversity in forest ecosystems. Cambridge: Cambridge University Press.
- Mesquita, R.C., P. Delamônica, and W.F. Laurance. 1999. "Effect of surrounding vegetation on edge-related tree mortality in Amazonian forest fragments." *Biological Conser*vation 91: 129–134.
- Pellissier, V., L. Bergès, T. Nedeltcheva, M.-C. Schmitt, C. Avon, C. Cluzeau, and J.-L. Dupouey. 2013. "Understory plant species show long-range spatial patterns in forest patches according to distance-to-edge." *Journal of Vegetation Science* 24: 9–24.
- Piessens, K., O. Honnay, R. Devlaeminck, and M. Hermy. 2006. "Biotic and abiotic edge effects in highly fragmented heathlands adjacent to cropland and forest." *Agriculture, Ecosystems and Environment* 114 (2): 335–342.
- Porté, A., F. Huard, and P. Dreyfus. 2004. "Microclimate beneath pine plantation, semi-mature pine plantation and mixed broadleaved-pine forest." *Agricultural and Forest Meteorology* 126: 175–182.
- Ranney, J.W., M.C. Bruner, and J.B. Levenson. 1981. "The importance of edge in the structure and dynamics of forest islands." In *Forest island dynamics in man-dominated landscapes*, edited by R.L. Burgess and D.M. Sharpe, 67–95. Berlin: Springer Verlag.
- Ries, L., and W.F. Fagan. 2003. "Habitat edges as a potential ecological trap for an insect predator." *Ecological Entomology* 28: 567–572.
- Ries, L., and T.D. Sisk. 2004. "A predictive model of edge effects." *Ecology* 85: 2917–2926.
- Ries, L., R.J. Fletcher Jr, J. Battin, and T.D. Sisk. 2004. "Ecological responses to habitat edges: mechanisms, models, and variability explained." *Annual Review of Ecology, Evolution, and Systematics* 35: 491–522.
- Ries, L., and T.D. Sisk. 2010. "What is an edge species? The implications of sensitivity to habitat edges." *Oikos* 119: 1636–1642.
- Strayer, D.L., M.E. Power, W.F. Fagan, S.T. Pickett, and J. Belnap. 2003. "A classification of ecological boundaries." *BioScience* 53: 723–729.

#### Appendix 1. Values of p for pairwise Student's t-tests

Pairwise Student's *t*-tests were used to compare (1) the species richness and (2) the Shannon species diversity between the forest and open habitats and their respective edges, in the three regions of France. In bold, significant tests at  $\alpha = 0.05$ .

		Rich	ness	Shannon		
Region	Contrast	Forest side	Open side	Forest side	Open side	
Aquitaine	High Low	0.4222	0.0576	<b>0.0319</b> 0.3329	0.1201	
Midi-Pyrénées	High	0.7674	0.0008	0.5185	0.0027	
Centre	Low High Low	0.7441 0.0580 <b>0.0005</b>	5.3e-05 5.8e-05 0.0156	0.6428 0.0385 0.0005	0.0001 2.8e-05 0.0065	

### Can we reliably estimate species richness with large plots? an assessment through calibration training

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**Abstract** The number of species (species richness) is certainly the most widely used descriptor of plant diversity. However, estimating richness is a difficult task because plant censuses are prone to overlooking and identification errors that may lead to spurious interpretations. We used calibration data from the

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E. Corcket UMR1202 BioGeCo, Université Bordeaux 1, Avenue des Facultés, F-33405 Talence, France French ICP-level II plots (RENECOFOR) to assess the magnitude of the two kinds of errors in large forest plots. Eleven teams of professional botanists recorded all plants on the same eight 100-m<sup>2</sup> plots in 2004 (four plots, eights teams) and 2005 (four plots, nine teams including six from 2004), first independently and then consensually. On average, 15.5% of the shrubs and trees above 2 m were overlooked and 2.3% not identified at the species level or misidentified. On average, 19.2% of the plant species below 2 m in

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J.-F. Dobremez Université de Savoie, Dynamique des Ecosystèmes d'Altitude, F-73376 Le Bourget du Lac Cedex, France e-mail: dobremez@univ-savoie.fr height were overlooked and 5.3% were misidentified and 1.3% were misidentified at the genus level (especially bryophytes). The overlooking rate also varied with plant species, morphological type, plot and team. It was higher when only one botanist made the census. It rapidly decreased with species cover and increased with plot species richness, the recording time of the census in the tree layer and the number of the censuses carried out during the day in the ground layer. Familiarity of the team with the local flora reduced the risk of overlooking and identification errors, whereas training had little impact. Differences in species richness (over space or time) in large plots should be cautiously interpreted, especially when several botanists participate in the survey. In particular, the quality of the data needs to be evaluated using calibration training and, if necessary, may be improved by involving more experienced botanists working in teams and by fixing a minimum recording time.

Keywords Calibration · Data quality · Long-term monitoring · Observer effect · Plant survey

#### Introduction

Plant communities may be investigated in forest ecosystems using large plots, in order to assess the

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impact of different land uses (e.g. Vellend et al. 2007), the temporal changes in the environment (e.g. van Tol et al. 1998) or the plant diversity itself (e.g. Thimonier et al. 1994). If the number of species (species richness) is certainly the most widely used descriptor of plant diversity, it has rarely been stated that the observed number of species might be a systematically biased, underestimate of true species richness because some species are unavoidably missed during the censuses (overlooking errors).

Non-exhaustiveness is a problem for any plant study; it is still more problematic in the case of biogeographical and monitoring studies as many botanists typically carry out the censuses over space and/or over time. Thus, differences in skill level among botanists may cause spurious spatial or temporal trends or may hide true trends. For instance, resampling of old relevés often shows an increase in species richness over time (e.g. Grabherr et al. 1994; Thimonier et al. 1994): this increase may simply result from a change in botanist, possibly because the botanists involved in the resampling searched for species more carefully than the former botanists (furthermore, they may have already known the list of past species, while the former botanists were not aware that their plots would be resampled).

Probably as a result of botanists' generally low awareness of this problem and/or willingness to

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accept this fact, relatively few studies have investigated the quality of plant censuses; however, all of them showed high levels of overlooking errors and significant differences between botanists (Nilsson and Nilsson 1985; Lepš and Hadincová 1992; Klimeš et al. 2001; Scott and Hallam 2002; Kercher et al. 2003; Archaux et al. 2006). Only three studies quantified the misidentification rate in plant censuses: Klimeš et al. (2001) found that misidentification was more important than overlooking, whereas Scott and Hallam (2002) and Archaux et al. (2006) found the contrary. However, the procedures used in the three studies to estimate identification errors are questionable since the botanists did not go back to the plots they had sampled to agree together on an accurate final list of species. Some factors influencing overlooking and misidentification have been evidenced, such as plant cover, morphological type and experience of the botanist (Klimeš et al. 2001; Scott and Hallam 2002; Archaux et al. 2006). However, some questions remain. Scott and Hallam (2002) apparently included a non-botanist manager and a student in their analyses, but we do not know whether differences between observers still persist among experienced botanists. Furthermore, many factors potentially impacting the census quality have never been investigated such as vertical layer, the number, training and fatigue of the botanists, although most botanists would acknowledge that these factors influence the quality of the plant censuses.

To reduce overlooking as much as possible, some monitoring programmes have been based on small plots, subdivided into subplots (e.g. Økland 1995 with  $1-m^2$  plots subdivided in 16 subplots). However, in temperate forests, only a very few species are usually found per m<sup>2</sup>, so that many monitoring programmes, including the only pan-European one, the ICP Forests Level II programme (international cooperative programme on assessment and monitoring of air pollution effects on forests), as well as many resampling studies and floristic databases (e.g. Gégout et al. 2005) are based on larger plots. For instance, 577 of the 708 ICP Forests Level II plots are 100 m<sup>2</sup> or more (de Vries et al. 2003).

In this context, our study aimed at:

1 Estimating the exhaustiveness of plant censuses carried out on large (100-m<sup>2</sup>) plots to assess whether differences in species numbers (over space or time) can be reliably interpreted using large plots,

2 Investigating the factors that may impact the quality of the censuses, such as plant coverabundance, plant morphology, vertical layer, number of botanists, their familiarity with the flora, fatigue and training. The identification of impacting factors may help improve sampling protocols.

#### Methods

Sampling design and relevés

We used the calibration training data gathered by the Quality Assurance (QA) procedure of the RENECO-FOR programme (Camaret et al. 2004). The RENECOFOR programme is the French part of the ICP Forests Level II programme and includes 102 permanent plots sampled every 5 years by professional botanists. Each plot is composed of eight  $2 \times 50$ -m subplots (four being fenced to exclude large herbivores). One of the objectives of the calibrations is to estimate the overlooking and misidentification rates for the teams involved in the monitoring of the permanent plots (11 teams in 1995, 10 in 2000 and 2005, 16 in total).

Calibrations were organised during two successive years in June. In each year, four  $2 \times 50$  m subplots were chosen so that they were as heterogeneous as possible in terms of species richness and composition: species numbers in the strata below 2 m in height ranged from 15 to 44 among the four subplots sampled in 2004 and from 37 to 67 in 2005 (Table 1). In 2004, calibration training took place near Issoudun (central France), and involved eight teams. In 2005, the calibration was located near Nancy (north-eastern France) and involved nine teams, including six teams who had participated in the calibration near Issoudun. The least experienced botanist had sampled plant communities since 1995, whereas most botanists had more than 15 years of experience (up to 30 years for three botanists).

In each team, the number of botanists varied from one to three (Table 1). On the first day of the calibration training, each team visited each subplot according to a random sequence. They walked **Table 1** Location, sampling year, main tree species andspecies richness in the ground layer (in italics, species richnessin the tree and shrub layers) of the eight subplots, ground-layervegetation overlooking and misidentification (in italics) rates

(%) for the 11 teams (in brackets the number of botanists in the team; only for team five, botanist number was one in 2004 but two in 2005)

Location	Main tree species	Species richness	Team's overlooking and misidentification rates										
and year			1 (1)	2 (2)	3 (1)	4 (2)	5 (1/ 2)	6 (2)	7 (3)	8 (2)	9 (1)	10 (1)	11 (1)
Issoudun	Quercus petraea, Pinus pinaster	15	26.7	-	-	33.3	40	20	13.3	20	26.7	_	53.3
2004		4	0			20	22.2	0	0	8.3	0		0
	Q. petraea	28	17.9	_	_	25	21.4	17.9	21.4	7.1	17.9	_	25
		4	0			9.5	0	4.3	9.1	0	0		0
	Q. robur, Carpinus betulus, Sorbus torminalis	44	11.4	_	_	20.5	11.4	13.6	22.7	2.3	20.5	_	25
		12	7.7			8.6	12.8	10.5	14.7	4.7	5.7		9.1
	Q. petraea	41	24.4	_	_	19.5	17.1	19.5	17.1	14.6	26.8	_	24.4
		8	3.2			6.1	8.8	3	14.7	2.9	3.3		6.5
Nancy 2005	Tilia cordata, C. betulus, Acer	64	_	14.1	28.1	25	20.3	7.8	_	17.2	18.8	23.4	_
	campestre	7		3.6	6.5	8.3	0	3.4		1.9	0	2	
	Q. petraea, C. betulus, S. aria	50	_	10	20	18	24	14	_	6	10	26	_
		11		0	10	22	2.6	0		2.1	6.7	2.7	
	Q. petraea, C. betulus, A. campestre	67	14.9	11.9	28.4	20.9	11.9	7.5	_	6	11.9	13.4	_
		9	3.5	0	8.3	13.2	1.7	0		1.6	6.8	12.5	
	Fagus sylvatica, Q. petraea, C. betulus	37	29.7	10.8	32.4	24.3	24.3	10.8	_	10.8	13.5	21.6	_
		7	3.8	6.1	0	17.9	0	0		3	12.5	3.4	

We included in the misidentifications the cases where a team correctly identified a species but also noted a second, wrong name from small/atypical specimens belonging to the same species

outside the subplots to avoid damaging the vegetation as much as possible but entered the subplots whenever necessary for identification. They recorded all vascular plants and terricolous bryophytes and estimated their cover-abundance in four vertical strata using the Braun-Blanquet semi-quantitative scale (r: cover less than 5%, only one individual; +: cover less than 5% and rare; 1: cover less than 5% and abundant; 2: 5-25% cover; 3: 25-50% cover; 4: 50-75% cover; 5: 75-100% cover). If plant identification (at the species or genus level) was doubtful, the teams added the *confere* (cf.) Latin prefix before the species or genus name. Time spent doing relevés was not controlled but was recorded. On the second day, to produce a consensual list of species in the subplots, all the teams surveyed the four plots again and together re-identified all the species they had individually recorded the day before. During the second day, very few new species were found (one in 2004 and two in 2005). Some plants could not be identified with certainty to the species level (small vegetative specimens). Teams noted the cause of the discrepancies between the consensual list and their own list (e.g. species considered outside the subplot by the team or the consensus, species overlooked, species misidentified, inversion of strata). In 2005, the teams also consensually estimated the plant cover during the second day. To get a similar consensual cover estimate for 2004 plant data, for each plant, we calculated the median of the reported Braun-Blanquet cover values for all the teams that recorded it. In the few cases where an equal number of teams recorded two different cover classes, we kept the most likely one. We weighted each observation by the probability that the cover reported by the team was correct. This probability was calculated for a given team and a given Braun-Blanquet class using 2005 data as the ratio between the number of times the team reported this Braun-Blanquet class correctly (i.e. the consensus agreed upon this cover class for the same plant) and the total number of times the team reported this cover class (correctly or not). The nomenclature is Flora Europaea (Tutin et al. 1968-1980, 1993).

#### Overlooking and misidentification rates

We considered four kinds of error: overlooking, misidentification at the species level, misidentification at the genus level and complex misidentification. The overlooking rate corresponds to the probability of missing a species during the census. The misidentification rate at the species level is the probability that a plant is misidentified (the species name is wrong); or identified at the genus only (the species name is lacking). Following Scott and Hallam (2002), we considered identifications at the genus level (incomplete identifications) as misidentifications at the species level (when the genus was correct), rather than analysing them separately, because there is a continuum between incomplete identifications and true misidentifications (i.e. the species name is wrong): facing the same seedling of Quercus robur, different teams could note Quercus sp., Quercus cf. petraea or Quercus petraea depending naturally on their level of experience, but also to some extent on their willingness to note incomplete names. None of the misidentifications at the species level for the tree layer were incomplete identifications, whereas incomplete identifications represented 48% of the misidentifications at the species level for the ground layer. The misidentification rate at the genus level is the probability that a plant is given a wrong genus name. Complex misidentifications correspond to cases where a team distinguishes two closely related species (for instance Tilia cordata and Tilia platyphyllos) when only one is present (e.g. Tilia cordata); complex misidentification rate is the probability of committing such an error.

The overlooking rate for a given team in a given plot was calculated as the ratio between the number of species overlooked by this team in this plot and the number of species consensually agreed by all teams in the same plot. The misidentification rate for a given team in a given plot was the ratio between the number of misidentifications made by this team in this plot and the number of species the team had recorded.

The number of cases of misidentification at the species or genus level in the tree layer (respectively, 10 and 3 out of 442 records) and of complex misidentification in the ground layer (18 out of 2,357 records) was too low to explore the factors explaining these errors (thus, only mean values are

reported). Therefore, factors affecting misidentification rates at the species and genus level were only analysed for ground vegetation.

#### Data selection

We analysed the error rates in two vegetation layers (ground and tree layers, respectively, below and above 2 m in height). As data initially comprised four vegetation layers (plus the bryophyte layer), we merged records of vascular species found in the two ground vegetation strata below 2 m and the two tree strata above 2 m and we kept the highest Braun-Blanquet cover value in each. The consensual lists of species retained in 2004 and 2005 are given in Appendix 1 in Supplementary Material (65% of the 148 species recorded were overlooked at least once and 40.5% misidentified at least once).

In the consensus list for each plot, we kept only the taxa that were identified at the species level and deleted those identified only at the genus level or with a cf. in the taxon name (on average 3.6 plants per plot for ground vegetation, representing 9.6% of the total number of taxa recorded; with a range over the eight plots of 0-6 plants, 0-21% of the total); thus, all species kept for analyses could be identified at the species level. Alternatively, we could have applied this data selection for the calculation of the misidentification rate only and kept all records for the overlooking rate but we preferred to use the same data set in all the analyses. We considered that the observations with a cf. in the taxon name corresponded to correct identifications if the species name recorded by the team was the one agreed upon by the consensus (e.g. team 1 noted Viola cf. riviniana and the consensus agreed on the presence of Viola riviniana).

#### Data analysis

Each observation was a binary variable (species recorded or overlooked for the overlooking rate; species identified or not for the misidentification rate) corresponding to a given species in a given subplot sampled by a given team. To relate these variables to sets of explanatory random and fixed factors, we applied generalised linear mixed-effect models (GLMEM) with a binomial logit link function using the lmer function from the R-package lme4

Strata	Ground	Tree			
Variables	Overlooking	Misid. species	Misid. genus	Overlooking	
Random effects					
Plant species	$1.45 \pm 0.14$	$1.12 \pm 0.04$	$1.91\pm0.07$	$1.30 \pm 0.24$	
Team	$0.46 \pm 0.12$	$0.48 \pm 0.12$	$0.36\pm0.02$	$0.00 \pm 0.00$	
Subplot	$0.27\pm0.03$	$0.09\pm0.00$	$0.00\pm0.00$	$0.00\pm0.00$	
Fixed effects					
Plant cover	$-2.31 \pm 0.17^{***}$	$-0.33 \pm 0.24$	$-0.88 \pm 0.68$	$-3.92 \pm 0.62^{***}$	
Bryophytes	$0.95\pm0.54$	$0.07\pm0.75$	$2.91 \pm 1.69$		
Forbs	$-1.27 \pm 0.42^{**}$	$0.31\pm0.46$	$1.24 \pm 1.41$		
Trees/Shrubs	$-0.93 \pm 0.43*$	$0.16\pm0.52$	$1.11 \pm 1.58$		
Number of botanists	$-0.45 \pm 0.27$	$-0.14 \pm 0.35$	$-0.80 \pm 0.85$	$-1.07 \pm 0.34^{**}$	
Order of the census	$0.14\pm0.07$	$-0.03 \pm 0.13$	$0.12 \pm 0.33$	$0.22\pm0.17$	
Recording time	$-0.01 \pm 0.01$	$-0.00 \pm 0.01$	$-0.03 \pm 0.03$	$-0.03 \pm 0.01*$	
Familiarity	$-0.35 \pm 0.10^{***}$	$-0.74 \pm 0.14^{***}$	$-1.02 \pm 0.34^{**}$	$-0.43\pm0.50$	
Training	$-0.03 \pm 0.03$	$0.14 \pm 0.05^{**}$	$0.13 \pm 0.11$	$0.08\pm0.05$	
Species richness	$0.03\pm0.02$	$0.01\pm0.02$	$-0.02 \pm 0.05$	$-0.08 \pm 0.02^{**}$	
Understory cover	$0.00\pm0.01$	$-0.01 \pm 0.01$	$0.02\pm0.02$		
Year/Plot	$-0.85\pm0.56$	$-1.26 \pm 0.68*$	$-0.21 \pm 1.76$	$1.06\pm0.69$	
Diff. identification	$-0.37 \pm 0.34$	$1.09 \pm 0.37^{**}$	$1.08\pm0.98$		
Freq. detection		$-0.05 \pm 0.84$	$-0.12 \pm 2.02$		

**Table 2** Mixed-effect models relating the error rates to random and fixed explanatory variables depending on the strata (Ground:below 2 m, Tree: above 2 m)

Coefficients for random effects are standard deviation and their standard deviation calculated from Monte Carlo Markov Chain sampling. Coefficients for fixed effects are estimates and their standard error. Italics: P < 0.1, \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. See Method section for explanation about the error rates and explanatory variables

(Bates et al. 2008; R Development Core Team 2008). GLMEM estimates *P*-values for fixed factors but not for random factors. To get an idea of the precision of the estimates and thus the magnitude of the random factors, we generated 1,000 Markov Chain Monte Carlo random effect estimates from the posterior distribution of the fitted GLMEM parameters using R function mcmcsamp; we then calculated the standard deviation of these 1,000 samples.

Explanatory variables included in the models were: plant species, morphological type and Braun-Blanquet class; subplot, subplot species richness, percentage cover of the ground vegetation in the subplot and plot/year (both being confounded); identity of the team (variable hereafter called "team"), recording time, order of the subplot survey in the day, training level of the team and familiarity with the plant species, difficulty to identify the plant, proportion of times the species was overlooked in the subplot. The last variable was naturally not included to model the overlooking rate. Variables used for the four error rates studied can be found in Table 2. Subplot, team and plant species were considered as random factors, all others as fixed factors. This allowed us to simultaneously consider (1) a random team effect and a fixed effect of the number of people in the team, and (2) a random subplot effect and fixed effects of the subplot species richness and percentage cover of the subplot ground vegetation. Plot/Year was considered to be a fixed factor to test the hypothesis that error rates were smaller in 2005 thanks to the 2004 calibration training (an informal test as plot and year were confounded variables).

Morphological type was defined as a four-class variable: bryophyte, graminoid (grasses and grasslike plants), other herbaceous species (forbs), ligneous species (lianas, shrubs and trees) and was used only for ground vegetation (because only ligneous species were recorded above 2 m). A preliminary inspection of the data revealed that species whose Braun-Blanquet cover was 2 or more were never overlooked. Therefore, we merged the Braun-Blanquet cover classes from 2 to 5 into a single 2+ class (see Fig. 1 for ground vegetation). This ordinal variable was coded from 1 to 4 in the models (1 for the r class, 2 for the +, 3 for the 1 and 4 for the classes over 1). We used the same transformation of Braun-Blanquet cover for the analysis of the misidentification rate since very few plants with Braun-Blanquet cover 2 or more were misidentified (Fig. 2), suggesting that the risk of misidentification for a plant is roughly the same, the plant Braun-Blanquet cover being 2, 3, 4 or 5.

The recording time increased with plot richness according to a seemingly log-linear relationship



**Fig. 1** Relationship between the overlooking rate (%) and the plant Braun-Blanquet cover value in the ground-layer vegetation, according to plant morphology (*B* Bryophyte, *G* Graminoid, *NG* Non-Graminoid herbaceous, *L* Ligneous species)



**Fig. 2** Relationship between the misidentification rate at the species level (%) and the plant Braun-Blanquet cover value in the ground-layer vegetation, according to plant morphology (*B* Bryophytes, *G* Graminoids, *NG* Non-Graminoid herbaceous, *L* ligneous plants)



Fig. 3 Relationship between recording time and subplot species richness (eight subplots, 8–9 teams per plot, regression line: time =  $40.12 \ln(richness) - 74.28$ ,  $R^2 = 0.51$ )

 $(R^2 = 0.51, \text{Fig. 3})$ . In order to distinguish the effect of plot richness from the effect of recording time on the overlooking and misidentification rates, we had to remove this relationship. Therefore, the recording time was entered into the models as the residual of the regression of the recording time on the logarithm of plot plant richness. By using this transformed variable, we effectively tested whether a team who spent more time than expected given the plot richness, detected and identified a greater proportion of the plants.

The variable "order" corresponded to the rank of visit, by each team, of the four plots during the first day of the calibration training (thus the order varied from 1 to 4).

The training of the team was defined as the number of RENECOFOR plots the team had sampled during the 3 months preceding the calibration exercise.

The familiarity of the team with plant species was defined as a four-level ordered class variable: (1) species never encountered before the calibration training; (2) species encountered very occasionally; (3) species that the team records in ca 1% of its censuses (not restricted to the censuses done for the RENECOFOR monitoring); (4) species that the team records in ca 5% or more of its censuses. We considered this variable to be continuous (varying from 1 for unknown species to 4 for well-known species). This information was obtained through a questionnaire filled in by the teams shortly after the 2005 calibration and was lacking for one team (team 5).

We defined the difficulty to identify a species in a plot as a two-state variable depending on whether at least one team (or no team) used the *cf*. prefix for the species in the plot, thus showing they were not sure they had correctly identified it. As a result, a given plant species may be "difficult" in some plots and not in others.

#### Results

#### Tree layer

The mean overlooking rate (over the 66 censuses) was  $15.5 \pm 2.3\%$  SE. The mean misidentification rates were  $2.3 \pm 0.9\%$  SE at the species level and  $0.9 \pm 0.5\%$  SE at the genus level. The model indicated high random variation among species and little variation between teams and subplots. The overlooking rate decreased with the plant cover, the number of botanists, the recording time and, surprisingly, the subplot species richness.

#### Ground vegetation layer

The mean overlooking rate over the 66 censuses was  $19.2 \pm 1.1\%$  SE. The mean overall misidentification rate at the species level was  $5.3 \pm 0.7\%$  (including complex misidentifications even if the teams reported the correct species name). Simple confusions—a species being given a single (wrong or incomplete) name—accounted for 102/120 = 85% of the misidentifications. The mean overall misidentification rate at the genus level was  $1.3 \pm 0.4\%$  SE.

The overlooking rate decreased with plant cover (less rapidly than for species in the tree layer), morphological type (bryophytes being more often overlooked than graminoids, graminoids being more often overlooked than other vascular plants) and familiarity with the species. More marginally, the overlooking rate was smaller (1) in plots with fewer plant species, (2) when teams included at least two people and (3) for censuses done early in the day. Important residual random variation was found among plant species, teams and subplots.

The probabilities of misidentification at the species and genus level were best explained by familiarity of the team with the species. Species for which at least one team reported a cf. in the name were more often misidentified at the species level. Unexpectedly, the training increased the risk of misidentification at the species level. The overall misidentification rate at the species level tended to be smaller in 2005 than in 2004, while bryophytes tended to be more often misidentified at the genus level than vascular plants.

#### Discussion

Sampling errors: mainly overlooking or mainly identification errors?

Our results show that about one out of five groundlayer plant species was missed on average. Although lianas, shrubs and trees in the tree layer were less often overlooked, about one out of six of these species was nonetheless overlooked. Such high overlooking rate values may question either the way we calculated them, the experience of the professional botanists who participated in the RENECOFOR programme and/or the method of survey (large plots, Braun-Blanquet relevés).

Some of the plants may have been missed during both the independent and consensual visits, so that the overlooking rate may actually be slightly higher than the value we estimated. However, very few species were found during the consensual visits done by eight or nine teams suggesting that this effect is likely to be small. A second potential source of bias in the data comes from the fact that some of the plants recorded by some teams could not be found again during the consensual visits, and were thus considered to be misidentifications, instead of overlooking errors. Considering the low level of misidentification errors, it is also unlikely that this phenomenon caused a major bias in the two rates. In addition, most of the botanists who participated in our study are professional botanists who have been recognised in their field for many years in France. Some previous plant studies have quantified observer effect but using a variety of indices, such as the pseudo-turnover rate PT (Nilsson and Nilsson 1985; Lepš and Hadincová 1992; Klimeš et al. 2001; Kercher et al. 2003), the Sørensen similarity index SI (Gray and Azuma 2005) and the agreement rate AR (Kirby et al. 1986; Scott and Hallam 2002. All these indices are closely related (indeed: PT = 1 - SI = (1 - AR)/(1 + AR)), so that the

Number of SI (%) Study Number of Quadrat Vegetation type botanists quadrats size Nilsson and Nilsson 1985 Swedish forested islands 2 0.03-2.19 ha 41 88.6 (80.6-95.8) 2 Kirby et al. 1986 British forests 0.20 ha 70.7 (55-79.5) 36 2 ca 0.025 Lepš and Hadincová 1992 Central European open land 40 87 Klimeš et al. 2001 5  $10 \text{ cm}^2 - 4 \text{ m}^2$ 7 Central European grassland (60 - 88)Scott and Hallam 2002 Range of British vegetation types 2  $0.16 \text{ m}^2$ 10 \* 10 76.5 Gray and Azuma 2003 Range of North American vegetation types 2 168 m<sup>2</sup> 48 \* 4 66.6 2  $1 \text{ m}^2$ North American herbaceous wetlands Kercher et al. 2003 12 \* 10 80.9 (69.5-90.7)  $100 \text{ m}^2$ This study French forests 2 \* 411 89.1 (70-97.8)

Table 3 Sørensen Similarity Index (SI = 2 \* number of paired records/total number of records) reported in and calculated from the literature

In brackets, range of SI values between observers and plots. For our study, we calculated the SI by comparing paired raw lists of species recorded by two teams in the same plot

results from these studies can be compared to our study (e.g. using Sørensen similarity index, Table 3). Although conditions varied greatly among studies (in terms of geographic region, vegetation types, number of teams/botanists, quadrat and sample sizes), the SI values are remarkably consistent among studies (incl. our study). It seems that higher values were found for larger plots or equivalently for forest plots (since larger plots are used in forests).

Our results are slightly lower than those reported by Klimeš et al. (2001) (ca 30%) and Archaux et al. (2006) (20–30%), possibly because:

- 1 Plant species may be more easily detected in the forest (mainly herbs/forbs) than in grasslands (mainly grasses),
- Mean sampling time was 74 min (for 100 m<sup>2</sup> quadrats), versus 60 min for 400-m<sup>2</sup> quadrats in Archaux et al. (2006),
- 3 We deleted all records not consensually identified at the species level. These records often referred to low-covering plants (often bryophytes). As low-covering plants are more often missed, our overlooking rate values are probably slightly optimistic.

About 5% of the ground layer species and 2% of the tree layer shrubs and trees were misidentified at the species level (ca 1% at the genus level for the two categories). These figures are very close to the ones reported in Scott and Hallam (2002) in various vegetation types where on average 2.7% of the plants were misidentified at the species level by experts and 0.2% at the genus level. Archaux et al. (2006) found a mean misidentification rate of 6.8% for 400-m<sup>2</sup> forest plots. The slightly higher value reported in the latter study may come from the fact that experts were less familiar with the local flora and/or potential misidentifications were inferred by comparing the team lists without confirmation in the field.

As found by Scott and Hallam (2002), Archaux et al. (2006) and suggested by Kirby et al. (1986), the misidentification rate at the species level was much lower than the overlooking rate. However, Klimeš et al. (2001) suggested that the observer discrepancies observed in small grassland quadrats resulted primarily from the misidentification of small specimens, rather than from overlooking errors. Although this might be true for very small quadrats where often only very few specimens per species are available for identification and/or although the risk of overlooking a species in small quadrats may be lower, this may not be true in general. Furthermore, it should be remembered that misidentifications are conditional on detection, so that we cannot estimate the misidentification rate of overlooked species, had they been detected; we may expect the misidentification rate to be higher for overlooked species, because these mostly include low-covering, infertile specimens. However, the risk of misidentification did not increase with the number of teams who overlooked the species in our study. Similarly, plants presenting a misidentification risk were not more often overlooked. Thus, the (non-estimatable) misidentification rate of overlooked species is probably close to the misidentification rate of detected plants.

### Plant and quadrat factors affecting the quality of the data

Plant cover was the main factor influencing the overlooking rate (Fig. 1 and 2), a result repeatedly found in plant studies (Lepš and Hadincová 1992; Klimeš et al. 2001; Archaux et al. 2006). On the contrary, the risk of misidentification was not significantly related to the cover of the plant, although Fig. 2 suggests this may hold for small, isolated graminoids. Scott and Hallam (2002) found that both the overlooking and misidentification rates varied greatly between species morphological types. In our study, the probability of missing a species was effectively greater for bryophytes than for graminoids, and greater for graminoids than for forbs and ligneous species. Bryophytes also tended to be misidentified at the genus level more often than vascular plants.

More marginally, species in the ground layer had a greater probability of being missed in rich plots (but not of being misidentified), but surprisingly, the reverse was found for trees and shrubs: botanists seem to more carefully screen the shrub and tree layers when the ground layer is species rich. This last result needs to be confirmed. Nonetheless, a bias of census exhaustiveness between rich and poor plots in favour of poor plots, similar to the one we found in the ground layer, had already been evidenced in temperate floristic relevés; this bias tended to vanish with longer censuses (Archaux et al. 2006).

Team-related factors affecting the quality of the data

We found strong, complex observer effects on both the overlooking and misidentification rates; these effects were related to the identity of the botanists, the familiarity with the local flora and, to a lesser extent, to the number of botanists in the team, training and fatigue. Observer effects were the main factor affecting the misidentification rates (together with the difficulty to identify the species for the probability of simple misidentification at the species level). Our results (and others) are contrary to the suggestion by Kirby et al. (1986)'s that overlooking is mainly a matter of chance and therefore, should be more or less constant across observers. Fewer species were missed when teams were composed of at least two people, certainly because the plot area effectively sampled increases with the number of people surveying the plot. The teams with at least two observers often included one expert plus one or two less-experienced observer(s): this probably explains why the number of observers had little impact on misidentification rates. Klimeš et al. (2001) recommended that at least three observers participate in plant censuses to guarantee data quality.

Experience has been repeatedly pointed out as one of the main factors affecting the quality of vegetation censuses (Kirby et al. 1986; Klimeš et al. 2001; Scott and Hallam 2002; Archaux et al. 2006). Scott and Hallam (2002) give figures showing that experts misidentify fewer species than less competent observers (misidentification rate of 2.7% for experts against 4.6% for competent observers and 14.1% for lessexperienced observers). It is not easy to evaluate the experience of an observer. In our case, we separated two aspects of experience: the familiarity with the local flora and the number of censuses carried out during the three months preceding the calibration exercises (training). Logically, the teams tended to more often misidentify the species they were less familiar with; a less expected result was that the teams also overlooked them more often: part of the overlooking errors concerning species teams are not familiar with are probably misidentifications in reality. This phenomenon probably explains why teams who misidentified a greater proportion of species also tended to miss more species (although the relationship is weak, Fig. 4). As a result, increasing the recording time would not necessarily reduce the differences in observed richness between teams; Archaux et al. (2006) effectively observed that differences in observed species richness between botanists do not decrease as the recording time increases.

Contrary to our expectation, training did not improve the quality of the censuses; on the contrary, we found that teams that carried out more censuses three months before the calibration trainings misidentified a greater proportion of plants in the ground layer. This would support the hypothesis that a kind of routine occurs in the course of a survey, i.e. trained teams may identify plants too quickly. Similarly, censuses done late in the day were generally less exhaustive in the ground layer than censuses done



**Fig. 4** Relationship between the overlooking rate and the misidentification rate for ground vegetation in 2004 (open symbols) and 2005 (closed ones) (Spearman's r = 0.40, z = 1.58, P = 0.11). Paired dots correspond to the six teams who were involved both years

early in the day. One explanation may be that the vegetation is progressively damaged by the visits of the teams, although the teams mainly stayed outside the subplots. A more likely explanation is that the quality of the censuses diminishes as teams become more tired.

Surprisingly, the recording time reduced only the overlooking rate in the tree layer, indicating that recording time essentially limits gross errors. The limited effect of recording time on the quality of census results is contrary to Archaux et al. (2006) but agrees with the statement by Klimeš et al. (2001) that some observers/teams of observers may be slower or faster at completing their censuses. However, neither our work, nor the study by Klimeš et al. (2001) investigated how recorded species accumulate during the censuses, contrary to Archaux et al. (2006); this difference may explain why conclusions differed between these studies.

Finally, plants for which certain teams expressed some doubt about identification were indeed more often misidentified than the average.

#### Recommendations for plant surveys

The comparison of our results with former studies shows that a significant and relatively constant proportion of plant species are missed or misidentified in vegetation surveys. To our knowledge, however, the study of the magnitude of the observer effect has been restricted to temperate and boreal ecosystems and has never been done in tropical ecosystems. In particular, the overlooking and misidentification rates in the tree layer are likely to be higher in tropical forests than in temperate and boreal forests, due to the far higher diversity in tree species. In temperate and boreal regions, our results question the use of large plots to track differences in species richness over space or time, especially if the expected differences in richness are small. For instance, plant communities usually evolve slowly during the forest succession (except during the youngest successional stages) (Aubert et al. 2003); thus long-term monitoring programmes such as ICP Forests may not allow us to reliably show such slow dynamics, unless plots are surveyed by a single team over time and team skills do not change over time (two options probably not realistic in the long term). Similarly, biogeographical studies based on large data sets gathered by different teams of botanists in different areas may suffer similar biases. For instance, in 2000, teams participating in RENECO-FOR were asked to record the presence of browsing or fraying indices in the plots in addition to the name and abundance of the plant species: the spatial distribution of the records of browsing and fraying exactly matches the spatial distribution of the teams (Camaret et al. 2004). Since the teams survey groups of plots, entire regions, sometimes with high abundance of large herbivores, seem erroneously free from herbivore impact.

There are obvious ways to reduce these errors such as involving only botanists familiar with the local flora, making only a few censuses per day and limiting their number during the vegetation season, and regularly enhancing team motivation (as species are much more often missed than misidentified). In our study, most botanists were not experts in bryology, which may explain why many bryophytes were overlooked and/or misidentified: we would recommend sampling vascular plants and bryophytes separately because (1) searching for bryophytes on the ground distracts the attention of the botanist from higher vegetation layers and vice versa, and (2) botanists often have different identification skills for the two taxonomic groups.

The overlooking rate could also be reduced using smaller plots by focusing the attention of the botanists on a smaller area. However, this may not necessarily be the case. For instance, Archaux et al. (2007) found the repeatability of species richness to be similar for 2, 4 or 400-m<sup>2</sup> plots (see also Table 3). Increasing the recording time may be more effective than reducing the plot size. For instance, on  $100-m^2$  forest plots, it may be necessary to spend at least 2 or 3 hours to get more acceptable levels of exhaustiveness (it my also be sensible to subdivide the plots into smaller subplots, e.g. 50 1 m × 2 m subplots). Fixing such a minimum recording time may also limit the bias of exhaustiveness we observed between species-rich and species-poor quadrats (cf Archaux et al. 2006).

A second significant improvement of plant surveys would be the use of teams of observers, even if all of them are not experts, as recommended by Klimeš et al. (2001). Teams composed of one expert in vascular plants and one bryologist would probably be an ideal configuration for plant studies. As species whose identification teams doubted were indeed more often misidentified, we strongly encourage teams involved in monitoring programmes to note their doubt in the field as these records could be more easily related to previous or future records on the same plots.

Finally, even though calibration exercises apparently failed to significantly reduce observer effects, we do think they help convince botanists of the necessity to spend enough time on the plots to carefully check all specimens of species prone to misidentification. Calibrations also have some limits: bringing all the teams together is costly and team behaviour during training is likely to be different from that in the field. Control visits are better adapted to obtain more realistic estimates of observer errors: during the survey, each team samples a few plots shortly after these plots have been sampled by other teams; observer effects can be estimated from these pairs of independent censuses done by different teams on the same plots. However, control visits cannot be used to distinguish between overlooking and identification errors (unless the controlled team accompanies the control team during the control visit to establish a consensual list). Thus, we recommend associating calibrations and control visits in plant surveys in general and in monitoring programmes in particular.

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#### References

- Archaux F, Bergès L, Chevalier R (2007) Are plant censuses carried out on small quadrats more reliable than on larger ones? Plant Ecol 188:179–190
- Archaux F, Gosselin F, Bergès L, Chevalier R (2006) Effects of sampling time, quadrat richness and observer on exhaustiveness of plant censuses. J Veg Sci 17:299–306
- Aubert M, Alard D, Bureau F (2003) Diversity of plant assemblages in managed temperate forests: a case study in Normandy (France). For Ecol Manage 175:321–337
- Bates D, Maechler M, Dai B (2008) The Ime4 Package. Available at: http://lme4.r-forge.r-project.org/
- Camaret S, Bourjot L, Dobremez JF (2004) Suivi de la composition floristique des placettes du réseau (1994/95– 2000) et élaboration d'un programme d'assurance qualité intensif. Office National des Forêts, Direction Technique, Fontainebleau
- de Vries W, Reinds G, Posch M, Sanz MJ, Krause G, Calatayud V, Renaud J, Dupouey J, Sterba H, Vel E, Dobbertin M, Gundersen P, Voogd J (2003) Intensive monitoring of forest ecosystems in Europe, Technical Report 2003. EC-UN/ECE, Brussels, Geneva
- Gégout JC, Coudun C, Bailly G, Jabiol B (2005) EcoPlant: a forest site database linking floristic data with soil and climate variables. J Veg Sci 16:257–260
- Grabherr G, Gottfried M, Pauli H (1994) Climate effects on mountain plants. Nature 369:448
- Gray AN, Azuma DL (2005) Repeatability and implementation of a forest vegetation indicator. Ecol Indic 5:57–71
- Kercher SM, Frieswyk CB, Zedler JB (2003) Effects of sampling teams and estimation methods on the assessment of plant cover. J Veg Sci 14:899–906
- Kirby KJ, Bines T, Burn A, Mackintosh J, Pitkin P, Smith I (1986) Seasonal and observer differences in vascular plant records from British woodlands. J Ecol 74:123–132
- Klimeš L, Dancák M, Hájek M, Jongepierová I, Kucera T (2001) Scale-dependent biases in species counts in a grassland. J Veg Sci 12:699–704
- Lepš J, Hadincová V (1992) How reliable are our vegetation analyses? J Veg Sci 3:119–124
- Nilsson IN, Nilsson SG (1985) Experimental estimates of census efficiency and pseudoturnover on islands: error trend and between-observer variation when recording vascular plants. J Ecol 73:65–70
- Økland RH (1995) Changes in the occurrence and abundance of plant species in a Norwegian boreal coniferous forest, 1988–1993. Nordic J Bot 15:415–438
- R Development Core Team (2008) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

- Scott WA, Hallam CJ (2002) Assessing species misidentification rates through quality assurance of vegetation monitoring. Plant Ecol 165:101–115
- Thimonier A, Dupouey JL, Bost F, Becker M (1994) Simultaneous eutrophication and acidification of a forest ecosystem in North-East France. New Phytol 126: 533–539
- Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (1968–1980, 1993) Flora Europaea. Cambridge University Press, 5 vols
- van Tol G, van Dobben HF, Schmidt P, Klap JM (1998) Biodiversity of Dutch forest ecosystems as affected by receding groundwater levels and atmospheric deposition. Biodiv Cons 7:221–228
- Vellend M, Verheyen K, Flinn KM, Jacquemyn H, Kolb A, van Calster H, Peterken G, Graae BJ, Bellemare J, Honnay O, Brunet J, Wulf M, Gerhardt F, Hermy M (2007) Homogenization of forest plant communities and weakening of species–environment relationships via agricultural land use. J Ecol 95:565–573



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### Respective influence of habitat conditions and management regimes on prealpine calcareous grasslands

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#### Abstract

The calcareous grasslands of the south-western French Alps have been poorly studied, although they provide suitable habitat for rare plant species and communities. The separate and combined effects on calcareous grassland communities of habitat conditions (lithology, soil moisture) and management regimes (grazing intensity, cutting regime) were studied using constrained ordination techniques (canonical correspondence analysis with variance partitioning). Among the explanatory variables considered, the most important factor determining floristic composition was lithology, which explained 11.9% of floristic variability, followed by grazing intensity (6.0%). Additive effects of management and lithology explained 23.9% of floristic variability. Species niche amplitude was measured by conditional variances of samples along main ordination axes, in order to define adequate conservation management for the rarest short-lived species with narrow niche breadth on both habitat and management gradients.

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#### 1. Introduction

European calcareous grasslands are species-rich communities providing useful examples for the study of disturbance effects on species  $\alpha$ -diversity and the mechanisms of species coexistence at the community level (During and Willems, 1984; Van der Maarel and Sykes, 1993; Gigon and Leutert, 1996; Thompson et al., 1996; Schläpfer et al., 1998; Dutoit et al., 1999). Important mechanisms explaining high levels of species coexistence in calcareous grassland communities include differences between regeneration niches of species (rather than differences between habitat niches of adult plants) (Van der Maarel and Sykes, 1993), spatio-temporal variations in opportunities for regeneration (Willems et al., 1993; Thompson et al., 1996) and for dispersal of species (Poschlod et al., 1998). Management regimes such as grazing and mowing have a central role in the establishment and maintenance of small-scale heterogeneity in grasslands, through patch dynamics (Gibson and Brown, 1991; Van der Maarel, 1996), together with geophysical factors such as geological underground, soil type and depth, or microtopography (Grime, 1990; Gigon and Leutert, 1996). Some previous studies focused on the interactions between stress (e.g. water and nutrient availability) and disturbance (e.g. farming management) (Fernandez Ales et al., 1993; Mc Intyre and Lavorel, 1994; Roche et al., 1998), or between several disturbance regimes (Chaneton and Facelli, 1991; Belsky, 1992; Noy Meir, 1995; Bergmeier, 1997; Dutoit et al., 1999). They demonstrated the importance of interactive and cumulative effects of different management regimes in the maintenance of species coexistence in grasslands, especially for conservation management purposes (Bullock and Pakeman, 1997; Willems, 2001; WallisdeVries et al., 2002).

Southern limestone French Prealps represent hotspots for the conservation of semi-natural dry calcareous grasslands due to the exceptionally large extension of those habitats in

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this submediterranean area, compared to northern Europe. However, calcareous grasslands of this area have received little attention by plant ecologists, and existing studies focus mostly on phytosociological classification of communities (Gaultier, 1989). In contrast with the situation in northern Europe where most of the remaining calcareous grasslands are no longer used for agriculture, calcareous grasslands of the Prealps are still currently used for low-intensity livestock farming supported by agri-environmental schemes (Barbaro et al., 2001).

Low-intensity farming systems allow the maintenance of large patches of calcareous grasslands (>100 ha) where dispersal of plants and insects is maintained by high habitat connectivity (Baudry and Merriam, 1988; Mortimer et al., 1998; Poschlod et al., 1998). An increasing risk of development towards woody landscapes through shrub encroachment of grasslands has been reported from several parts of the southern French and Swiss Prealps where more than 50% of the dry limestone grasslands have already disappeared since 1950 (Léouffre and Leclerc, 1996; Stampfli and Zeiter, 1999). As a result, many species with particular life traits and high habitat specialisation have been lost recently in calcareous grasslands of central Europe due to a decrease in dispersal and establishment opportunities at the landscape level (Fischer and Stöcklin, 1997; Poschlod and Bonn, 1998).

However, there is a lack of knowledge concerning the influence of particular management regimes (i.e. current low-intensity agro-pastoral practices, often the combination of cutting and grazing) on calcareous grassland dynamics (Stampfli and Zeiter, 1999). The various grassland management regimes controlled by livestock farming in the Vercors Prealps offer an interesting context for the study of their effects on the species composition of calcareous grasslands. Moreover, such management regimes occur in very particular habitat conditions, due to the biogeography (i.e. a bioclimatic gradient between submediterranean and northern prealpine regions), and geomorphology (i.e. the Urgonian limestone tableland within the study area).

In the present study, the following questions are asked: (i) What are the respective influence of habitat conditions and management regimes on the floristic composition of prealpine calcareous grasslands? (ii) What are their combined effects, and which combination has the largest impact on floristic variability? (iii) Is the measurement of species niche amplitude in the above-defined ecological gradients a useful tool to identify target species for conservation?

#### 2. Materials and methods

#### 2.1. Study area and data collection

The Vercors is one of the most western massifs of the external belt of the Alps. It extends to the northern subalpine range of the French Alps, which are sedimentary mountains oriented along a NW–SE direction, with a relief structured by two deep limestone layers (Debelmas and Kerckhove, 1980). Geomorphological investigations have shown that the Vercors may be divided into several 'litho-systems' ('landscape units' *sensus* Barthès and Bornand, 1987) such as 'pure limestone systems' including the Urgonian tableland, 'marly limestone and marl systems', and 'slopes below cliff systems' which include slope screes and colluvium (Boissier, 2000).

The Vercors is located at the bioclimatic limit between the central European and Mediterranean regions (Fig. 1).



Fig. 1. Localisation of the study area (in grey: municipality territories where the 102 calcareous grasslands were sampled).

The Mediterranean influence is important at the latitude of Die (400 m, Drôme district, 5°22′06″E, 44°45′25″N), in the southern part of the Vercors tableland, with a mean annual rainfall of 900 mm  $yr^{-1}$  and a mean annual temperature of 11 °C. By contrast, the prealpine influence is predominant in the north-eastern part of the range, in Villard-de-Lans  $(1040 \text{ m}, \text{Isère district}, 5^{\circ}33'05''\text{E}, 45^{\circ}04'19''\text{N})$ , with a mean annual rainfall of  $1200 \text{ mm yr}^{-1}$  and a mean annual temperature of 7 °C. The soils are mostly shallow Rendosols, stony Lithosols, Brunisols and Colluviosols (Corcket, 2000). Decarbonatation occurs above ca. 1000 m of elevation due to increasing rainfall, and depends strongly on geological underground and topographical position. Habitat conditions within the study area range from xeric conditions (Urgonian limestone of the tableland) to mesoxeric (marl and marly limestone of hillsides) and mesic conditions (colluvium and clays of the lower part of slopes and valleys).

A stratified sampling of 102 calcareous grasslands, according to the main agro-ecological variables, was performed after preliminary classifications of Vercors grasslands (Barbaro and Cozic, 1998). The species composition of these 102 calcareous grasslands was measured in 1996 and 1997, by the use of a 10-m long line transect which was established randomly within each grassland in order to take into account the small-scale heterogeneity of the vegetation, especially in mosaics of grassland and shrubland patches. Along this transect, we sampled 50 point-quadrats separated by 20-cm intervals. In each of these 50 points, we counted the number of individuals of each taxa touching a 30-cm long needle established perpendicular to the line and the soil where the transect was established. Daget and Poissonet (1995) and Sutherland (1996) have stressed that 50 points are enough to characterise the floristic composition and structure of grassland and meadow vegetation. Thus, the number of pins of each species is proportional to the species cover and the species frequency used in the statistical analyses is the frequency of occurrence of a given species on 50 points within a given sample.

Lithology and soil moisture were retained as the best explanatory variables (Table 1) according to their relative contributions in preliminary multivariate analyses of a table of 8 habitat condition variables (Barbaro et al., 2000). These variables included elevation, slope, aspect, topography, mean annual temperature and decarbonatation level (reaction of top soil horizon to HCl). According to the ordination axes of a correspondence analysis on floristic data, climatic variables and elevation ranked third in importance, after edaphic stress and management, respectively (Barbaro and Cozic, 1998). This is probably due to the fact that we restricted our study to grasslands located under 1200 m a.s.l., where mountain plant species are still scarce compared to higher elevations. There is also a relationship between lithology and elevation, since Urgonian limestones are situated at higher elevations, and marly limestones and colluvium at lower ones.

Table 1	
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Categories of habitat and management variables

	Categories	Frequency (%		
Habitat variables				
1. Lithology	a. Urgonian limestone	22.5		
	b. Slope scree	14.7		
	c. Marly limestone	25.5		
	d. Marl	16.7		
	e. Colluvium and clay	20.6		
2. Soil moisture (mm)	a. <20	34.3		
	b. 20–40	29.4		
	c. >40	36.3		
Management variables				
1. Cutting regime	a. Regular cutting (annual)	36.3		
0 0	b. Irregular cutting (pluriannual)	35.3		
	c. Uncut	28.4		
2. Grazing intensity (L.Udays $ha^{-1}$ )	a. <50	23.5		
· · · /	b. 50–200	35.3		
	c. 200–400	21.6		
	d. >400	19.6		

The percentage frequency of each category in the total sample is given (n = 102).

A quantitative estimation of soil moisture was calculated as follows, based on simple measures of thickness, rockiness and texture from a soil profile description for each of the 102 grasslands (d'Epenoux, 1992):

$$SMI = \sum SMI(H_i)$$

 $SMI(H_i) = text(H_i) \times thick(H_i) \times (1 - rock(H_i))$ 

SMI( $H_i$ ) is soil moisture index of horizon *i* (in mm), text( $H_i$ ) is moisture coefficient for a given texture, e.g. 2 for silty clay texture (in mm per cm of thickness), thick( $H_i$ ) is thickness of horizon *i* (in cm) and rock( $H_i$ ) is rockiness of horizon *i* (in %).

Management regimes were characterised with several variables obtained from both farmer inquiries and field observations during a 6 year period (Barbaro et al., 2001). As for habitat variables, we retained the two best explanatory variables (Table 1) to describe management regimes (i.e. cutting or shrub-clearing frequency and grazing intensity, in Livestock-Units-days ha<sup>-1</sup>). These two variables were also retained after preliminary multivariate analyses of a table of eight management variables, which included paddock area, livestock type, grazing season and duration, number of livestock units per paddock and instantaneous stocking rate (Alard et al., 1994).

#### 2.2. Data analysis

Direct gradient analysis (i.e. canonical correspondence analysis (CCA)) was performed on the data set in order to quantify the relationships between explanatory variables and the floristic table (118 species-appendix  $1 \times 102$ samples). This method allows to study simultaneously complex relations between species, and between species and their environment (Ter Braak and Prentice, 1988; Økland, 1996; Van der Maarel, 1996). CCA (and its unconstrained form, correspondence analysis) is the most adequate ordination method, in accordance with the data table structure (i.e. unimodal response curves for most of the species) and because of its modelling properties (Ter Braak and Prentice, 1988; Prodon and Lebreton, 1994). The basic principle of CCA is to constrain the ordination axes to be linear combinations of explanatory variables (Lebreton et al., 1991).

Moreover, the respective and combined influence of habitat conditions (i.e. lithology and soil moisture) and management regimes (i.e. cutting regime and grazing intensity) can be studied by partial CCA or CCA with variance partitioning (Ter Braak, 1988; Sabatier et al., 1989; Lebreton et al., 1991; Økland and Eilertsen, 1994; Roche et al., 1998; Økland, 1999). It allows to remove statistically the effect of a predominant factor masking the effects of secondary factors still interesting to study (Yoccoz and Chessel, 1988).

Different combinations of the categorical variables were used to partition the variance in different effects (i.e. separate, additive and combined effects), based on sample projections in different orthonormal bases (Sabatier et al., 1989; Chessel, 1997). Statistical significance of the effect of each variable or combination of variables was tested according to a Monte-Carlo permutation test with 2000 permutations (Chessel, 1997). Moreover, the ratio of total inertia (I) of each separate CCA to total inertia of unconstrained CA can be considered as a Multiple Correlation Ratio (MCR, in %) (Sabatier et al., 1989). It allows to evaluate the percentage of total floristic variation explained by a given variable or combination of variables (Lebreton et al., 1991). The ratio of total inertia to the number of factors (i.e. the number of categories of explanatory variables), noted I/F, was calculated in order to compare the explanatory power of variables and combination of variables with different numbers of categories (3-5 in this study).

Multivariate analyses were performed in three steps: (i) analysis of the effect of habitat variables and their combined effects, (ii) analysis of the effect of management variables and their combined effects, (iii) analysis of the additive effect of various combinations of habitat and management variables, in order to obtain the best possible multivariate model for predicting floristic composition of calcareous grasslands (i.e. with both the highest possible MCR and the lowest number of factors compared to the number of samples).

Niche amplitude of each species along ordination axes can be calculated after CA (Chessel et al., 1982; Balent, 1991) or CCA (Mc Intyre and Lavorel, 1994; Alard and Poudevigne, 2000). It is measured, for each ordination axis, by the conditional variance of sample scores where a given species is present (Chessel et al., 1982), and allows to quantify species response to the main agro-ecological factors (main CA or CCA ordination axes). All the multivariate analyses were performed with ADE-4 software (Thioulouse et al., 1997).

#### 3. Results

# 3.1. Separate and combined effect of lithology and soil moisture

Analyses of the separate effects of habitat conditions showed that lithology was the most determinant factor with both highest total inertia (0.595), inertia per factor (0.119) and MCR (11.9%) (Table 2).

The separate effect of soil moisture explained 5.2% of total floristic variability, and the additive effect of lithology + soil moisture 14.5%, both significant at  $p \leq$ 0.001 according to a Monte-Carlo test with 2000 permutations. The combined effect of habitat conditions (lithology  $\times$  soil moisture) was also significant at  $p \leq$ 0.001 and explained 22.9% of the floristic ordination of samples in unconstrained CA (Fig. 2). As a result, the structure of floristic composition of prealpine calcareous grassland communities could be largely predicted by these soil variables only. This floristic structure was characterised by an ordination from mesic to xeric communities on axis 1, and on axis 2, from open short grasslands occurring preferentially on Urgonian limestone and colluvium, to grassland-scrub mosaics occurring preferentially on marly limestone and marl (Fig. 2).

#### 3.2. Separate and combined effect of management regimes

The separate influence of management regimes on floristic composition was 4.7% for cutting regime and 6.0% for grazing intensity, and the inertia for these factors was close (0.078 and 0.075, respectively); both were significant at  $p \le 0.001$  (Table 2) The additive effect of cutting regime + grazing intensity explained 9.1% of total floristic variability and the combined effect of cutting regime  $\times$  grazing intensity 14.5%. Management was less influential than habitat in explaining species composition but was still important, although partially masked by distinct habitat conditions (mostly water and nutrient availability). Axis 1 of the corresponding CCA was a management intensity gradient combining the influence of both cutting and grazing regimes (Fig. 3). As a result, taking only into account the management variables was not sufficient to predict the floristic composition of calcareous grassland communities mostly determined by lithology. As plant species have distinct habitat requirements and occur in particular grassland types more than others, their response
#### Table 2

Variance partitioning of canonical correspondence analysis and partial CCA

	Ι	F	I/F	MCR (%)	р
(1) Separate and combined effects of habitat					
Lithology	0.595	5	0.119	11.9	***
Soil moisture	0.258	3	0.086	5.2	***
Lithology + soil moisture	0.721	8	0.090	14.5	***
Habitat = lithology $\times$ soil moisture	1.145	15	0.077	22.9	***
(2) Separate and combined effects of management					
Cutting regime	0.233	3	0.078	4.7	***
Grazing intensity	0.298	4	0.075	6.0	***
Cutting regime + grazing intensity	0.453	7	0.065	9.1	***
Management = cutting regime $\times$ grazing intensity	0.724	12	0.060	14.5	***
(3) Additive effects of habitat and management					
Cutting regime + lithology	0.733	8	0.092	14.7	***
Grazing intensity + lithology	0.832	9	0.092	16.7	***
Management + lithology	1.191	17	0.070	23.9	***
Cutting regime + habitat	1.266	18	0.070	25.4	***
Grazing intensity + habitat	1.347	19	0.071	27.0	***
Management + habitat	1.671	27	0.062	33.5	***

*I* is total inertia of CCA, *F* is the number of factors (i.e. the number of categories per variable), MCR is a multiple correlation ratio measuring the quality of the prediction of sample scores by explanatory variables (% of total variance explained), it is calculated by the ratio: total inertia of CCA/total inertia of unconstrained CA, which is 4.99; *p* is the significance level according to the Monte-Carlo test with 2000 permutations, with \*\*\* $p \le 0.001$ .

to management can be masked by distinct habitat conditions. But even if the part of variance explained by management alone was less important than that explained by habitat conditions alone, species were ordinated according to their direct response to management irrespective of habitat conditions.

# 3.3. Additive effect of habitat conditions and management regimes

Since CCA remains a robust method as long as the number of factors is ca 10% of the number of samples (Prodon and Lebreton, 1994), several combinations of



Fig. 2. Sample ordination by CCA with combined effect of habitat (lithology × soil moisture). 1a = Urgonian limestones, 1b = Slope screes, 1c = Marly limestones, 1d = Marls, 1e = Colluvium and clays. 2a = Soil moisture < 20 mm, 2b = 20-40 mm, 2c = >40 mm. Each circle is located at the barycentre of samples occurring in each category.



Fig. 3. Sample ordination by CCA with combined effect of management (cutting regime × grazing intensity). 1a = Regular cutting, 1b = Irregular cutting, 1c = No cutting, 2a = Grazing intensity <50 L.U.-days  $ha^{-1}$ , 2b = 50-200 L.U.-days  $ha^{-1}$ , 2c = 200-400 L.U.-days  $ha^{-1}$ , 2d = >400 L.U.-days  $ha^{-1}$ .

habitat and management factors were attempted in order to obtain the best multivariate model of floristic variability (i.e. with both a high MCR and a low number of factors). Results showed that the highest percentage of variability (33.5%) was explained by the additive effects of habitat + management (i.e. (lithology × soil moisture) + (cutting regime × grazing intensity)), but with a high number of factors compared to the number of samples (27 versus 102). Thus, the best model was obtained with the additive effects of management + lithology, with a MCR of 23.9% and 17 factors (significant at  $p \le 0.001$ ), since the additive effects of grazing + habitat and cutting + habitat did not strongly increase the MCR (Table 2).

Species ordination by CCA of the additive effect of management + lithology (Fig. 4) is very close to that obtained by unconstrained CA. Thus, it indicated that the combination of cutting and grazing regimes that allows the maintenance of open short grassland communities with low shrub encroachment (Table A2) was found preferentially on colluvium and Urgonian limestones, mainly because of favourable topographical location. By contrast, mesoxeric grasslands occurring on marl and marly limestones were generally characterised by higher shrub encroachment (mostly with Genista cinerea, Juniperus communis and Pinus sylvestris), because of lower grazing intensity and absence or irregularity of mechanical cutting (Fig. 4; Table A2). The relationship between grazing intensity and lithology tested by one-factor ANOVA was significant at  $p \le 0.001$  (*F*-ratio = 5.472, n = 102).

Significant differences between mean grazing intensities under the different lithologies were tested with a Fischer's Least-Square-Difference test (Fig. 5). It indicated that significantly ( $p \le 0.002$ ) higher grazing intensities occur on Urgonian limestone (377.8 ± 51.1 L.U.-days ha<sup>-1</sup>) and colluvium (379.6 ± 53.5), whereas significantly lower ones occur on marly limestone (154.3 ± 48.1) and marl (126.9 ± 59.5).

# 3.4. Species responses to habitat conditions and management regimes

We established species niche amplitude along the first and second axis of the CCA with additive effects of management + lithology, i.e. respectively the gradient of edaphic stress (Fig. 6) and management intensity (Fig. 7). Species turnover along agro-ecological gradients was summarised, and it was possible to find indicator species for each stage of both gradients (i.e. species with both narrow niche amplitude and high correlation for a given factor). Thus, the idea of niche-sharing in oligotrophic grazed communities was illustrated by the high degree of niche overlap along gradients.

Moreover, species can be classified according to their response to habitat conditions and management regimes. On the edaphic stress gradient (Fig. 6), species restricted to xeric oligotrophic grasslands on very shallow soils had both isolation at the higher part of the gradient and narrow niche breadth along this axis (*Aira caryophyllea*,



Fig. 4. Species ordination by CCA with additive effect of management + lithology.

Trinia glauca, Bupleurum baldense, Bombycilaena erecta). By contrast, species widespread and often dominant under low to intermediate level of water and nutrient availability (e.g. Thymus serpyllum, Teucrium chamaedrys, Festuca ovina, Bromus erectus, Carex hallerana) had a large amplitude on the higher part of axis 1, as well as rarer species such as Trifolium scabrum, Petrorhagia prolifera or Argyrolobium zanonii. Species tolerating oligotrophy but not xericity and occurring preferably or exclusively on marls and marly limestones (Hieracium pilosella, Brachypodium pinnatum, Briza media, Ranunculus bulbosus, Lotus corniculatus) had a large habitat amplitude on the lower part of this axis. Species restricted to marly bedstones in the southern submediterranean part of the study area had a narrow niche breadth (e.g. Dorycnium pentaphyllum, Chamaecytisus supinus, Lavandula angustifolia, Psoralea bituminosa, Catananche caerulea). At the end of this axis were located species favoured by nutrient enrichment (Achillea millefolium, Dactylis glomerata, Poa pratensis, Lolium perenne, Bellis perennis, Taraxacum officinale).

Species of highly disturbed grasslands occurred at the higher part of the gradient of management intensity, with narrow niche amplitude (Fig. 7). They had high tolerance to trampling (*L. perenne*), grazing (*B. erecta*, *A. caryophyllea*) or cutting disturbance (*Sherardia arvensis*), irrespective of their water or nutrient requirements. Species tolerating regular cutting and high to intermediate grazing pressure had narrow to large niche in the higher part of the gradient (e.g. *Koeleria pyramidata, T. scabrum, B. baldense, Dianthus sylvestris, P. prolifera*). Species occurring preferably under moderate to low grazing pressure included typical taxa of meso-acidophilous grasslands (*Dianthus monspessulanus, Carlina acaulis ssp caulescens,* 



Fig. 5. Mean ( $\pm$  standard deviation) grazing intensities per lithological categories. 1 = Urgonian limestones, 2 = Slope screes, 3 = Marly limestones, 4 = Marls, 5 = Colluvium and clays. Different letters denote significant differences between categories according to a pairwise mean comparison test (Fischer's L.S.D. test at  $p \le 0.002$ ).



Fig. 6. Species niche amplitude along habitat condition gradient (CCA axis 1). For each species, dots are located at their mean position along the ordination axis and the size of the dot is proportional to species weight in CCA. Horizontal bars indicate the variance of sample scores where a given species is present.

Danthonia decumbens, Centaurium erythraea), and several forbs with morphological defences to grazing (e.g. Eryngium campestre, Carlina spp, Cirsium acaule, Ononis spinosa). Finally, at the end of this gradient were located typical species of dense undisturbed grasslands which tolerate very low grazing pressure or total absence of management, with both large (e.g. B. pinnatum) or narrow habitat amplitude (e.g. Vincetoxicum hirundinaria, Hieracium murorum, Hieracium bifidum, C. supinus).

# 4. Discussion

# 4.1. Effects of lithology and soil moisture

Among the explanatory variables considered in the present study, the most important factor determining floristic composition of prealpine calcareous grassland communities was lithology, which was twice as important a determining factor as the second most important factor, grazing intensity (respectively 12 and 6%). However, the two other factors taken into account, soil moisture and cutting regime, were comparable in rank to grazing (ca. 5% each). Although the structure of the floristic composition showed a strong dependence on lithology, with groups of species restricted to one particular lithology, an important part of this structure was influenced by other factors, especially management regimes.

In oligotrophic grassland communities, the effect of management is often masked by edapho-climatic factors, so that its influence can be under-evaluated. In this study, the combined effects of habitat conditions (lithology  $\times$  soil moisture) explained ca. 23% of total floristic variability, although the combined effect of management still explained 14.5%, which is close to the results obtained by similar studies (Mc Intyre and Lavorel, 1994). However, the percentage of variation in floristic composition explained by the effect of lithology was more important than the one reported by these authors for Australian grasslands, where elevation was predominant. In the case of Vercors prealpine



Fig. 7. Species niche amplitude along management intensity gradient (CCA axis 2).

grasslands, this higher influence of lithology can be related to its important correlations with other geophysical factors, because of the geomorphological structure of the study area.

As a result, lithology can be considered as the predominant habitat variable for calcareous grasslands of the Vercors, at least at the community level, and can be used as a background for the study of management effects. The effect of habitat conditions, as measured by the combined effect of lithology  $\times$  soil moisture was more important than the effect of management regimes. This differs from Belsky's conclusions (1992), who suggested a stronger effect of disturbance than physical stress or competition in structuring some grassland communities. However, it has been shown that the patterns of floristic variability observed are scale-dependent and that the sampling design has a strong influence on the dominance hierarchy of ecological factors (Chaneton and Facelli, 1991).

## 4.2. Effects of management regimes

Lithology is also significantly correlated with management variables, and especially grazing, which is a frequent case in semi-natural grasslands as demonstrated by similar studies (Alard and Poudevigne, 2000). Regular cutting and high grazing intensities currently occur mostly on colluvium and Urgonian limestone of the Vercors tableland, where mechanical cutting is easier than on the slopes. There are distinct and complementary influences of ploughing, mowing, shrub-clearing and grazing on calcareous grassland communities (During and Willems, 1984; Dolman and Sutherland, 1994; Mitchlev and Willems, 1995; Schläpfer et al., 1998; Dutoit et al., 1999). Three main combinations of grazing and cutting regimes occur in the Vercors: (i) a combination of irregular, pluri-annual shrub-clearing and grazing intensity under 50 L.U.-days  $ha^{-1}$ ; (ii) a combination of regular, annual mowing and/or mechanical shrub-clearing and grazing intensity above 400 L.U.-days  $ha^{-1}$ ; (iii) intermediate grazing intensities (between 50 and 400 L.U.-days ha<sup>-1</sup>) with mostly no cutting or shrubclearing.

Each combination of management regimes is related preferentially to one or two lithologies, which determine for a large part the species pool that may occur on corresponding grasslands (Gigon and Leutert, 1996). For example, irregular cutting and low grazing intensities favour tall grasses and forbs intolerant to grazing (Ward and Jennings, 1990; Mitchley and Willems, 1995; Schläpfer et al., 1998). In the Vercors,

it is the case for Lathyrus pratensis, Vicia cracca, Arrhenatherum elatius, Pimpinella saxifraga, Viola hirta or Clinopodium vulgare, occurring mostly on deeper soils (located on marl and colluvium). By contrast, regular cutting and high grazing intensities on colluvial soils favour short graminoids and legumes adapted to frequent defoliation, such as L. perenne and Trifolium repens (Bullock et al., 1994; Watt et al., 1996), rosette species such as B. perennis or Leontodon hispidus (Ward and Jennings, 1990; Mitchley and Willems, 1995), as well as annuals (Verkaar et al., 1983; Gibson and Brown, 1991). On Urgonian limestones, the same management (especially with autumn and winter grazing) favours rare annuals such as A. caryophyllea, B. erecta or T. scabrum, because it increases seedling establishment within rocky, bare ground or short sward patches (Bullock et al., 1994; Watt et al., 1996; Bergmeier, 1997). Finally, intermediate grazing intensities without cutting on slope screes, marly limestones and marls favour short mediterranean or thermophilic chamephytes such as D. pentaphyllum, A. zanonii, Genista pilosa ssp jordani, Coronilla minima or Linum tenuifolium.

## 4.3. Implications for conservation management

As pointed out by Schläpfer et al. (1998), it is imperative for calcareous grassland conservation to define precisely the responses of species to particular management regimes in order to avoid any losses of species by inadequate changes in agro-pastoral management (e.g. by grazing a long-term mown grassland or mowing a longterm grazed grassland, see Kahmen et al. (2002)). The combination of management regimes generally increases the occurrence of rare species within grassland communities because of interactive and cumulative effects of two particular disturbances, e.g. grazing and flooding (Chaneton and Facelli, 1991), grazing and burning (Noy Meir, 1995; Bergmeier, 1997), grazing and mowing (Bullock and Pakeman, 1997), or mowing and ploughing (Dolman and Sutherland, 1994; Dutoit et al., 1999). By contrast, the use of mowing without grazing cannot always restore species-rich calcareous grassland (Stampfli and Zeiter, 1999), notably in cases where there is a lack of adjacent seed sources (Poschlod et al., 1998).

In the calcareous grasslands of the Vercors, the combination of grazing and regular cutting of oligotrophic xeric grasslands established on Urgonian limestones lead to both high species richness and rarity, with several annuals of conservation interest. Small-scale heterogeneity in sward structure created by the cumulative effect of cutting and grazing enhances the opportunities for establishment and regeneration of new species, since it is a major mechanism of species coexistence in calcareous grassland communities (Van der Maarel and Sykes, 1993; Willems et al., 1993; Thompson et al., 1996). Additive effects of trampling and random dung deposition

throughout large paddocks increase respectively bare ground patches and ungrazed fertilised patches, in combination with microtopography variation, so that it allows coexistence of species with very different light and nutrient requirements (During and Willems, 1984; Mitchley and Willems, 1995; Schläpfer et al., 1998). For example, species such as *B. erecta* and *Cynosurus* cristatus can coexist in the same plots within xeric submediterranean grasslands of southern Vercors (Ononido-Carlinetum, Gaultier, 1989). Moreover, the influence of grazing animal and grazing season (Bullock et al., 1994; Watt et al., 1996; Bullock and Pakeman, 1997), despite their lower importance compared to grazing intensity and cutting frequency, have to be taken into account as far as the conservation of a particular target species is concerned, especially for rare orchids (Willems and Melser, 1998; Barbaro et al., 2003).

# 5. Conclusion

The main factors influencing floristic variability within calcareous grassland communities of the Vercors are the additive effect of management (cutting regime × grazing intensity) and lithology, which is the most important abiotic factor of variation for the study area. Approximately 25% of total floristic variability is explained by only three variables that one can easily obtain from field surveys in large areas such as the southern French Prealps. Other potential explanatory factors of the remaining unexplained variation include the influence of landscape structure on dispersal processes (Poschlod et al., 1998; Pärtel et al., 1999; Burnside et al., 2002), and the history of the management practices (Chaneton and Facelli, 1991; Schläpfer et al., 1998; Dutoit et al., 1999, 2003).

Changes in landscape structure surrounding calcareous grasslands are probably crucial to explain current patterns of rare species occurrence (Poschlod et al., 1998; Pärtel et al., 1999; Stampfli and Zeiter, 1999), since most mesoxeric grasslands occurring on marls, slope screes and marly limestones are now surrounded by woods and dense shrublands within the study area. When grasslands are surrounded by wooded areas and isolated from other grasslands acting as potential seed sources, there is a lack of species dispersal for re-colonisation of grasslands restored by shrub-clearing and grazing (Barbaro et al., 2001). By contrast, mesic and xeric grasslands have been successfully restored by current management regimes because these grasslands are surrounded by other grazed or mown grasslands (Barbaro et al., 2001).

Finally, analysis of the functional responses of species on the basis of the relationship between species life traits and environmental factors can be a useful tool for management (Belsky, 1992; Fernandez Ales et al., 1993; Thompson et al., 1996; Lavorel et al., 1998; Barbaro et al., 2000). For example, in the Vercors most of the short-lived rare species no longer occur on lithologies other than Urgonian limestones of the tableland, and to a lesser extent on deeper colluvial soils when unfertilised. We suggest that measuring the niche breadth of species along constrained ordination axes corresponding to habitat and management gradients allows to hierarchize conservation priorities between species within a given biogeographic area, and to focus conservation strategies on species with particular life traits (Barbaro et al., 2000, 2003). Further research on the influence of current agro-pastoral practices and landscape structure on prealpine calcareous grasslands are needed, in order to define precisely adequate conservation management of endangered species and communities, according to their particular responses to management regimes (Schläpfer et al., 1998; Willems and Melser, 1998).

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# Appendix

Tables A1 and A2.

Table A1 Species list in alphabetical order

#### Species

Achillea millefolium L. Agrimonia eupatoria L. Agrostis capillaris L.

Aira caryophyllea L. Anthoxanthum odoratum L. Anthyllis vulneraria L. Arabis hirsuta (L.) Scop. Argyrolobium zanonii (Turra) P.W. Ball. Arrhenatherum elatius (L.) J. et C. Presl Asperula cynanchica L. Avenula pratensis (L.) Dum. Bellis perennis L.

Juniperus communis L. Knautia arvensis (L.) Coulter Koeleria pyramidata (Lam.) P. Beauv. Lathyrus pratensis L. Lavandula angustifolia Mill. Leoontodon hispidus L. Leucanthemum vulgare Lam. Linum tenuifolium L.

Lolium perenne L.

Lotus corniculatus L. Luzula campestris (L.) DC. Medicago lupulina L.

Table A1 (continued)	
Species	
Blackstonia perfoliata (L.) Hudson	Onobrychis vicifolia Scop.
Bombycilaena erecta (L.) Smolj.	Ononis repens L.
<i>Brachypodium pinnatum</i> (L.) P. Beauv.	Ononis spinosa L.
Briza media L.	Origanum vulgare L.
Bromus erectus Huds.	<i>Petrorhagia prolifera</i> (L.) Ball et Heywood.
Bupleurum baldense Turra	Pimpinella saxifraga L.
Buxus sempervirens L.	Plantago lanceolata L.
Campanula rotundifolia L.	Plantago media L.
Carex flacca Schreb.	Poa bulbosa L.
Carex hallerana Asso	Poa compressa L.
Carlina acaulis sep caulascons I	Polyada calcaraa
Carina acauns ssp cauescens L.	F W Schultz
Catananche caerulea L	Polygala vulgaris L
Centaurea jacea s 1	Potentilla tabernaemontani
Centurieu fueeu sii.	Asch.
Centaurium ervthraea Rafn	Prunella laciniata (L.) L.
Cerastium pumilum Curtis	Prunella vulgaris L.
Chamaecytisus supinus	Psoralea bituminosa L.
(L.) Link	
<i>Chamaespartium saggitale</i> (L.) P. Gibbs	Quercus pubescens Wild.
Cirsium acaule (L.) Web	Ranunculus acris L.
Clinopodium vulgare L.	Ranunculus bulbosus L.
Coronilla minima L.	Rhinanthus alectorolophus (Scop.) Pollich
Coronilla varia L.	Rosa canina L.
Crataegus monogyna Jacq.	Salvia pratensis L.
Cynosurus cristatus L.	Sanguisorba minor Scop.
Dactylis glomerata L.	Scabiosa columbaria s.l.
Danthonia decumbens	Sedum acre L.
(L.) DC	Sadum album I
Dianthus monspassulanus	Sedum reflexum I
Dianthus sylvestris Wulf	Sherardia arvensis L
Dorycnium pentaphyllum s 1	Taraxacum officinale Web
Ervngium campestre L.	Teucrium chamaedrys L.
Festuca arundinacea	Teucrium montanum L.
Schreb.	
Festuca glauca s.l.	Teucrium polium L.
Festuca ovina s.l.	Thesium divaricatum Jan.
Festuca rubra L.	Thymus serpyllum s.l.
Galium mollugo L.	Trifolium campestre Schreb.
Galium pumilum s.l.	Trifolium medium L.
Genista cinerea DC.	Trifolium montanum L.
Genista pilosa L.	Trifolium pratense L.
Globularia punctata	Trifolium repens L.
Lapeyr.	Trifel' in a show I
Heliantnemum nummularium	Trijouum scabrum L.
(L.) Mill. Historium hifdum Vit	Trivia alawaa (L.) Dum
Hieracium Dijiaum Kit.	Trina glauca (L.) Duni. Trisatum flavascans
	(L) Decury
Hieracium murorum L.	
Hieracium murorum L.	(L.) Beauv. Verronica chamaedrys L
Hieracium murorum L. Hieracium pilosella L. Hippocrepis comosa L.	(L.) Beauv. Verronica chamaedrys L. Vicia cracca L.
Hieracium murorum L. Hieracium pilosella L. Hippocrepis comosa L. Holcus lanatus L.	(L.) Beauv. Verronica chamaedrys L. Vicia cracca L. Vincetoxicum hirundinaria Med.

Table A2

Factorial co-ordinates of species on the first two axes extracted from the canonical correspondence analysis

Species	Axis 1 (stress)	Species	Axis 2 (management)
Trinia glauca	1.82	Lolium perenne	1.59
Buxus sempervirens	1.79	Sedum album	1.54
Aira caryophyllea	1.77	Sherardia arvensis	1.53
Bombycilaena erecta	1.75	Aira caryophyllea	1.49
Bupleurum baldense	1.65	Ranunculus acris	1.28
Teucrium polium	1.60	Bombycilaena erecta	1.24
Koeleria pyramidata	1.58	Holcus lanatus	1.21
Dianthus sylvestris	1.49	Trinia glauca	1.17
Sedum acre	1.45	Koeleria pyramidata	1.03
Sedum album	1.41	Taraxacum officinales	1.02
Festuca glauca	1.40	Festuca glauca	0.96
Anthyllis vulneraria	1.36	Trifolium scabrum	0.91
Teucrium montanum	1.35	Trifolium repens	0.87
Poa bulbosa	1.28	Poa bulbosa	0.81
Genista cinerea	1.26	Bellis perennis	0.79
Petrorhagia prolifera	1.23	Trifolium pratense	0.78
Trifolium scabrum	1.05	Bupleurum baldense	0.72
Campanula rotundifolia	1.03	Veronica chamaedrys	0.69
Potentilla tabernaemontani	1.01	Trifolium campestre	0.68
Arabis hirsuta	1.00	Rhinanthus alectorolophus	0.67
Sherardia arvensis	0.92	Anthyllis vulneraria	0.65
Rhinanthus alectorolophus	0.87	Trisetum flavescens	0.63
Ononis spinosa	0.87	Teucrium polium	0.61
Galium pumilum	0.82	Campanula rotundifolia	0.60
Avenula pratensis	0.81	Genista cinerea	0.60
Danthonia decumbens	0.69	Onobrychis viciifolia	0.58
Sedum reflexum	0.68	Agrimonia eupatoria	0.56
Eryngium campestre	0.68	Sedum acre	0.56
Trifolium campestre	0.64	Agrostis capillaris	0.55
Thesium divaricatum	0.63	Medicago lupulina	0.54
Argyrolobium zanonii	0.62	Lathyrus pratensis	0.54
Teucrium chamaedrys	0.60	Petrorhagia prolifera	0.53
Asperula cynanchica	0.55	Plantago lanceolata	0.51
Thymus serpyllum	0.52	Arabis hirsuta	0.50
Chamaespartium saggitale	0.51	Dianthus sylvestris	0.50
Linum tenuifolium	0.40	Cynosurus cristatus	0.46
Onobrychis viciifolia	0.39	Salvia pratensis	0.46
Helianthemum nummularium	0.39	Luzula campestris	0.45
Cerastium pumilum	0.38	Achillea millefolium	0.42
Festuca ovina	0.37	Avena pratensis	0.40
Globularia punctata	0.26	Leotondon hispidus	0.38
Sanguisorba minor	0.22	Festuca arundinacea	0.37
Luzula campestris	0.21	Buxus sempervirens	0.35
Hypericum perforatum	0.21	Poa compressa Sumania antina a	0.35
Dorycnium pentapnylium	0.18	Sanguisorba minor	0.33
Bromus erectus	0.10	Arrhenatherum etatius	0.32
Carex nallerana	0.10	Poa pratensis	0.31
	0.09	Trunella Vulgaris	0.30
Quercus pubescens	0.06	Detentille tekense en enteni	0.29
Sumperus communis	0.06	Polenilla labernaemoniani Plantas e media	0.28
Cartina acauits Polyogla oglograg	0.05	rianiago media Lotus compiculatur	0.20
r orygala calcarea	0.03	Calium pumilum	0.25
nippocrepis comosa	0.02	Anthorauthur adaration	0.24
r ou compressu Plaakstonia parfaliata	0.02	Aninoxaninum odoranum Conastium munilum	0.19
Biacksionia perfonata Hiaragium pilogolla	0.00	Cerasium pumium	0.12
nieracium piloseila Primolla laoirista	- 0.01	Empointe activitation and a second activity of the second activity o	0.12
Franctia taciniata	- 0.03	Eryngium campestre	0.00
Chamaaoptisus supirus	- 0.08	Daucus carota	0.09
Chamaecyusus supinus	-0.11	Duncus caroid	0.09

(continued on next page)

# Table A2 (continued)

Species	Axis 1 (stress)	Species	Axis 2 (management)
Briza media	-0.11	Thymus serpyllum	0.08
Hieracium murorum	-0.11	Asperula cynanchica	0.07
Carex flacca	-0.13	Ranunculus bulbosus	0.06
Ranunculus bulbosus	-0.13	Festuca rubra	0.06
Knautia arvensis	-0.13	Galium mollugo	0.04
Vincetoxicum hirundinaria	-0.14	Origanum vulgare	0.04
Pimpinella saxifraga	-0.14	Prunella laciniata	0.04
Agrostis cappilaris	-0.15	Danthonia decumbens	0.02
Centaurium erythraea	-0.15	Knautia arvensis	0.01
Genista pilosa	-0.15	Sedum reflexum	-0.01
Cirsium acaule	-0.16	Polygala vulgaris	-0.03
Brachypodium pinnatum	-0.16	Ononis repens	-0.06
Trifolium pratense	-0.20	Chamaespartium sagittale	-0.07
Trifolium montanum	-0.22	Carlina acaulis ssp caulescens	-0.07
Carlina acaulis ssp caulescens	-0.22	Bromus erectus	-0.08
Catananche caerulea	-0.22	Leucanthemum vulgare	-0.11
Lavandula angustifolia	-0.24	Briza media	-0.11
Cynosurus cristatus	-0.24	Thesium divaricatum	-0.14
Hieracium bifidum	-0.26	Trifolium montanum	-0.15
Salvia pratensis	-0.27	Hieracium pilosella	-0.17
Rosa canina	-0.28	Ononis spinosa	-0.20
Psoralea bituminosa	-0.30	Centaurea jacea	-0.26
Trifolium medium	-0.31	Carex hallerana	-0.27
Origanum vulgare	-0.32	Genista pilosa	-0.30
Scabiosa columbaria	-0.33	Centaurium erythraea	-0.32
Crataegus monogyna	-0.33	Cirsium acaulis	-0.33
Polygala vulgaris	-0.34	Viola hirta	-0.34
Festuca rubra	-0.37	Trifolium medium	-0.34
Lotus corniculatus	-0.37	Helianthemum nummularium	-0.34
Medicago lupulina	-0.38	Clinopodium vulgare	-0.35
Clinopodium vulgare	-0.38	Argyrolobium zanonii	-0.36
Daucus carota	-0.42	Pimpinella saxifraga	-0.37
Plantago media	-0.44	Coronilla varia	-0.39
Leoontodon hispidus	-0.44	Rosa canina	-0.39
Leucanthemum vulgare	-0.44	Carex flacca	-0.40
Viola hirta	-0.45	Crataegus monogyna	-0.41
Vicia cracca	-0.46	Hypericum perforatum	-0.42
Plantago lanceolata	-0.52	Scabiosa columbaria	-0.46
Coronilla varia	-0.52	Vincetoxicum hirundinaria	-0.54
Achillea millefolium	-0.54	Vicia cracca	-0.56
Ononis repens	-0.55	Polygala calcarea	-0.56
Anthoxanthum odoratum	-0.59	Brachypodium pinnatum	-0.57
Dactylis glomerata	-0.63	Teucrium chamaedrys	-0.58
Centaurea jacea	-0.71	Linum tenuifolium	-0.67
Galium mollugo	-0.72	Globularia punctata	-0.68
Prunella vulgaris	-0.75	Juniperus communis	-0.69
Trifolium repens	-0.90	Hieracium bifidum	-0.75
Trisetum flavescens	-0.91	Hieracium murorum	-0.76
Poa pratensis	-0.92	Festuca ovina	-0.79
Lathyrus pratensis	-0.94	Coronilla minima	-0.84
Festuca arundinacea	-0.96	Hippocrepis comosa	-0.89
Verronica chamaedrys	-0.98	Carlina acaulis	-0.95
Bellis perennis	-0.99	Blackstonia perfoliata	-0.99
Arrhenatherum elatius	-1.09	Chamaecytisus supinus	-1.00
Ranunculus acris	-1.18	Dorycnium pentaphyllum	-1.02
Agrimonia eupatoria	- 1.34	Catananche caerulea	- 1.15
Lolium perenne	-1.37	Psoralea bituminosa	-1.17
Taraxacum officinale	- 1.37	Lavandula angustifolia	-1.51
Holcus lanatus	- 1.42	Quercus pubescens	- 1.61

#### References

- Alard, D., Poudevigne, I., 2000. Diversity patterns in grasslands along a landscape gradient in northwestern France. Journal of Vegetation Science 11, 287–294.
- Alard, D., Bance, J.F., Frileux, P.N., 1994. Grassland vegetation as an indicator of the main agro-ecological factors in a rural landscape: consequences for biodiversity and wildlife conservation in central Normandy. Journal of Environmental Management 42, 91–109.(doi:10.1006/jema.1994.1063).
- Balent, G., 1991. Construction of a reference frame for studying changes in species composition in grasslands: the example of an old-field succession. Options Méditerranéennes 15, 73–81.
- Barbaro, L., Cozic, P., 1998. Organisation agro-écologique des pelouses et landes calcicoles du Parc naturel régional du Vercors (Rhône-Alpes, France). Ecologie 29, 443–457.
- Barbaro, L., Corcket, E., Dutoit, T., Peltier, J.P., 2000. Réponses fonctionnelles des communautés de pelouses calcicoles aux facteurs agro-écologiques dans les Préalpes françaises. Canadian Journal of Botany 78, 1010–1020.
- Barbaro, L., Dutoit, T., Cozic, P., 2001. A six-year experimental restoration of biodiversity by shrub-clearing and grazing in calcareous grasslands of the French Prealps. Biodiversity and Conservation 10, 119–135.
- Barbaro, L., Dutoit, T., Grossi, J.L., 2003. Influence des facteurs agroécologiques sur les assemblages d'orchidées dans les pelouses calcicoles du Vercors (Préalpes, France). Botanica Helvetica 113/1, 63–79.
- Barthès, J.P., Bornand, M., 1987. Cartographie des sols en moyenne montagne calcaire sèche. Les Colloques de l'INRA 39, 95–127.
- Baudry, J., Merriam, H.G., 1988. Connectivity and connectedness: functional versus structural patterns in landscapes. In: Schreiber, K.F., (Ed.), Connectivity in Landscape Ecology. Proceedings of the Second International Seminar I.A.L.E., Münster, pp. 23–28.
- Belsky, A.J., 1992. Effects of grazing, competition, disturbance and fire on species composition and diversity in grassland communities. Journal of Vegetation Science 3, 187–200.
- Bergmeier, E., 1997. Combined effects of fire and grazing on phrygana vegetation—a case study in SW Crete (Greece). Ecologia Mediterranea 23, 1–10.
- Boissier, J.M., 2000. Catalogue des Types de Stations Forestières du Massif du Vercors et de ses Bordures. Centre de Biologie Alpine, Université Joseph Fourier, Grenoble.
- Bullock, J.M., Pakeman, R.J., 1997. Grazing of lowland heath in England: management methods and their effects on heathland vegetation. Biological Conservation 79, 1–13.
- Bullock, J.M., Clear Hill, B., Dale, M.P., Silvertown, J., 1994. An experimental study of the effects of sheep grazing on vegetation change in a species-poor grassland and the role of seedling recruitment into gaps. Journal of Applied Ecology 31, 493–507.
- Burnside, N.G., Smith, R.F., Waite, S., 2002. Habitat suitability modelling for calcareous grassland restoration on the South Downs, United Kingdom. Journal of Environmental Management 65, 209–221.(doi:10.1006/jema.2002.0546).
- Chaneton, E.J., Facelli, J.M., 1991. Disturbance effects on plant community diversity: spatial scales and dominance hierarchies. Vegetatio 93, 143–155.
- Chessel, D., 1997. Ordination sous Contraintes. Documentation de la Programmathèque ADE-4. Université Lyon I, Lyon.
- Chessel, D., Lebreton, J.D., Prodon, R., 1982. Mesures symétriques d'amplitude d'habitat et de diversité intra-échantillons dans un tableau espèces-relevés: cas d'un gradient simple. Comptes Rendus de l'Académie des Sciences, série III, Sciences de la Vie 295, 83–88.
- Corcket, E., 2000. Catalogue des Types de Stations Forestières du Diois et des Baronnies Drômoises. Centre de Biologie Alpine, Université Joseph Fourier, Grenoble.

- Daget, P., Poissonet, J., 1995. Méthodes d'étude de la végétation des pâturages. In: Daget, P., Godron, M. (Eds.), Pastoralisme. Troupeaux, Espaces et Sociétés. Hatier, Paris, pp. 87–100.
- Debelmas, J., Kerckhove, Cl., 1980. Les Alpes franco-italiennes. Géologie Alpine 56, 21–58.
- Dolman, P.M., Sutherland, W.J., 1994. The use of soil disturbance in the management of Breckland grass heaths for nature conservation. Journal of Environmental Management 41, 123–140.(doi:10.1006/ jema.1994.1039).
- During, H.J., Willems, J.H., 1984. Diversity models applied to a chalk grassland. Vegetatio 27, 103-114.
- Dutoit, T., Roche, P., Alard, D., 1999. Influence de perturbations anthropiques sur la composition et la diversité botanique des pelouses calcicoles de la vallée de la Seine en Haute-Normandie (France). Canadian Journal of Botany 77, 1–12.
- Dutoit, T., Buisson, E., Roche, P., Alard, D., 2003. Land use history and botanical changes in the calcareous hillsides of Upper-Normandy (North-western France): new implications for their conservation management. Biological Conservation 115, 1–19.
- d'Epenoux, F., 1992. Relations milieu-production: application au pin noir d'Autriche dans les Alpes externes méridionales. PhD Thesis, University of Grenoble I, Grenoble.
- Fernandez Alés, R., Laffarga, J.M., Ortega, F., 1993. Strategies in Mediterranean grassland annuals in relation to stress and disturbance. Journal of Vegetation Science 4, 312–322.
- Fischer, M., Stöcklin, J., 1997. Local extinctions of plants in remnants of extensively used calcareous grasslands 1950–1985. Conservation Biology 11, 727–737.
- Gaultier, C., 1989. Relations entre pelouses eurosibériennes (Festuco-Brometea) et groupements méditerranéens (Ononido-Rosmarinetea): étude régionale (Diois) et synthèse sur le pourtour méditerranéen Nord-Occidental. PhD Thesis, Université Paris XI, Orsay.
- Gibson, C.W.D., Brown, V.K., 1991. The effects of grazing on local colonisation and extinction during early succession. Journal of Vegetation Science 2, 291–300.
- Gigon, A., Leutert, A., 1996. The dynamic keyhole-key model of coexistence to explain diversity of plants in limestone and other grasslands. Journal of Vegetation Science 7, 29–40.
- Grime, J.P., 1990. Mechanisms promoting floristic diversity in calcareous grasslands. In: Hillier, S.H., Walton, D.W.H., Wells, D.A. (Eds.), Calcareous Grasslands: Ecology and Management. Bluntisham Books, Huntingdon, pp. 51–56.
- Kahmen, S., Poschlod, P., Schreiber, K.F., 2002. Conservation management of calcareous grasslands. Changes in plant species composition and response of functional traits during 25 years. Biological Conservation 104, 319–328.
- Lavorel, S., Touzard, B., Lebreton, J.D., Clément, B., 1998. Identifying functional groups for response to disturbance in an abandoned pasture. Acta Oecologica 19, 227–240.
- Lebreton, J.D., Sabatier, R., Banco, G., Bacou, A.M., 1991. Principal component and correspondence analyses with respect to instrumental variables: an overview of their role in studies of structure–activity and species–environment relationships. In: Devillers, J., Karcher, W. (Eds.), Applied Multivariate Analysis in SAR and Environmental Studies. Kluwer, Dordrecht, pp. 85–114.
- Léouffre, M.C., Leclerc, B., 1996. Dynamique paysagère et évolution des risques d'incendie: l'exemple d'une vallée des Préalpes. Etudes et Recherches sur les Systèmes Agraires et le Développement 29, 153–167.
- Mc Intyre, S., Lavorel, S., 1994. How environmental and disturbance factors influence species composition in temperate Australian grasslands. Journal of Vegetation Science 5, 373–384.
- Mitchley, J., Willems, J.H., 1995. Vertical canopy structure of Dutch chalk grasslands in relation to their management. Vegetatio 117, 17–27.
- Mortimer, S.R., Hollier, J.A., Brown, V.K., 1998. Interactions between plant and insect diversity in the restoration of lowland calcareous

grasslands in southern Britain. Applied Vegetation Science 1, 101-114.

- Noy Meir, I., 1995. Interactive effects of fire and grazing on structure and diversity of Mediterranean grasslands. Journal of Vegetation Science 6, 701–710.
- Økland, R.H., 1996. Are ordination and constrained ordination alternative or complementary strategies in general ecological studies? Journal of Vegetation Science 7, 289–292.
- Økland, R.H., 1999. On the variation explained by ordination and constrained ordination axes. Journal of Vegetation Science 10, 131–136.
- Økland, R.H., Eilertsen, O., 1994. Canonical correspondence analysis with variation partitioning: some comments and an application. Journal of Vegetation Science 5, 117–126.
- Pärtel, M., Mändla, R., Zobel, M., 1999. Landscape history of a calcareous (alvar) grassland in Hanila, western Estonia, during the last three hundred years. Landscape Ecology 14, 187–196.
- Poschlod, P., Bonn, S., 1998. Changing dispersal processes in the central European landscape since the last ice age: an explanation for the actual decrease of plant species richness in different habitats? Acta Botanica Neerlandica 47, 27–44.
- Poschlod, P., Kiefer, S., Tränkle, U., Fischer, S., Bonn, S., 1998. Plant species richness in calcareous grasslands as affected by dispersability in space and time. Applied Vegetation Science 1, 75–90.
- Prodon, R., Lebreton, J.D., 1994. Analyses multivariées des relations espèces-milieu: structure et interprétation écologique. Vie et Milieu 44, 69–91.
- Roche, P., Tatoni, T., Médail, F., 1998. Relative importance of abiotic and land use factors in explaining variation in woody vegetation in a French rural landscape. Journal of Vegetation Science 9, 221–228.
- Sabatier, R., Lebreton, J.D., Chessel, D., 1989. Principal component analysis with instrumental variables as a tool for modelling composition data. In: Coppi, R., Bolasco, S. (Eds.), Multiway Data Analysis. Elsevier, Amsterdam, pp. 341–352.
- Schläpfer, M., Zoller, H., Körner, C., 1998. Influences of mowing and grazing on plant species composition in calcareous grassland. Botanica Helvetica 108, 57–67.
- Stampfli, A., Zeiter, M., 1999. Plant species decline due to abandonment of meadows cannot easily be reversed by mowing. A case study from the southern Alps. Journal of Vegetation Science 10, 151–164.
- Sutherland, W.J., 1996. Ecological Census Techniques. Cambridge University Press, Cambridge.

- Ter Braak, C.J.F., 1988. Partial canonical correspondence analysis. In: Bock, H.H., (Ed.), Classification and Related Methods of Data Analysis. North-Holland, Amsterdam, pp. 551–558.
- Ter Braak, C.J.F., Prentice, I.C., 1988. A theory of gradient analysis. Advances in Ecological Researches 18, 271–317.
- Thioulouse, J., Chessel, D., Dolédec, S., Olivier, J.M., 1997. ADE-4: a multivariate analysis and graphical display software. Statistics and Computers 7, 75–83.
- Thompson, K., Hillier, S.H., Grime, J.P., Bossard, C.C., Band, S.R., 1996. A functional analysis of a limestone grassland community. Journal of Vegetation Science 7, 371–380.
- Van der Maarel, E., 1996. Vegetation dynamics and dynamic vegetation science. Acta Botanica Neerlandica 45, 421–442.
- Van der Maarel, E., Sykes, M.T., 1993. Small-scale plant species turnover in a limestone grassland: the carousel model and some comments on the niche concept. Journal of Vegetation Science 4, 179–188.
- Verkaar, H.J., Schenkeveld, A.J., Brand, J.M., 1983. On the ecology of short-lived forbs in chalk grasslands: micro-site tolerances in relation to vegetation structure. Vegetatio 52, 91–102.
- WallisdeVries, M.F., Poschlod, P., Willems, J.H., 2002. Challenges for the conservation of calcareous grasslands in northwestern Europe: integrating the requirements of flora and fauna. Biological Conservation 104, 265–273.
- Ward, L.K., Jennings, R.D., 1990. Succession of disturbed and undisturbed chalk grassland at Aston Rowant National Nature Reserve: details of changes in species. Journal of Applied Ecology 27, 913–923.
- Watt, T.A., Treweek, J.R., Woolmer, F.S., 1996. An experimental study of the impact of seasonal sheep grazing on formerly fertilized grassland. Journal of Vegetation Science 7, 535–542.
- Willems, J.H., 2001. Problems, approaches, and results in restoration of Dutch calcareous grassland during the last 30 years. Restoration Ecology 9, 147–154.
- Willems, J.H., Melser, C., 1998. Population dynamics and life-history of *Coeloglossum viride* (L.) Hartm.: an endangered orchid species in the Netherlands. Botanical Journal of the Linnean Society 126, 83–93.
- Willems, J.H., Peet, R., Bik, L., 1993. Changes in chalk-grassland structure and species richness resulting from selective nutrient additions. Journal of Vegetation Science 4, 203–212.
- Yoccoz, N., Chessel, D., 1988. Ordination sous contrainte de relevés d'avifaune: élimination d'effets dans un plan d'observation à deux facteurs. Comptes Rendus de l'Académie des Sciences, Série III, Sciences de la Vie 307, 189–194.

COSCIENCE

# Habitat amelioration and associational defence as main facilitative mechanisms in Mediterranean grasslands grazed by domestic livestock<sup>1</sup>

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> Abstract: Facilitative interactions alter species dynamics through the mechanisms of habitat amelioration, associational defence against herbivores, and release of competitive interactions. However, the relative importance of each mechanism remains poorly known. This study attempts to fill that gap for systems grazed by domestic livestock, where abiotic stress, grazing, and competition potentially limit plant performance. In southern France, two light-demanding trees, *Pimus sylvestris* and Pinus nigra, invade grasslands and often become established near shrubs. We tested the effects of habitat amelioration, associational defence against herbivores and release of herb competition by shrubs on the establishment of pine seedlings by sowing and transplantation experiments. We also assessed the effect of shrubs on seed predation and sapling growth. Shrubs increased seed predation and depressed seedling emergence and sapling growth, but they facilitated the survival of pine seedlings by increasing shade and soil moisture and by protecting seedlings against sheep grazing. There was no effect of herb competitive release. This study points out that habitat amelioration and associational defence are both highly important in dry grasslands grazed by domestic livestock because these mechanisms enhance seedling survival, a major life stage for tree dynamics. This situation, in which two facilitative mechanisms are important, is not taken into account by current models of facilitation and is probably due to the coupling of domestic herbivore grazing and stressful abiotic conditions. *Keywords:* competitive release, direct and indirect effects, facilitation, livestock grazing, *Pinus*, woody plants.

> Résumé : Les interactions de facilitation modifient la dynamique des espèces par les mécanismes d'amélioration de l'habitat, de défense par association contre les herbivores et de suppression des interactions compétitives. Cependant, l'importance relative de chaque mécanisme reste peu connue. Cette étude a pour but de combler cette lacune pour les systèmes pâturés par des herbivores domestiques, dans lesquels le stress abiotique, le pâturage et la compétition réduisent potentiellement la performance des plantes. Dans le Sud de la France, deux arbres héliophiles, Pinus sylvestris et Pinus nigra, envahissent les prairies et s'installent souvent à proximité des buissons. Nous avons testé les effets de l'amélioration de l'habitat, de la défense contre les herbivores et de la réduction de la compétition herbacée par les buissons sur l'établissement des plantules de pins par des expérimentations de semis et de transplautation. Nous avons aussi évalué les effets des buissons sur la prédation des graines et la croissance des juvéniles. Les buissons ont fait augmenté la prédation des graines, réduit l'émergence des plantules et la croissance des juvéniles, mais ont facilité la survie des plantules en augmentant l'ombrage et l'humidité du sol et en protégeant les plantules contre le pâturage ovin. Il n'y a pas eu d'effet de réduction de la compétition herbacée. Cette étude met en évidence que deux mécanismes, l'amélioration de l'habitat et la défense contre les herbivores, sont très importants dans les prairies sèches pâturées par des herbivores domestiques en augmentant la survie des plantules, un stade de vie déterminant pour la dynamique des arbres. Cette situation, dans laquelle 2 mécanismes de facilitation sont importants, n'est pas prise en compte par les modèles actuels de facilitation et est probablement due au couplage entre du pâturage par des herbivores domestiques et des conditions abiotiques stressantes.

> Mots-clés : effets directs et indirects, facilitation, pâturage par des herbivores domestiques, Pinus, plantes ligneuses, réduction de la compétition.

Nomenclature: Coste, 1937.

## Introduction

Facilitation, i.e., the positive effect of a plant on the performance of another one, has been highlighted in a large

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number of studies (Callaway, 1995; Brooker & Callaghan, 1998) and recognized as essential to understanding and predicting community structure, species dynamics, and invasion success (Bruno, Stachowicz & Bertness, 2003). Several direct and indirect mechanisms can explain the facilitative effects of a neighbouring plant on a target one (Figure 1) (Bruno, Stachowicz & Bertness, 2003). Direct positive

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effects result from microhabitat amelioration, including reduction of osmotic stress, heat, desiccation, or wind and increase of soil moisture or nutrients (Figure 1, effect 1) (Franco & Nobel, 1989; Gómez-Aparicio et al., 2004; Baumeister & Callaway, 2006). The indirect effect of associational defence is due to herbivore deterrence or physical protection against herbivores provided by unpalatable or spiny plants for more palatable plants growing under them or in their neighbourhood (Figure 1, effect 2) (McAuliffe, 1986; Brooker et al., 2006). A neighbouring plant can also indirectly facilitate the target plant if its negative effect on the competitors of the target plant is higher than its direct competitive effect on the target plant itself (Figure 1, effect 3) (Levine, 1999; Kunstler et al., 2006). Surprisingly, studies that have tested the occurrence of several of these facilitative mechanisms are scarce (but see Callaway, 1992; Rousset & Lepart, 2000), and to our knowledge no study has explicitly tested the occurrence and importance of each of these 3 mechanisms in a given system (Figure 1). We have attempted to fill that gap, with the example of pine seedling establishment in Mediterranean grasslands extensively grazed by sheep.

Current conceptual models of facilitation predict that habitat amelioration occurs in communities submitted to harsh abiotic conditions, whereas associational defence dominates in communities submitted to high grazing pressure (Bertness & Callaway, 1994; Bruno, Stachowicz & Bertness, 2003). These models assume that wild herbivore pressure and abiotic stress are negatively correlated, most probably because of their indirect link with community productivity. Therefore, they predict that habitat amelioration and associational defence cannot be of similar importance in a given community. Competitive release effect is not explicitly included in these conceptual models (Bertness



FIGURE 1. Summary of indirect positive effects of a neighbouring plant on a target plant: (1) habitat amelioration, (2) associational defence, and (3) competitive release. Solid lines indicate direct effects, and dashed-dotted lines indicate indirect positive effects.

& Callaway, 1994), although it has been found to occur in communities of relatively high (Li & Wilson, 1998; Siemann & Rogers, 2003) as well as low productivity (Kunstler et al., 2006). Initially developed for unmanaged systems, it is questionable whether the predictions of these models still hold in systems grazed by domestic livestock. Indeed, these systems are characterized by the coupling of high herbivore pressure and strong abiotic stress due to low temperature or shallow soils. These systems are commonly encroached by trees, whose establishment can be facilitated by nurse plants (Debussche, Lepart & Dervieux, 1999; Kunstler et al., 2006). We could thus expect that facilitation in these systems, unlike that in unmanaged systems, results both from habitat amelioration and associational defence in grazed places and from the release of herb competition in the absence of grazing.

We tested these predictions in the Causse du Larzac, southern France, where the native Pinus sylvestris and the introduced Pinus nigra ssp. nigra invade grasslands. Both species often become established near shrubs, but they do not establish under the shrub canopy, where light levels are too low. In grassland, the establishment of tree seedlings can be severely restricted by drought, especially in the Mediterranean area (Castro et al., 2005a), as well as by grazing or herb competition (Rousset & Lepart, 2000; Jurena & Archer, 2003). For each pine species, we performed sowing experiments during 2 y and sapling trans-plantation to test for the occurrence of habitat amelioration and associational defence by shrubs in a grazed area. In the absence of grazing, artificial vertical shading was used to test for the occurrence of positive effects of shade and herb competitive release. Because interactions vary with the ontogeny of the target plant (Callaway & Walker, 1997; Miriti, 2006), we further explored the effects of shrubs on pine seed predation and sapling growth.

#### Methods

### STUDY AREA AND SPECIES

This study was conducted in the Causse du Larzac (about 44.00° N and 3.30° E), a 1000-km<sup>2</sup> plateau, 560 to 920 m high, on the southern margins of the Massif Central, France. Soils are usually shallow, with a low field capacity (Cadillon, 1970), and climate is characterized by oceanic and Mediterranean influences. Annual precipitation varies between 710 and 1730 mm, with a maximum in winter and early spring and minimum in summer (Meteo France, La Cavalerie, 1988-2005). The landscape consists of calcareous grasslands dominated by Bromus erectus with scattered shrubs, mainly the unpalatable Buxus sempervirens and Juniperus communis, woodlands of the native Pinus sylvestris, and plantations of the introduced Pinus nigra ssp. nigra. These 2 pioneer heliophilous pine species invade grasslands (Marty et al., 2002; Caplat, Lepart & Marty, 2006). Their non-dormant seeds are dispersed by wind between January and April and germinate soon afterwards. Whereas P. sylvestris is extremely widespread in Eurasia (Willis, Bennett & Birks, 1998), P. nigra ssp. nigra is native to the Balkans (Barbéro et al., 1998).

Sites used for the experiments were grasslands located in the northern part of the Causse du Larzac (between 43.92° and 44.13° N and 3.12° and  $3.30^{\circ}$  E). The 2 experimental years (2005 and 2006) were dry in late spring and early summer, with respectively 51 and 32 mm of rain in June and 26 and 12 mm in July (median values from 1988 to 2005 were 60 and 40 mm, respectively for June and July). Seeds used in the experiments came from local pine populations. Cones of both species collected in several pine populations were opened by air-drying for about 4 d at 35 °C. Seeds were dewinged to prevent wind transport after sowing. For *P. sylvestris* and *P. nigra*, mean seed weights were respectively 7.7 and 22 mg and mean germination rates were 94 and 92% in controlled conditions (20 °C during the day and 10 °C at night).

## EXPERIMENTS

#### EFFECT OF SHRUBS ON SEED PREDATION

The effect of shrubs on seed predation was tested using 3 sites (43.92° N, 3.28° E; 43.95° N, 3.12° E; 44.04° N, 3.12° E), within each of which 10 blocks were randomly established. In each block, 2 Petri dishes of 5.5 cm diameter filled with sand, one containing 10 seeds of P. sylvestris, the other 10 seeds of P. nigra, were fixed to the soil at the periphery of the canopy of an isolated shrub about 2 m high ("shrub" habitat). Two others were installed in a nearby grassland area, at least 2 m away from each shrub ("open" habitat). For each of 3 blocks at each site, 4 control dishes protected by a 15-mm wire mesh, excluding predation by small mammals and birds, and filled with 10 seeds of either P. sylvestris or P. nigra, were added in both habitats. Predation was recorded by counting seeds 4 to 7 d after the Petri dishes were set up. The experiment was repeated 3 times in March 2006, during natural pine seed dispersal. A total of 3600 seeds were submitted to predation, and 360 seeds were used for control Petri dishes. To identify seed predators, imprints were collected within 15-cm-diameter dishes filled with fine clay. A total of 12 dishes were installed in shrub and open habitats in April 2006 in 3 blocks at each site. Half of them were filled with seeds of *P. sylvestris*; the other half with *P. nigra*. Dishes were checked for predation and filled with new seeds and imprints were identified every 12 h for 2 d.

#### HABITAT AMELIORATION AND ASSOCIATIONAL DEFENCE BY SHRUBS

Ten blocks, each comprising 1 isolated Juniperus communis shrub about 2 m high and a nearby plot of grassland, were located in a 10-ha grazed grassland area (43.92° N, 3.28° E). In each block, four  $0.3 - \times 0.3 - m^2$  plots were established at the northern periphery of the shrub canopy ("shrub" habitat) to maximize the shade effect and 4 others were established in the grassland ("open" habitat). Half of the plots, 2 in each habitat, were protected from sheep grazing by a 1-m-high fence (grazing treatment). In each combination of habitat and grazing treatments, one plot was randomly sown with P. nigra and the other with P. sylvestris. During the winters of 2005 and 2006, seeds were sown on the ground to mimic natural anemochorous dispersal, with 1 seed every 3 cm, for a total of 100 seeds per plot and 4000 seeds per species and experimental year. To prevent vertebrate seed predation, each plot was protected by a 1.5-cm wire mesh, which was removed after seedling emergence. Emerged seedlings were counted in April, May, and

June and surveyed until September. Plots were watered in mid June and July 2006 with an equivalent of 20 mm and 40 mm, respectively, because of the rainfall deficit.

We further tested the occurrence of associational defence by following direct impacts of livestock on transplanted 1-y-old pine saplings in a 15-ha pasture area (43.95° N, 3.12° E). In 2006, we recorded herbivory damage on pine saplings during the autumn grazing season (290 ewes over 8 d). In each of 3 blocks, we established 4 transects of about 20 m: 2 in the grassland, at least 5 m away from shrubs, and 2 following the periphery of the shrub canopy. In each block, 20 P. sylvestris saplings were transplanted every metre in 2 transects: one in the grassland and the other at the shrub periphery. The same was done for P. nigra, for a total of 240 transplanted saplings. Sheep livestock were introduced 1 week later. Herbivory on each sapling was assessed 2, 4, and 8 d after sheep introduction by recording whether the plant was intact, lightly grazed with leaves eaten but apical bud intact, heavily grazed with apical bud eaten and a few leaves intact, or totally eaten with only a part of the stem remaining. Livestock were removed after 8 grazing days because of low herb height, an indicator of the end of the grazing season.

### DIRECT EFFECT OF SHADING AND HERB COMPETITION RELEASE

We identified 10 blocks, each comprising 8 plots of  $0.3 \times 0.3 \text{ m}^2$ , in a 2500-m<sup>2</sup> grassland area (44.04° N, 3.23° E). A vertical pan of 50 cm height and 8 m length, made of 80% light-intercepting tissue, was placed against the southern side of 4 plots per block ("shade" habitat; "open" habitat otherwise) in order to mimic the shading effect of shrubs at their periphery. Glyphosate treatment was applied randomly in 2 plots per habitat to suppress herb competition, and litter was then removed. In winter 2006, in each combination of habitat and herb competition treatments, one plot was randomly sown with P. nigra and the other with P. sylvestris. One hundred seeds were sown on the ground of each plot, 1 seed every 3 cm, for a total of 4000 seeds for each species. Plots were protected from vertebrate predation by a 1.5-cm wire mesh. Emerged seedlings were counted in April and May, and their survival was followed until September. Plots were watered in mid June and July with 20 mm because of the high rainfall deficit.

#### EFFECT OF SHRUBS ON SAPLING GROWTH

The effect of shrubs on sapling growth was tested in 3 sites  $(43.92^{\circ} \text{ N}, 3.28^{\circ} \text{ E}; 44.05^{\circ} \text{ N}, 3.30^{\circ} \text{ E}; 44.13^{\circ} \text{ N}, 3.30^{\circ} \text{ E})$ . A total of 43 pine saplings established in the grassland and 53 at the shrub periphery were randomly sampled and cut at ground level for annual whorl length and ring thickness measurement (LINTAB and TSAP software, Frank Rinn®, Heidelberg, Germany).

#### MICROENVIRONMENTAL VARIABLES

Soil water content  $(\%, \forall_v)$  at 15 cm depth was measured in each block between February and September near shrubs and in grassland, and next to the vertical pans and in grassland, with a time domain reflectometer (Minitrase 6050X3K1, Soil Moisture Equipment Corp., Santa Barbara, California, USA). Air temperature and relative humidity at 20 cm height and soil temperature at 5 cm depth were

recorded at the shrub periphery and in grassland every 30 min from February to September 2006 with a data logger (CR10X, Campbell Scientific, Leicester, UK) in 3 blocks. Air humidity and temperature were used to calculate the vapour pressure deficit (VPD), which indicates evapotranspiration demand. In June 2006, soil cores of 10 cm depth and 3 cm diameter were collected near shrubs and in grassland in the 10 blocks for pH and mineralized nitrogen (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) standard analysis. In June, the herbaceous above-ground biomass next to the vertical pans and in grassland was estimated on two 0.4-m<sup>2</sup> squares in each of the 10 blocks.

#### STATISTICAL ANALYSES

For the seed predation experiment, and for each site and treatment (Petri dishes submitted to predation or control dishes), the proportions of missing seeds were analyzed with general linear models (GLMs) that included the effects of block, habitat (open/shrub periphery), pine species, and interactions between these terms. To take the split-plot design into account, the effect of habitat was tested against the habitat  $\times$  block interaction.

For each sowing experiment, seedling emergence rates were analyzed with GLMs that included block, habitat (open/shrub periphery or open/artificial shade, depending on the experiment), treatment (weeded/unweeded, only for the experiment with artificial shade), pine species, and all interactions. Year of experiment (only for the experiment with shrubs) and two-way interactions with previous terms were also added as independent variables. Error terms were specified to take the split-plot design into account. Habitat × block interaction was used as an error term for habitat and block effects, and treatment × habitat × block interaction was used as an error term for treatment effect and the treatment × habitat interaction. For each year, the difference in timing of emergence between shrub and grassland habitats was tested using a GLM, with the number of emerged seedlings in April, May, and June as independent variables, time as a repeated factor, and habitat, block, and their interaction as independent variables.

In each sowing experiment, survival rates from emergence to the end of each experimental year, i.e., the ratios of living seedlings recorded at each time t and t-1, were modelled with logistic regressions (Egli & Schmidt, 2001). Deviance ratios were computed by taking the split-plot design into account, as previously explained for emergence. The same effects and interactions as for emergence were tested. Logarithms of the length of each measurement interval were used as an offset, and the number of seedlings at risk (number of seedlings at t-1) was used as a frequency variable. The effect of shrubs on sapling herbivory at the end of the grazing season was analyzed with a logistic regression, with block, habitat, species, and all interactions as dependent variables. Block and habitat were tested against the block × habitat interaction, and the species and its interactions with block and habitat were tested against the block × habitat × species interaction.

The effect of shrubs and artificial shade on soil relative humidity and air VPD was analyzed with GLMs and F-tests using time as a repeated factor and habitat, block, and treatment (weeded/unweeded, only for the experiment with artificial shade) as independent variables, error terms being specified as previously to take the split-plot design into account. GLMs were also performed at each measurement date. Differences in soil pH, nitrate and ammonium contents, and herb biomass between habitats were analyzed with Student's *t*-tests. The effect of shrubs on sapling height and diameter growth, *i.e.*, the cumulated length of whorls and ring thickness, were analyzed with GLMs and *F*-tests, with age as a repeated polynomial factor and habitat and site as independent variables.

Hypotheses of residual normality and homoscedasticity for GLMs, and sphericity tests for analyses including a repeated factor, were checked. All analyses were performed with SAS V3.1.

#### Results

#### EFFECT OF SHRUBS ON SEED PREDATION

Seeds were more predated near shrubs than in grassland for non-protected dishes ( $F_{1,9} = 116$ , 27, and 25 for each site, respectively; P < 0.001), with no difference for protected dishes ( $F_{1,9} = 0.7$ , 0.5, and 1 for each site, respectively; P > 0.1). Mean predation rates, which were 55.6% in grassland and 83.5% near shrubs, did not differ between pine species ( $F_{1,89} = 0.7$ , 0.05, and 1 for each site, respectively; P > 0.05). Of the 27 imprints, 26 were of rodents, identified as being *Apodemus sylvaticus*, and 1 imprint was from a passerine species.

HABITAT AMELIORATION AND ASSOCIATIONAL DEFENCE BY SHRUBS

Environmental conditions were significantly buffered at the shrub periphery, with lower soil temperature during the pine germination period and enhanced soil relative humidity in June, when most pine seedling mortality occurred (Table I; Figure 2). VPD, pH, and ammonium contents were not affected, whereas soil nitrate content was reduced at the shrub periphery (Table 1).

The total emergence rate was generally higher in grassland than at the shrub periphery, with a difference between years (Table II). The mean emergence rate did not differ between habitats in 2005 (27.3% ± 1.72 [SE] and  $30.3\% \pm 2.18$  at the shrub periphery and in grassland, respectively), but it was significantly lower near shrubs (11.5% ± 1.82) than in grassland (52.9% ± 1.59) in 2006. Seedling emergence was delayed at the shrub periphery, the difference between shrubs and grassland in the number of emerged seedlings decreasing with time (habitat × time interaction in 2005:  $F_{1, 120} = 7$ , P < 0.05; and 2006:  $F_{1, 120} = 120$ , P < 0.001). The mean emergence rate was higher in 2005 for *Pinus nigra* than for *Pinus sylvestris* (33.2% ± 1.98 and 24.4% ± 1.70, respectively), but not in 2006 (31.7% ± 3.56 and 32.7% ± 3.90, respectively) (Table II).

Seedling survival was very low, especially in 2005, but was higher near shrubs, especially in 2006 (0.00 and 0.86% in 2005, and 0.056% and 19.3% in 2006, respectively in grassland and at the shrub periphery) (Figure 2; Table II). Most mortality occurred during the spring and

TABLE I. Effect of *Juniperus communis* on microenvironmental variables at the limit of its canopy (means at shrub periphery and in open habitat,  $\pm$  SE). Soil temperature was measured at 5 cm depth, soil relative humidity in the upper 15 cm, vapour pressure deficit at 20 cm height, and soil pH and ammonium and nitrate content in the upper 10 cm (ns P > 0.05; \*\*\* P < 0.001).

Abiotic variable				Significance
	Date of measurement	Shrub periphery	Open	of shrub effect
Soil temperature (°C)	Daily means in April-May 2006	$T_{min} 9.1 \pm 0.37$ $T_{max} 12.1 \pm 0.41$	$\begin{array}{c} T_{min} \ 9.9 \pm 0.42 \\ T_{max} \ 18.6 \pm 0.63 \end{array}$	***
Soil relative humidity (1/1,8%)	June 2005	15.5±0.39	$12.2\pm0.69$	***
•	June 2006	$12.8 \pm 0.76$	$10.1 \pm 0.48$	****
Vapour pressure deficit (mBar)	Daily mean at 1400 for May–July 2006	$21.2 \pm 1.21$	$22.9 \pm 1.26$	ns
Soil pH	June 2006	$7.2\pm0.059$	$\textbf{7.3} \pm \textbf{0.026}$	ns
Soil ammonium content (ppm)	June 2006	$1.29 \pm 0.13$	$0.97 \pm 0.17$	ns
Soil nitrate content (ppm)	June 2006	0.43 ± 0.056	$1.08 \pm 0.15$	***



FIGURE 2. Survival rates of *Pinus nigra* in (a) 2005 and (d) 2006 and *Pinus splvestris* in (b) 2005 and (e) 2006 and soil water content in (c) 2005 and (f) 2006 as a function of time (julian days). Survival rates are shown for open (white dots) or shrub (black triangles) habitats, in grazed (dotted lines) or non-grazed (solid lines) conditions. Soil water content is shown for open (white dots) and shrub (black triangles) habitats (\*\*\* P < 0.001). Vertical arrows show livestock introduction, and vertical bars show standard errors.

summer drought, with similar survival of the 2 pine species (Figure 2). At the shrub periphery, grazing further reduced the survival of seedlings by 33.3% and 59.0% for *P. sylvestris* and 64.3% and 74.1% for *P. nigra*, respectively in 2005 and 2006 (Figure 2), but the large mortality of seedlings induced by drought in open habitat prevented characterization of the grazing effect in open habitat, so the indirect effect of shrubs on pine herbivory could not be evaluated.

The protection by shrubs against herbivores was thus further recorded for 1-y-old transplanted pine saplings. Grazing led to the removal of the apical bud in 94% of the grazed saplings; thus, most of the grazed saplings are expected to die (Figure 3). We did not observe damage by trampling. Sapling survival in grassland was very low, especially for *P. nigra* (4.84%  $\pm$  2.75, compared to 21.7%  $\pm$  4.41 for *P. sylvestris*), but remained quite high at the shrub

a biological meaning are	$r \operatorname{snown}(\operatorname{ns} P > $	· 0.1; = P < 0	9.1; <b>**</b> <i>F</i> ≤	0.01; *** / <	0.001).			
		Emergence m	ites		S	urvival rate		
Effect	SS	đf	I <sup>P</sup> tes	st	Deviance change	đť	Quasi-,	F <sup>*</sup> test
Shrub	19 802.5	1	36.42	***	5278.7	1	8.0	**
Grazing	81.2	1	0.38	ns	440.9	1	1.4	ns
Shrub × grazing	10.0	1	0,05	ns	175.6	1	0.6	ns
Species	616.2	1	11.05	***	526.3	1	2.8	ns
Species × shrub	25.6	1	0.46	ns	929.8	I	4.9	*
Species × grazing	15.6	1	0.28	DS .	13.5	1	0.1	ns
Species × shrub × grazing	25.6	1	0.68	ns	262.7	1	1,4	ns
Year	455.6	1	9.32	***	2437.6	1	159.3	***
Year × shrub	147.5	1	301.59	***	3415.6	1	223.2	由非年
Year × grazing	9.0	1	0.18	ns	23.4	1	1.5	ns
Year × species	970.2	1	19.84	****	3142.2	1	205.3	长老女

TABLE II. Results of the general linear model (emergence) and logistic regression (survival) for effects of shrub, grazing, pine species, and experimental years on pine seedling emergence and survival rates. Interactions with blocks were included in the model, but only effects with



FIGURE 3. Rates of herbivory on 1-y-old transplanted seedlings of Pinus nigra and Pinus sylvestris at the end of the grazing season, in grassland and at the shrub periphery. In black: seedlings very intensively grazed (leaves and apical bud eaten); in dark grey: seedlings intensively grazed (apical bud eaten, a few leaves intact); in light grey: seedlings lightly grazed (apical bud intact and a few leaves eaten). Vertical bars show standard errors.

periphery (68.3%  $\pm$  6.67 and 50.1%  $\pm$  4.93 for *P. sylvestris* and P. nigra, respectively). Saplings situated in grassland were significantly more grazed than those situated near shrubs (quasi- $F_{1,2} = 84$ , P = 0.012) (Figure 3). Pinus nigra saplings were more grazed than *P. sylvestris* ones (quasi- $F_{1,2} = 33, P = 0.029$ ).

# DIRECT EFFECT OF SHADING AND HERB COMPETITION RELEASE

Soil relative humidity tended to be higher at the limit of artificial shade than in grassland ( $F_{1,9} = 4$ , P = 0.07), especially at the end of June and July ( $F_{1,9} = 4.6$ , P = 0.06, and  $F_{1,9} = 6.7$ , P = 0.03, respectively). It was also higher in the weeded treatment than in intact herbaceous cover  $(F_{1,18} = 5.3, P = 0.033)$ , especially at the end of June and July (Figure 4). Herbaceous biomass was not affected by artificial shade  $(192.0 \text{ g} \cdot \text{m}^{-2} \pm 14.2 \text{ compared to } 174.6 \text{ g} \cdot \text{m}^{-2} \pm 11.1,$ respectively with and without shade,  $T_{18} = 0.96$ , P > 0.1).

Habitat amelioration was highlighted by the higher

survival of pine seedlings at the shrub periphery than in grassland in relation to higher soil moisture (Figure 2) and to the direct effect of shading, as suggested by the positive effect of the artificial vertical pan on pine seedling survival (Figure 4). This result is consistent with other studies documenting the facilitation of tree establishment by shrubs in Mediterranean environments (Gómez-Aparício et al., 2004; 2005). The higher facilitative effect of shrubs in 2006 than in 2005 (Figure 2) suggests that habitat amelioration is likely to be maximal when rainfall is low to moderate (Greenlee & Callaway, 1996; Kitzberger, Steinaker & Veblen, 2000). Habitat amelioration is probably very low under high rainfall, when seedling survival in grassland is fairly high (Debain, Curt & Lepart, 2005), and under very low rainfall (as in 2005), as all seedlings die whatever their establishment site (Figure 2).

Associational defence was also an important facilitative mechanism (Figure 3), as already highlighted in systems

The mean seedling emergence was 20.6%, with no difference between pine species, habitats, or herb cover treatments (Table III). Seedling survival was null in unweeded plots, whatever the shade (Figure 4). It varied between  $27.5\% \pm 1.38$  for P. nigra and  $2\overline{2}.8\% \pm 1.14$  for P. sylvestris in weeded plots (Figure 4), with larger values in shaded plots  $(65.7\% \pm 5.76 \text{ and } 38.1\% \pm 7.30 \text{ for } P. nigra \text{ and } P. sylvestris,$ respectively) than in open habitats (26.6%  $\pm$  6.46 and  $22.5\% \pm 6.56$  for *P. nigra* and *P. sylvestris*, respectively). Pinus nigra had a higher survival rate than P. sylvestris in weeded and shaded plots (Figure 4; Table III).

## EFFECT OF SHRUBS ON SAPLING GROWTH

Diameter growth of pine saplings, which did not differ between sites  $(F_{2,97} = 0.7, P > 0.1)$ , was higher for pines established in grassland compared to those at the shrub periphery ( $F_{1,97} = 4.7$ , P = 0.03). Sapling height growth was not affected by site or location ( $F_{2,88} = 1.7$ , P = 0.2 and  $F_{1,88} = 2.8, P = 0.1$ , respectively).

# Discussion

This study aimed at testing the relative influence of habitat amelioration, associational defence, and herb competitive release by shrubs on pine sapling establishment in communities grazed by domestic livestock (Figure 1).

grazed by wild herbivores (McAuliffe, 1986; Brooker et al., 2006) and domestic herbivores in natural reserves (Kuiters & Slim, 2003; Bossuyt, De Fre & Hoffmann, 2005) or agricultural systems (Rousset & Lepart, 2000; Rebollo et al., 2002; Bakker et al., 2004; Callaway et al., 2005). Pine saplings were protected from herbivory when established near unpalatable shrubs, probably through herbivore deterrence. However, the positive effect was lower for these shade-avoiding species than for shade-tolerant saplings that can grow under the canopy of shrubs (Rousset & Lepart, 2000).

The negative effect of unweeded vegetation on seedling survival (Figure 4) confirms the occurrence of competition between herbs and tree seedlings (Harrington, 1991; Jurena & Archer, 2003). Competition probably occurred mainly for



FIGURE 4. Survival rates of (a) *Pinus sylvestris* and (b) *Pinus nigra*, and (c) soil relative humidity (%, v/v), as a function of time (julian days) in 2006, in open (dots) or shade (triangles) habitats, in unweeded (black symbols with solid line) or weeded (white symbols with dotted line) plots. Vertical bars show standard errors (for soil water content,  $\dagger P < 0.1$ ; \* P < 0.01).

water since little or no difference in seedling survival was detected between weeded and undisturbed plots during a wet year (Debain, Curt & Lepart, 2005). However, we did not find evidence that shrubs released herb competition, as shading, mimicking the effect of the shrub canopy, did not improve seedling survival in herb cover (Figure 4). Light interception at the shrub periphery is probably too low to reduce herb competition, in contrast to light conditions under trees or shrubs where shade-tolerant woody seedlings can successfully become established (Li & Wilson, 1998; Pagès et al., 2003; Siemann & Rogers, 2003; Kunstler et al., 2006). Nevertheless, it is rather puzzling that pine seedling survival was not improved in undisturbed vegetation by the artificial shade (Figure 4), whereas it remained quite high at the shrub periphery (Figure 2). This could be explained by a stronger competitive effect of herbs in the ungrazed site compared with the grazed one or by an effect of herb competitive release by shrubs induced by a mechanism other than shading, perhaps by litter accumulation.

The co-occurrence of habitat amelioration and associational defence in our system contrasts with the predictions of the facilitation models (Bertness & Callaway, 1994; Bruno, Stachowicz & Bertness, 2003), which were designed for wild herbivores. These models predict that the 2 mechanisms occur at opposite ends of a continuum between communities submitted to high abiotic stress, where habitat amelioration occurs, and those submitted to high herbivore pressure, where associational defence occurs. Experimental studies support that the importance of habitat amelioration increases with environmental stress (Greenlee & Callaway, 1996; Maestre, Bautista & Cortina, 2003; Sthultz, Gehring & Whitham, 2007) and that the importance of associational defence increases with herbivore pressure (Rebollo, Milchunas & Noy-Meir, 2005). For unmanaged systems, the hypothesis of a negative correlation between wild herbivore pressure and abiotic stress is linked to vegetation productivity: high abiotic stress leads to low-productive communities, whereas herbivore density tends to increase with productivity (Leibold, 1996; Crête, 1999). These relationships result in the prediction that habitat amelioration and associational defence should occur in different communities. This prediction is supported by the predominance of a single facilitative mechanism, habitat amelioration or associational defence, in systems grazed by wild herbivores or by domestic herbivores but at very low grazing pressure (Callaway, 1992; Levine, 2000; Rebollo et al., 2002). However, in systems grazed by domestic livestock, grazing pressure is managed

TABLE III. Results of the general linear model (emergence) and logistic regression (survival) for the effects of shade, herb competition treatment, and pine species on emergence and survival rates. Interactions with blocks were included in the model, but only effects with a biological meaning are shown (ns P > 0.05; \*\* P < 0.01; \*\*\* P < 0.001).

		Emergence rate	\$	Ş	urvival rate	:5	
Effect	<b>S</b> S	df	F test	Deviance change	df	Quasi-F	test
Shade	27.6	1	0.54 ns	9880.7	1	19.6	***
Herb	1.5	1	0.02 ns	46 196.5	1	17 415.0	***
Herb × shade	248.5	1	3.90 ns	25.8	1	9.7	**
Species	154.0	1	2.40 ns	1383.4	1	521.5	***
Species × shade	122.5	I	1.90 ns	696.4	1	262.5	***
Species × herb	90.3	t	0.30 ns	693.8	ĩ	261.5	***
Species × shade × herb	63.0	1	1.00 ns	84.7	i	31,9	***

by farmers and is usually higher and more stable in space and time than in systems grazed by wild herbivores. Forage supplementation, water supply, and seasonal transhumance allow domestic herbivores to cope with annual and interannual variability in primary resources (Van de Koppel & Rietkerk, 2000). Therefore, even in stressful environments, grazing pressure due to domestic livestock can be high. In managed grasslands, the combination of high grazing pressure and severe environmental conditions can lead to the occurrence of habitat amelioration and associational defence in the same place. This exception to the model of Bertness and Callaway (1994) could be widespread as a significant part of herbaceous ecosystems in the world are grazed by domestic herbivores in stressful conditions.

Our results confirm the importance of facilitative interactions in plant invasion (Bruno, Stachowicz & Bertness, 2003). The introduced and native pine species studied here present some subtle differences in their regeneration niches. Pinus nigra seedlings are more resistant to herbaceous competition in nutrient-rich conditions (Debain, Curt & Lepart, 2005) and survive better than those of P. sylvestris in shaded and weeded plots. Combined with a longer mean distance of seed dispersal (Debain et al., 2007), these findings suggest that P. nigra could invade open habitats faster than the native pine species. However, in grazed grassland, P. nigra sapling survival was about 4.5 times lower than that of P. sylvestris (Figure 3), which could be due to the lower height of P. sylvestris seedlings, inducing a lower probability of being grazed in the herbaceous cover. Surprisingly, we did not find a difference in seedling drought tolerance between the 2 species (Figures 2 and 4), despite the more Mediterranean distribution and larger drought tolerance of P. nigra (Martinez-Vilalta & Piñol, 2002). Pinus sylvestris is near the southern limit of its distribution range, and seedling establishment is limited by summer drought in the relict populations present at high altitudes in Spain (Castro et al., 2004; 2005). The decrease by shrubs of the deleterious drought effects on pine seedling establishment could become of increasing importance in the face of increasing water deficit, Indeed, in the study site, mean June rainfalls are expected to decrease by 20% (predicted evolution for 2050-2100, Meteo France, ARPEGE model).

Besides their facilitative effects, shrubs also had negative effects on pine establishment and growth, but these were of minor importance compared with the positive effects allowing some seedlings to survive under grazed or drought conditions. Seed predation was 1.5 times higher in the shrub vicinity than in grassland, probably because shrubs provided shelter for seed consumers (Hulme, 1998). The reduction of emergence in the shrub vicinity was probably linked to the delayed emergence rates. This was due to the lower soil temperature in the shrub vicinity, which increases desiccation risks, as suggested by Castro *et al.* (2005b) for explaining similar effects of *Juniperus communis* on *P. sylvestris* germination. Lastly, light interception by shrubs also slightly reduced the growth of the saplings established in their neighbourhood.

In conclusion, our study pointed out the importance of both habitat amelioration and associational defence in dry grasslands grazed by domestic livestock. This combination of positive mechanisms, which is not included in conceptual models of facilitation in unmanaged systems (Bertness & Callaway, 1994; Bruno, Stachowicz & Bertness, 2003), could be widespread in systems grazed by domestic herbivores. Better knowledge of the mechanisms that trigger facilitative interactions is necessary to understand and predict species dynamics in the face of global change, including climatic and anthropogenic factors.

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#### Literature cited

- Bakker, E. S., H. Olff, C. Vandenberghe, K. De Maeyer, R. Smit, J. M. Gleichman & F. W. M. Vera, 2004. Ecological anachronisms in the recruitment of temperate light-demanding tree species in wooded pastures. Journal of Applied Ecology, 41: 571–582.
- Barbéro, M., R. Loisel, P. Quézel, F. Médail, D. M. Richardson & F. Romane, 1998. Pines of the Mediterranean Basin. Pages 153-170 in D. M. Richardson (ed.). Ecology and Biogeography of *Pinus*. Cambridge University Press, Cambridge.
- Baumeister, D. & R. M. Callaway, 2006. Facilitation by *Pinus flexilis* during succession: A hierarchy of mechanisms benefits other plant species. Ecology, 87: 1816–1830.
- Bertness, M. D. & R. Callaway, 1994. Positive interactions in communities. Trends in Ecology & Evolution, 9: 191–193.
- Bossuyt, B., B. De Fre & M. Hoffmann, 2005. Abundance and flowering success patterns in a short-term grazed grassland: Early evidence of facilitation. Journal of Ecology, 93: 1104–1114.
- Brooker, R. W. & T. V. Callaghan, 1998. The balance between positive and negative plant interactions and its relationship to environmental gradients: A model. Oikos, 81: 196-207.
- Brooker, R. W., D. Scott, S. C. F. Palmer & E. Swaine, 2006. Transient facilitative effects of heather on Scots pine along a grazing disturbance gradient in Scottish moorland. Journal of Ecology, 94: 637-645.
- Bruno, J. F., J. J. Stachowicz & M. D. Bertness, 2003. Inclusion of facilitation into ecological theory. Trends in Ecology & Evolution, 18: 119-125.
- Cadillon, M., 1970. Les sols du Causse du Larzac. Université Montpellier II, Montpellier.
- Callaway, R. M., 1992. Effect of shrubs of *Quercus douglasii* and *Quercus lobata* in California. Ecology, 73: 2118–2128.
- Callaway, R. M., 1995. Positive interactions among plants. Botanical Review, 61: 306-349.
- Callaway, R. M. & L. R. Walker, 1997. Competition and facilitation: A synthetic approach to interactions in plant communities. Ecology, 78: 1958–1965.
- Callaway, R. M., D. Kikodze, M. Chiboshvili & L. Khetsuriani, 2005. Unpalatable plants protect neighbours from grazing and increase plant community diversity. Ecology, 86: 1856–1862.

- Caplat, P., J. Lepart & P. Marty, 2006. Landscape patterns and agriculture: Modelling the long term effects of human practices on *Pinus sylvestris* spatial dynamics (Causse Mejean, France). Landscape Ecology, 21: 657–670.
- Castro, J., R. Zamora, J. A. Hodar & J. M. Gomez, 2004. Seedling establishment of a boreal tree species (*Pinus sylvestris*) at its southernmost distribution limit: Consequences of being in a marginal Mediterranean habitat. Journal of Ecology, 92: 266-277.
- Castro, J., R. Zamora, J. A. Hodar & J. M. Gomez, 2005a. Alleviation of summer drought boosts establishment success of *Pinus sylvestris* in a Mediterranean mountain; An experimental approach. Plant Ecology, 181: 191–202.
- Castro, J., R. Zamora, J. A. Hodar & J. M. Gomez, 2005b. Ecology of seed germination of *Pinus sylvestris* L. at its southern, Mediterranean distribution range. Investigación Agraria: Sistemas y Recursos Forestales 14:143-152.
- Coste, H., 1937. Flore descriptive et illustrée de la France, de la Corse et des contrées limitrophes. Librairie des Sciences et des Arts, Paris.
- Crête, M., 1999. The distribution of deer biomass in North America supports the hypothesis of exploitation ecosystems. Ecology Letters, 2: 223–227.
- Debain, S., T. Curt & J. Lepart, 2005. Indirect effects of grazing on the establishment of *Pinus sylvestris* and *Pinus nigra* seedlings in calcareous grasslands in relation to resource level. Écoscience, 12: 192-201.
- Debain, S., J. Chadoeuf, T. Curt, G. Kunstler & J. Lepart, 2007. Comparing effective dispersal in expanding population of *Pinus sylvestris* and *Pinus nigra* in calcareous grassland. Canadian Journal of Forest Research, 37: 705–718.
- Debussche, M., J. Lepart & A. Dervieux, 1999. Mediterranean landscape changes: Evidence from old postcards. Global Ecology and Biogeography, 8: 3-15.
- Egli, P. & B. Schmid, 2001. The analysis of complex leaf survival data. Basic and Applied Ecology, 2: 223-231.
- Franco, A. C. & P. S. Nobel, 1989. Effect of nurse plants on the microhabitat and growth of eacti. Journal of Ecology, 77: 870-886.
- Gómez-Aparicio, L., R. Zamora, J. M. Gomez, J. A. Hodar, J. Castro & E. Baraza, 2004. Applying plant facilitation to forest restoration: A meta-analysis of the use of shrubs as nurse plants. Ecological Applications, 14: 1128-1138.
- Gómez-Aparicio, L., J. M. Gomez, R. Zamora & J. L. Boettinger, 2005. Canopy versus soil effects of shrubs facilitating tree seedlings in Mediterranean montane ecosystems. Journal of Vegetation Science, 16: 191-198.
- Greenlee, J. T. & R. M. Callaway, 1996. Abiotic stress and the relative importance of interference and facilitation in montane bunchgrass communities in western Montana. American Naturalist, 148: 386–396.
- Harrington, G. N., 1991. Effects of soil moisture on shrub seedling survival in a semiarid grassland. Ecology, 72: 1138–1149.
- Hulme, P. E., 1998. Post-dispersal seed predation: Consequences for plant demography and evolution. Perspectives in Plant Ecology, Evolution and Systematics, 1: 32-46.
- Jurena, P. N. & S. Archer, 2003. Woody plant establishment and spatial heterogeneity in grasslands. Ecology, 84: 907–919.
- Kitzberger, T., D. F. Steinaker & T. F. Veblen, 2000. Effects of climatic variability on facilitation of tree establishment in northern Patagonia. Ecology, 81: 1914–1924.

- Kuiters, A. T. & P. A. Slim, 2003. Tree colonisation of abandoned arable land after 27 years of horse-grazing: The role of bramble as a facilitator of oak wood regeneration. Forest Ecology and Management, 181: 239-251.
- Kunstler, G., T. Curt, M. Bouchaud & J. Lepart, 2006. Indirect facilitation and competition in tree species colonization of sub-Mediterranean grasslands. Journal of Vegetation Science, 17: 379–388.
- Leibold, M. A., 1996. A graphical model of keystone predators in food webs: Trophic regulation of abundance, incidence, and diversity patterns in communities. American Naturalist, 147: 784–812.
- Levine, J. M., 1999. Indirect facilitation: Evidence and predictions from a riparian community. Ecology, 80: 1762-1769.
- Levine, J. M., 2000. Complex interactions in a streamside plant community. Ecology, 81: 3431-3444.
- Li, X. D. & S. D. Wilson, 1998. Facilitation among woody plants establishing in an old field. Ecology, 79: 2694–2705.
- Maestre, F. T., S. Bautista & J. Cortina, 2003. Positive, negative, and net effects in grass-shrub interactions in Mediterranean semiarid grasslands. Ecology, 84: 3186-3197.
- Martinez-Vilalta, J. & J. Piñol, 2002. Drought-induced mortality and hydraulic architecture in pine populations of the NE Iberian Peninsula. Forest Ecology and Management, 161: 247–256.
- Marty, P., E. Pélaquier, B. Jaudon & J. Lepart, 2002. Spontaneous reforestation in a peri-Mediterranean landscape: History of agricultural systems and dynamics of woody species. International Conference. Environmental Dynamics and History in Mediterranean Regions, Paris, France.
- McAuliffe, J. R., 1986. Herbivore-limited establishment of a Sonoran desert tree: Cercidium microphyllum. Ecology, 67: 276-280.
- Miriti, M. N., 2006. Ontogenetic shift from facilitation to competition in a desert shrub. Journal of Ecology, 94: 973–979.
- Pagès, J. P., G. Pache, D. Joud, N. Magnan & R. Michalet, 2003. Direct and indirect effects of shade on four forest tree seedlings in the French Alps. Ecology, 84: 2741–2750.
- Rebollo, S., D. G. Milchunas & I. Noy-Meir, 2005. Refuge effects of a cactus in grazed short-grass steppe. Journal of Vegetation Science, 16: 85-92.
- Rebollo, S., D. G. Milchunas, I. Noy-Meir & P. L. Chapman, 2002. The role of a spiny plant refuge in structuring grazed shortgrass steppe plant communities. Oikos, 98: 53-64.
- Rousset, O. & J. Lepart, 2000. Positive and negative interactions at different life stages of a colonizing species (*Quercus humilis*). Journal of Ecology, 88: 401–412.
- Siemann, E. & W. E. Rogers, 2003. Changes in light and nitrogen availability under pioneer trees may indirectly facilitate tree invasions of grasslands. Journal of Ecology, 91: 923-931.
- Sthultz, C. M., C. A. Gehring & T. G. Whitham, 2007. Shifts from competition to facilitation between a foundation tree and a pioneer shrub across spatial and temporal scales in a semiarid woodland. New Phytologist, 173: 135-145.
- Van de Koppel, J. & M. Rietkerk, 2000. Herbivore regulation and irreversible vegetation change in semi-arid grazing systems. Oikos, 90: 253-260.
- Willis, K. J., K. D. Bennett & H. J. B. Birks, 1998. The late Quaternary dynamics of pines in Europe. Pages 107-121 in D. M. Richardson (ed.). Ecology and Biogeography of *Pinus*. Cambridge University Press, Cambridge.

# **ESSAY REVIEW**

# Facilitation in plant communities: the past, the present, and the future

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# Summary

1. Once neglected, the role of facilitative interactions in plant communities has received considerable attention in the last two decades, and is now widely recognized. It is timely to consider the progress made by research in this field.

**2.** We review the development of plant facilitation research, focusing on the history of the field, the relationship between plant–plant interactions and environmental severity gradients, and attempts to integrate facilitation into mainstream ecological theory. We then consider future directions for facilitation research.

**3.** With respect to our fundamental understanding of plant facilitation, clarification of the relationship between interactions and environmental gradients is central for further progress, and necessitates the design and implementation of experiments that move beyond the clear limitations of previous studies.

4. There is substantial scope for exploring indirect facilitative effects in plant communities, including their impacts on diversity and evolution, and future studies should connect the degree of non-transitivity in plant competitive networks to community diversity and facilitative promotion of species coexistence, and explore how the role of indirect facilitation varies with environmental severity.

**5.** Certain ecological modelling approaches (e.g. individual-based modelling), although thus far largely neglected, provide highly useful tools for exploring these fundamental processes.

**6.** Evolutionary responses might result from facilitative interactions, and consideration of facilitation might lead to re-assessment of the evolution of plant growth forms.

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7. Improved understanding of facilitation processes has direct relevance for the development of tools for ecosystem restoration, and for improving our understanding of the response of plant species and communities to environmental change drivers.

**8.** Attempts to apply our developing ecological knowledge would benefit from explicit recognition of the potential role of facilitative plant–plant interactions in the design and interpretation of studies from the fields of restoration and global change ecology.

**9.** Synthesis: Plant facilitation research provides new insights into classic ecological theory and pressing environmental issues. Awareness and understanding of facilitation should be part of the basic ecological knowledge of all plant ecologists.

**Key-words:** competition, disturbance, ecological theory, environmental change, environmental gradients, facilitation, plant communities, positive plant interactions, review, stress

# Introduction

'He grew low palms, for their spreading leaves shaded his plants from the sun which otherwise might in that stark valley wither them.'

- T. E. Lawrence

Plants interact in many different ways, both negative and positive. They compete for light, nutrients, space, pollinators and water, but at the same time protect one another from the impacts of herbivores, potential competitors or extremes of climate, and provide additional resources through canopy leaching, microbial enhancement, mycorrhizal networks and hydraulic lift.

There has been a particular resurgence of interest in those positive, non-trophic interactions that occur between physiologically independent plants and that are mediated through changes in the abiotic environment or through other organisms (both plant and animal; for reviews see Hunter & Aarssen 1988; Callaway 1995; Bruno et al. 2003; Flores & Jurado 2003). Substantial recent research has examined such interactions, exploring in detail the mechanisms by which they take place (Holzapfel & Mahall 1999; Maestre et al. 2003a), the way in which they control the structure and function of communities (Tirado & Pugnaire 2003; Kikvidze et al. 2005), and their implications for classic ecological theory (Bruno et al. 2003; Lortie et al. 2004). Furthermore, plant facilitation research is making links to some of the most important current ecological issues, including the relationship between biodiversity and ecosystem function, and the impacts of global change (Hooper et al. 2005; Brooker 2006).

It is perhaps a suitable time for reviewing progress. Has the recent research effort taken this field forward? Given our current understanding, what gaps in our knowledge of facilitative interactions most urgently need to be addressed? Can we understand their role in mediating the impact of environmental change drivers or use this knowledge to mitigate such impacts? This paper will attempt to answer these questions.

As a necessary starting point we provide a brief overview of some key papers (several of which are themselves reviews) that helped to revitalize interest in plant facilitation. We also discuss how their conclusions have been the impetus for recent developments in facilitation research (examples in Table 1; see also Table S1 in Supplementary Material). We then suggest areas where we consider there to be substantial opportunities for future research, and the approaches that might be used. We focus our review in particular on interactions between vascular plants, as they have been the focus of the bulk of plant facilitation studies.

# Recent developments in plant facilitation research

Until recently, many ecologists only encountered facilitative plant–plant interactions within a particular context: facilitation of one successional stage by the preceding stage was a recognized, albeit underestimated, component of some theories of succession (Clements 1916; Connell & Slatyer 1977) and had been demonstrated in a number of ecosystems, for example in the classic studies of plant succession in Glacier Bay (Crocker & Major 1955; Chapin *et al.* 1994; for a review of the role of facilitation in primary succession see Walker & del Moral 2003).

However, in the late 1980s and throughout the 1990s a number of papers (e.g. Hunter & Aarssen 1988; Bertness & Callaway 1994; Callaway 1995, 1997; Brooker & Callaghan 1998) pointed out that facilitative interactions operated to regulate plant success and community composition in stable, non-successional communities, and were not merely important during successional change. Although some of these reviews (e.g. Hunter & Aarssen 1988) considered relatively well-recognized types of positive interactions, e.g. the attraction of pollinators (Thomson 1978), the positive impact of shrub species on soil nitrogen availability (García-Moya & McKell 1970), the capacity for resource sharing through common mycorrhizal networks (Chiariello et al. 1982), and classic nurse plant effects (Went 1942; Fig. 1), they also discussed a developing body of research examining non-trophic interactions between neighbouring plants that either did not fit into these well-recognized categories, or that demonstrated that some facilitative effects (e.g. the nurse plant effect) were more widespread than was previously thought.

From these papers some common themes emerged. Evidence of facilitative effects between plants tended to come from severe environments, such as deserts, arctic or alpine

Author(s)	Topic of paper	Key development or finding
Integrating facilitation into mainstrea	m ecological theory	
Bruno <i>et al.</i> (2003)	The need to include facilitation into mainstream ecological theory and the proposition that this process will 'challenge some of our most cherished paradigms'.	Revision of theory to include: potential for expansion of the realized niche by facilitation, positive density-dependence at high population densities, inclusion of facilitation in the diversity-invasibility paradigm, the role of dominant species in regulating local diversity.
Michalet et al. (2006)	Revision of Grime's (1973) model to incorporate facilitative interactions in plant communities.	Explicit consideration of facilitation in one of the central theories of plant community ecology.
Facilitation and environmental gradies	nts	
Tielbörger & Kadmon (2000a)	Temporal environmental variation between competition and facilitation in desert plants.	Increasing annual rainfall produced varying responses in impact of desert shrubs on annuals. Increased rainfall changed the effect of shrubs from negative to neutral, or neutral to positive depending on the species. Findings contradict prediction of increased facilitation with increased environmental severity.
Choler et al. (2001)	Examination of the relative importance of competitive and facilitative interactions along elevational and topographical gradients in alpine environments.	Facilitation increases with increasing altitude or exposure, but particularly strong for species at their upper altitudinal or physiological limit. Facilitation may therefore be promoting niche expansion into severe environments.
Callaway et al. (2002)	Multi-site examination of relationship between environmental severity and plant–plant interactions in arctic–alpine environments.	General shift from competition to facilitation as average community interaction with increasing altitude, and demonstration of large-scale relationship between dominant type of interaction and environmental severity.
Maestre & Cortina (2004)	Test of stress gradient hypothesis in semi-arid steppe environment.	Hump-backed relationship between interactions and accumulated rainfall (environmental severity) – competitive interactions dominate at both extremes of the severity gradient.
Maestre et al. (2005)	Meta-analysis of field and common garden experiments evaluating the effect of abiotic stress on the net outcome of plant-plant interactions in arid and semi-arid environments.	The measure of plant performance and experimental approach strongly influence the observed relationship between stress and net plant–plant interactions. Conclude that the role of facilitation does not increase with abiotic stress.
Cavieres et al. (2006)	Examination of the relative frequency of facilitative interactions at the community level along elevational gradients in Mediterranean-type alpine environments	Facilitative interactions did not increase with elevation. They were more frequent at lower elevations where environmental stress is higher due to water limitation, thus supporting the SGH. Stress-tolerant species (e.g. annuals, shrubs) were not facilitated.
Lortie & Callaway (2006)	Critique of Maestre <i>et al.</i> 's (2005) meta-analysis.	Conclude that study selection for Maestre <i>et al.</i> 's analysis was not rigorous, and that differences in stress gradient lengths between studies could have a considerable impact on results.
Indirect interactions and facilitation		
Levine (1999)	Experimental study of indirect facilitation in a riparian community.	Experimental demonstration of indirect facilitation. Proposition of a general hypothesis that indirect facilitation among competitors will be important in assemblages where species vary in competitive mechanism.

Table 1. Advances in facilitation research since the publication of Bertness & Callaway (1994) and Callaway (1995), and examples of papers that have addressed these issues including their author(s), topics, and key development or finding. For a more extensive list of studies see Table S1

#### Table 1. continued

Author(s)	Topic of paper	Key development or finding
Development of ecological models incorp	porating plant facilitation	
Travis et al. (2005, 2006)	Development of a simple patch occupancy model to simulate the dynamics of two species, a mutualist and cheater, along an imposed environmental gradient.Use of simulation modelling to ex in the role of plant–plant interact environmental gradients and their the spatial distribution of species.	
Brooker <i>et al.</i> (2006)	Development of patch-occupancy model rom Travis <i>et al.</i> (2005, 2006) to simulate mpacts of climate change on a simple model ystem including two main plant strategies. Demonstration that species distributi environmental gradients, as determin facilitative and competitive interaction with dispersal ability to determine ran dynamics and species survival.	
Connecting facilitation to evolution		
Scheffer & van Nes (2006)	Examination of the evolutionary processes involved in self-organized similarity.	Indirect facilitation may be one of the processes driving the evolution of niche convergence.
Valiente-Banuet et al. (2006)	Facilitation of Tertiary plant lineages in Mediterranean-climate ecosystems by modern Quaternary species.	A large number of ancient Tertiary plant lineages, which evolved under wetter climatic conditions than found currently, are preserved by facilitative nurse plant effects from modern Quaternary species. Facilitation is a source of stabilizing selection for the regeneration niches of Tertiary species.
Facilitation and ecosystem restoration		
Maestre et al. (2001)	Potential for using nurse plant effect of grasses to promote shrub establishment in degraded semiarid steppe ecosystems.	Facilitative effect of dominant grass on introduced shrubs, related to improved water potential. Evidence of potential use of facilitative interactions for the restoration of semi-arid steppes.
Castro <i>et al.</i> (2004)	Use of shrubs as nurse plants to promote reforestation in Mediterranean-type mountain ecosystems.	Technique proven – proposed to have the added advantage of utilizing natural successional processes, and thus reducing impact on the studied community.
Gómez-Aparicio et al. (2004)	Meta-analysis of the use of shrubs as nurse plants for reforestation.	Consistent evidence of facilitative effect but strength varied between environment and species. Pioneer shrubs can positively influence restoration efforts in Mediterranean mountains.

tundra systems, or salt marshes. It was even suggested that 'fascination with competition has focused attention on communities where competition is conspicuous' (Bertness & Callaway 1994). These reviews also proposed that the severity of the environment influenced the balance of the numerous positive and negative interactions that occur between interacting plants. For example, plants that compete for nutrients can have simultaneous positive effects through the provision of shelter or protection from herbivory. Increased environmental severity appeared to increase either the potential for, or strength of, positive interactions, relative to negative interactions, thus shifting the observable net interactions toward facilitation in extreme environments (Hunter & Aarssen 1988; Bertness & Callaway 1994; Callaway & Walker 1997; Brooker & Callaghan 1998).

Competition still dominates consideration of plant-plant interactions within the ecological literature (Fig. 2). However, since these earlier papers, recent studies have helped to deal with 'the mistaken notion that positive interactions are not well demonstrated with field experiments' (Callaway 1995), and have explored the issues raised in detail (Table 1). One topic in particular has received considerable recent attention: the relationship between plant–plant interactions and environmental severity.

Although Hunter & Aarssen (1988) discussed the balance between positive and negative plant–plant interactions, they did not suggest any generic relationships between this balance and environmental gradients. Bertness & Callaway's (1994) paper took such a step, proposing that increases in either the degree of physical stress or consumer pressure would increase the frequency of positive interactions. Callaway & Walker (1997) discussed the possible regulatory effects of climatic conditions and life-history stage (see also Callaway 1995) on the balance between positive and negative interactions. Brooker & Callaghan (1998) explicitly framed their model within the context of Grime's (1974, 1977, 1979) definitions of stress and disturbance, concentrating on changes in interactions along gradients of disturbance because of unresolved



Fig. 1. Examples of mechanisms of plantplant facilitation. (a) Buffered substrate and air temperature, enhanced soil moisture and nutrient content. Cushion of Azorella monantha harbouring native and invasive species (e.g. the Andean cauliflower Nastanthus agglomeratus and the field chickweed Cerastium arvense, respectively) at the upper limit of vegetation (3600 m a.s.l) in the high Andes of central Chile. Photo: Lohengrin A. Cavieres (see Cavieres et al. 2005, 2007). (b) Protection from drought. Adult individual of the tussock grass Stipa tenacissima facilitating a sapling of Pinus halepensis in a semi-arid steppe, south-east Spain. Photo: Fernando T. Maestre (see Maestre et al. 2001, 2003a). (c) Protection from browsing. Quercus pubescens seedling within unpalatable Buxus sempervirens shrubs, southern France. Photo: Georges Kunstler (see Kunstler et al. 2006). (d) Protection from browsing and drought. Facilitation by Gymnocarpos decander of annual vegetation in a semi-arid environment, Jordan. Photo: Pierre Liancourt.

debate concerning gradients of stress (Tilman 1988; Grace 1991, 1993; Reader *et al.* 1994; Brooker *et al.* 2005), although their approach was not without criticism (Bertness 1998).

Overall, an amalgam of these models has become known as the stress gradient hypothesis (SGH) – something of a misnomer considering that the commonly cited model of Bertness & Callaway (1994) included both stress and consumer pressure. Many recent studies have attempted to test the SGH, and have taken a 'high vs. low' approach, i.e. they consider two or perhaps three levels of environmental severity (disturbance and/or stress), and have frequently found that with increasing severity the beneficial impacts of neighbours increase (e.g. Greenlee & Callaway 1996; Pugnaire & Luque 2001; Maestre *et al.* 2003a; Gómez-Aparicio *et al.* 2004; Brooker *et al.* 2006; Kikvidze *et al.* 2006b; Callaway 2007). However, such studies often focus on interactions that are clearly facilitative, and examine one particular species pairing (but see Cavieres *et al.* 2002, 2006 for community-level studies).

A few studies have taken a broader approach, examining patterns across gradients. Choler *et al.* (2001) found that increasing altitude was associated with increasing frequency of facilitative interactions. They also found that facilitation depended on species identity – facilitated species were commonly at the extreme ends of their environmental tolerance (see also Liancourt *et al.* 2005) – and led to range expansion (as discussed by Bruno *et al.* (2003) with respect to niche theory). Callaway *et al.* (2002) found a generic shift in the average type of interaction along a large-scale climatic gradient, with facilitative interactions in colder environments and increasingly competitive interactions in warmer environments. In an aridity gradient study, Holzapfel *et al.* (2006) found a steady and consistent shift from net positive or neutral effects to net



Fig. 1. continued

negative effects of desert shrubs on annual species with increasing water availability. In contrast, but in a study conducted on only one species pair, Maestre & Cortina (2004) found a switch from competition to facilitation and back to competition along a gradient of decreasing rainfall in a semiarid steppe system. Similarly, Tielbörger & Kadmon (2000a) found that the effect of desert shrubs on annuals shifted from negative to neutral or neutral to positive (depending on the species) with increasing annual rainfall. Pennings *et al.* (2003) failed to find support for predictions from the SGH in a study conducted over a large-scale geographical gradient in salt marshes.

Simple individual-based models have also been used to examine the SGH. Travis *et al.* (2005) found that facilitative interactions were restricted to the most severe environmental conditions when the stress gradient acted upon reproduction, but competitive interactions were again prevalent in severe conditions when it acted upon mortality (Travis *et al.* 2006). These results match the conclusions of Goldberg *et al.*'s (1999) review of field studies that the observed relationship between environmental severity and success depends on the measure of plant performance used. However, Goldberg *et al.*'s review (and studies therein) may themselves be biased by use of inappropriate indices to test the hypotheses examined (Brooker *et al.* 2005; Gaucherand *et al.* 2006).

A number of factors have been proposed to influence the outcome of studies testing predictions from the SGH. These include relatively short-term experiments, a lack of adequate control for the occurrence of multiple stress factors or ontogenetic effects (but see Armas & Pugnaire 2005; Miriti 2006; Schiffers & Tielbörger 2006), or the effects of resource vs. non-resource stress factors (Michalet 2007). Maestre *et al.* 



Fig. 2. The number of papers examining facilitation (open bars) and competition (closed bars) as a percentage of the total number of papers published in five leading plant ecology journals (*American Naturalist*, *Ecology, Journal of Ecology, Oikos* and *Oecologia*) between 1995 and 2006. Data obtained from ISI Web of Knowledge (http://portal.isiknowledge.com/; 1 March 2007) using the search strings [('positive interaction\*' OR facilitation) AND plant\*] or alternatively [('negative interaction\*' OR competition) AND plant\*], specifying English language articles only. After Dormann & Brooker (2002).

(2005), in a meta-analysis of experiments from arid environments, concluded that there was no generic relationship between environmental severity and plant interactions, and that the approach used - e.g. experiments vs. observational studies - had a substantial impact on interpretation of the relationship (see also Dormann & Brooker 2002; Michalet 2006). Maestre et al.'s (2005) study led to considerable debate; Lortie & Callaway (2006) concluded that study selection for the analysis was not rigorous, and that differences in stress gradient lengths between studies could have a considerable impact on results - criticisms that have been disputed by Maestre et al. (2006). It is therefore difficult to conclude whether the current uncertainty surrounding the SGH is the result of the analytical approach used (either in the field or statistically), the scale at which processes are examined (i.e. within a community through time, or across a local- or continental-scale environmental gradient) or the type of severity gradient used (resource vs. non-resource or temporal vs. spatial).

Progress in understanding this relationship is crucial as it underlies key theories within plant ecology and is central to integrating facilitation into mainstream ecological theory. When interactions have been addressed in theories of plant community or population ecology (e.g. Grime 1977; Tilman 1988) they have tended to be negative, competitive interactions (except for the handful of well-recognized examples mentioned above). However, given that facilitative interactions are widespread and can regulate the success of individuals and the composition of communities, there is a clear need to explore how facilitation might fit within or modify these classic theories. Bruno et al. (2003) discussed revision of niche theory to include the potential for expansion of the realized niche by facilitation (as empirically demonstrated by Hacker & Gaines 1997; Choler et al. 2001), positive density-dependence at high population densities, inclusion of facilitation in the diversity-invasibility paradigm, and the role of dominant species in regulating local diversity.

However, Bruno *et al.* (2003) did not discuss in detail one of the classic theories of plant community ecology to which the SGH is directly relevant – the hump-backed diversity model (Grime 1973; Huston 1979). One of the first attempts to explore the relevance of facilitation to this model was that of Hacker & Gaines (1997), who suggested a conceptual scheme in which the positive effects of facilitation on biodiversity (species richness) increase from intermediate to very high environmental severity, in line with predictions from the SGH. Hacker & Bertness (1999) demonstrated the importance of facilitation for increasing diversity at intermediate levels of environmental severity. Michalet et al. (2006) further developed these ideas, suggesting that facilitation promotes diversity at medium to high environmental severity by expanding the range of stress-intolerant competitive species into harsh physical conditions (as discussed by Bruno et al. 2003 and demonstrated by Choler et al. 2001), but that when environmental conditions become extremely severe the positive effects of the benefactors wane and diversity is reduced, indicating that biotic interactions shape both sides of the humped-back curve of diversity. Perhaps one of the most striking examples of the potential importance of facilitation for the maintenance of biodiversity is that described by Valiente-Banuet et al. (2006). They found that a large number of ancient Tertiary plant lineages, which evolved under much wetter climatic conditions than found in the Mediterraneanclimate ecosystems where they now live, have been preserved by facilitative nurse plant effects from modern Quaternary species. In the absence of such interactions the flora of these regions would be significantly altered, and facilitative interactions have therefore played 'a central role in the preservation of global biodiversity' (Valiente-Banuet et al. 2006).

# Looking to the future

Recent research has clearly built upon the synthesis papers that reignited interest in plant facilitation. This research, as well as continuing to explore in detail the mechanisms of individual facilitative interactions (see Table S1), has attempted to test the broader scale synthetic predictions and models derived from these classic papers. The accuracy of these broader syntheses has become a topic of considerable debate, but it has also become clear that resolving such debate is directly relevant to a number of ecological issues, not least the integration of facilitation into mainstream ecological theory. Our review therefore highlights the significant potential for future research into plant facilitation. In addition, there are a number of research fields that have, perhaps surprisingly, so far failed to make links to facilitation research, despite their clear relevance. In this section we thus discuss what we consider to be some of the most interesting topics for future research and, where possible, the approaches by which they could be taken forward.

# IMPROVING OUR UNDERSTANDING

# Facilitation and environmental gradients

One important step to understanding the shifting balance between positive and negative interactions along environmental gradients is the design and implementation of experiments that move beyond the limitations of previous studies. These should, whenever possible, include at least one of the following aspects: (i) several co-occurring stress factors (e.g. temperature and water availability in arid areas and Mediterranean mountains), (ii) several levels within each stress factor, ideally accounting for a wide range in the variation of each factor, and (iii) an appropriate control of species-specific responses in competitive and facilitative ability and tolerance to stress (e.g. Tielbörger & Kadmon 1995; Callaway 1998; Choler et al. 2001; Gómez-Aparicio et al. 2004; Liancourt et al. 2005). Of particular interest in testing the SGH are experiments conducted in situations where benefactors may themselves be limited, such as at the extreme end of severity gradients. Experiments should also attempt to isolate the environmental factors affected by potential benefactors (to provide a mechanistic understanding of severity interaction relationships, e.g. Holmgren et al. (1997), Holmgren (2000)), evaluate different performance variables (e.g. survival, growth, physiological status and fitness), attempt to account for factors such as initial biomass effects (Gibson et al. 1999; Goldberg et al. 1999; Kikvidze et al. 2006a), provide a fine characterization of abiotic conditions, and be conducted over time periods long enough to cover different life stages in the studied species (e.g. Gómez-Aparicio et al. 2004; Miriti 2006; Schiffers & Tielbörger 2006). Taking into account these issues will be challenging and (comparatively) costly. However, we also recommend complementing them with observational studies conducted at the community level in multiple sites. When combined with appropriate statistical tools – such as structural equation models - these studies would allow us to assess multi-species interactions over broad geographical gradients, and to tease out the relative influence of different stress factors on them (Kikvidze et al. 2005).

In parallel with this rejuvenated research effort, we must also address the issue of the importance of facilitative interactions. A detectable process may not necessarily play a predominant role. Community composition is commonly seen as being regulated by filters, including chance biogeographical

events, local abiotic conditions and interactions with other species (Grime 1998; Diaz et al. 1999; Lortie et al. 2004). The relative impact of each filter is not fixed, and there is a general assumption that filters operate to regulate distribution at different scales, e.g. chance biogeographical events and biotic interactions are considered most important for regulating distributions at the regional and local scales, respectively (but see Callaway & Ridenour 2004 and Valiente-Banuet et al. 2006 for examples of interactions determining ranges at a global scale). Central to these discussions, but frequently ignored, are the issues of the importance and intensity of interactions (Welden & Slauson 1986; Grace 1991; Corcket et al. 2003a; Brooker et al. 2005; Gaucherand et al. 2006; Grime 2007). Although the intensity of plant-plant interactions may remain unaltered along an environmental gradient, their impact relative to other processes (i.e. their importance) may vary. When and where, therefore, are facilitative interactions not only detectable but playing a key role, and does the importance of their role vary depending upon whether we are considering (for example) community composition or evolutionary processes?

The few studies to have considered the response of both the importance and the intensity of interactions demonstrate that they might not change in parallel along environmental gradients (Brooker et al. 2005; Gaucherand et al. 2006), and that a detectable effect on biomass need not necessarily translate into a fitness effect (Goldberg et al. 1999). Evaluating the effects of facilitation on the survival and fitness of individuals is therefore crucial if we want to understand when and where facilitation acts as a filtering process in plant communities. We predict that facilitation is likely to be important relative to other filters when climate stress (e.g. aridity) or disturbance (e.g. grazing) is high but not excessive so that well-fitted benefactor species (e.g. 'nurses') are able to grow and significantly ameliorate stress or provide protection from disturbances. Important facilitation would be indicated when these interactions enable a considerable number of beneficiary species to survive and reproduce when growing adjacent to the benefactor species (see Development of models incorporating plant *facilitation* for discussion of the related concept of a plant's interaction 'kernel'). Low or extremely high environmental severity would prevent facilitation from having a significant role, either in regulating the composition of the community or as a selective force. Field studies need to test these simple predictions by considering explicitly the importance of interactions along environmental gradients (e.g. Gaucherand et al. 2006).

#### Indirect interactions and facilitation

Interest in the effects of indirect interactions among species that occupy different trophic levels has been ongoing and has increased in recent years (Root 1973; Menge 1976; Hay 1986; Wooton 1994; Rousset & Lepart 2000; Corcket *et al.* 2003b; Seifan & Kadmon 2006). However, complex indirect interactions *within* a trophic level, or among competitors (Aarssen 1992; Miller 1994; Levine 1999; Callaway & Pennings 2000;

Tielbörger & Kadmon 2000b; Callaway 2007) have received much less attention. The outcomes of pair-wise interactions therefore shape our thinking and thus community theory (Connolly et al. 2001), with pair-wise studies of competitive interactions leading to the perspective that competitive hierarchies in plant communities are transitive, or linear, with each species out-competing all those that are lower in the hierarchy. From this we might conclude that the only outcome of strong competition in communities at equilibrium is the exclusion of some or most members. However, this overlooks potential positive effects that occur in multi-species communities due to non-transitive 'networks' of interactions (Aarssen 1992). When interactions take place among many species at the same time, the competitive suppression of one species (B) by another (A) can decrease the latter's competitive effect on a third species (C) (Fig. 3a), thus leading to the phenomenon of indirect facilitation.

Few studies have considered that the occurrence of indirect facilitation may depend on complementarities in the interactions between species and therefore the identities or traits of the organisms involved (Huisman & Weissing 1999; Huisman et al. 2001). For example, in our model system (Fig. 3) indirect facilitation is most likely when pairs of plants (A-B, B-C) compete for different resources, for example light for the first species pair, and water or nutrients for the second (Levine 1999; Pagès & Michalet 2003; Siemann & Rogers 2003; Kunstler et al. 2006). Plant characteristics other than resource uptake ability (such as the production of allelopathic chemicals) may create such species-specific interactions, and thus non-transitive interaction networks and indirect interactions (Pagès et al. 2003; Callaway & Howard 2006; Kunstler et al. 2006; Callaway 2007). If the existence of multiple interaction mechanisms among coexisting species increases the potential for strong indirect interactions, indirect facilitation should be more common in communities where several limiting factors co-occur with similar strengths. In contrast, in environments with one dominant limiting factor, such as xeric, N-poor or low light conditions, indirect facilitation should be less important.

Surprisingly few studies have analysed how gradients of diversity will influence, and be influenced by, indirect facili-

tative interactions. Indirect facilitation should be more common in species-rich communities (Miller 1994; Dodds 1997). Indeed, it may have the potential to sustain the coexistence of high species diversity by reducing the potential for competitive exclusion (Czaran *et al.* 2002; Laird & Schamp 2006) and may be the dominant facilitative process in more productive environments (given the predicted reduction in direct facilitative effects). Indirect facilitation among competitors therefore challenges assumptions about competition consistently leading to exclusion.

There is therefore a notable dearth of studies exploring the role of indirect facilitative effects in plant communities, including their impacts on diversity and evolution. Future studies, using artificial manipulations of diversity or modelling approaches (see Development of models incorporating *plant facilitation*), should: (i) attempt to assess the conditions under which we might expect the greatest level of non-transitivity within plant communities; (ii) connect the degree of non-transitivity in plant competitive networks to community diversity and facilitative promotion of species coexistence; (iii) explore how the role of non-transitivity and indirect facilitation varies along gradients both of diversity and of system productivity, and in relation to direct facilitative effects; and (iv) explore, through multi-species evolutionary modelling, the potential for indirect facilitative effects to drive or limit niche differentiation.

# Development of ecological models incorporating plant facilitation

Although a broad suite of modelling approaches is available, and despite their likely relevance and utility, they have not yet been widely employed in furthering our understanding of the role of facilitative plant interactions. This represents a significant missed opportunity for promoting the development of theory in this field.

Recent work illustrates the potential for relatively simple models to provide generic insights into the dynamics of communities structured with a mixture of positive and negative interactions. For example, Yamamura *et al.* (2004) used a



**Fig. 3.** Indirect (dotted line) and direct (solid line) interactions in a complex system. (a) Species A suppresses species B, which affects the potential negative effect of species B on C. As suggested by Levine (1976), if the benefit from suppression is higher than the direct negative effects, indirect facilitation occurs in the community. However, the benefit from suppression can be outweighed by direct negative effects, resulting in no net indirect facilitation (Levine 1999; Pagès *et al.* 2003). (b) Non-transitive interactions (A > B, B > C, C > A). The suppression of species B by A causes an indirect facilitation of species C, which increases its negative effect on A. In such a system the species ameliorate each other's effects and contribute to long-term coexistence (Czaran *et al.* 2002; Callaway & Howard 2006).

spatially explicit patch occupancy model to study the evolution of mutualisms, highlighting the importance of local spatial structure in determining the outcome of interactions. Their modelling approach represented space as a regular grid, each cell of which can be in one of two states, occupied or unoccupied. Rules determine the way in which neighbouring plants interact, reproduce, disperse their propagules, evolve and die. Yamamura *et al.* highlighted the importance of local spatial structure in determining the outcome of interactions among a mutualistic and a cheater species. Although having great potential, spatially explicit simulation models of this type have only recently been applied to facilitation.

Travis *et al.* (2005) extended the model of Yamamura *et al.* to consider plant facilitation specifically, demonstrating that positive interactions are most likely towards the harsh end of environmental gradients (although this depends on whether the gradient primarily influences reproduction or survival; Travis *et al.* 2006). Such models can therefore readily provide hypotheses suitable for testing with field experiments. In addition they can also be used to run experiments. Brooker *et al.* (2007) used the spatial model of Travis *et al.* (2005, 2006) to explore the impact of interactions on the capacity of species to track a moving 'climate envelope'. They found that the spatial arrangement of species with particular traits (as determined initially by interactions), along with the rate of climate change and level of long-distance dispersal, interacted to regulate the capacity of species to track their climate envelope.

Such developments in facilitation modelling are at an early stage. Although these spatially explicit models are a move in the right direction, they do not yet properly represent facilitation as most empirical plant ecologists have described it. In the mutualism models adapted for facilitation research the interaction term is commonly +/+ (Odum 1968), i.e. reciprocal benefit. However, although mutualistic interactions between neighbouring plants are possible, and may be more common than currently expected (Pugnaire et al. 1996), the impact of the beneficiary on the benefactor may range from positive to zero (+/0 commensalism), and some interactions might even be classified as parasitic (+/-). Future modelling should therefore start to distinguish clearly between mutualism and facilitation. Such a distinction would, for example, be important in determining the outcome of evolutionary modelling studies - facilitative interactions may have very different evolutionary impacts from mutualistic interactions, and may also evolve under different circumstances.

There is also a need for modelling approaches that accommodate variation in the role of facilitative interactions along environmental gradients, both in space and time, and at a local as well as landscape scale. If the spatial extents of positive and negative effects differ, the net outcome of interactions will be highly dependent on the spatial relation of the individual plants. Appropriate models would include the key elements of neighbourhood models (currently used to examine competitive plant interactions), i.e. individuals with an explicit location and basal extension where no other plant can exist, and a zone of competitive influence in which negative interactions occur (Stoll & Weiner 2000). Facilitation could be included through a zone of facilitation, although such zones of influence typically assume that all neighbours within a certain distance of a focal plant experience the same negative impact from competition. This is clearly unrealistic. More sophisticated approaches using interaction kernels, which describe the relationship between the distance of a neighbour from a focal individual and the competitive impact of that individual, have recently been developed (e.g. Murrell & Law 2003). However, few, if any, experiments in the literature provide the parameters necessary for such models.

Models thus extended to incorporate facilitation have the potential for a wide range of applications. For example, by allowing evolution of competition and facilitation kernels we may systematically test under which circumstances (frequency of interactions, gene flow, etc.) facilitation might evolve as an evolutionary stable strategy. Modelling could also explore the potential for different life-history characteristics (such as dispersal, dormancy, time to first reproduction, self-compatibility) to evolve in response to the balance between facilitation and competition present within a local community. Furthermore, the need to investigate interactions in multi-species assemblages is increasingly being recognized (e.g. Weigelt et al. 2007) and two-species models (Travis et al. 2006; Brooker et al. 2007) could readily be extended to multispecies models. This would enable, for example, the dynamics of indirect interactions to be examined in detail, including predicting the conditions under which this type of effect is likely to be observed in natural systems, and how its community and evolutionary impacts might compare with those of direct facilitative effects.

# Connecting facilitation to evolution

Recent studies indicate that facilitation may act as an evolutionary force: Scheffer & van Nes (2006) predicted that indirect facilitation may drive convergence of species' niches, and Valiente-Banuet *et al.* (2006) suggested that facilitation is a source of stabilizing selection for the regeneration niches of ancient Tertiary species within Mediterranean-climate ecosystems. However, although the possible relationships between facilitation and evolution have been discussed previously (Hunter & Aarssen 1988; Brooker & Callaghan 1998; Callaway 2007), an evolutionary focus is absent from almost all recent work in the facilitation field.

We can readily visualize the 'competitor' syndrome that might evolve in response to strong and consistent negative interactions in productive environments: a high, dense canopy of wide-spreading leaves, rapid potential RGR (relative growth rate), high morphological plasticity, etc. (a classic C-strategy species; Grime 1977). However, it may not be straightforward to predict the evolutionary response to facilitative interactions, as both facilitative and competitive mechanisms may evolve in response to facilitation. For example, if species A is facilitated by species B, any negative effects of species A could select for those B individuals that are weaker facilitators (either by selecting against the key facilitation trait or in favour of an avoidance mechanism in species B). However, an alternative second selective force might favour individuals of species A with lower negative impacts on their benefactor.

One key trait that may come under such selection within the context of an assemblage comprising facilitators and facilitated is dispersal. Selection might favour dispersal kernels in the facilitated species that map onto those of the facilitator, for example through the convergence of dispersal agents. Conversely, selection of the facilitator might favour dispersal kernels enabling escape from the negative consequences of competition (unless the level of competition is itself reduced). Similarly, selection acting on characteristics such as the balance between reproduction by clones and seed may vary depending on the nature or balance of interactions - reproduction by seed may aid dispersal and be favoured by avoidance of competition, whereas clonal growth may be selected for by facilitation in the immediate vicinity of the parent plant. It is interesting to speculate that the reduced competitive effect or enhanced clonality of stress-tolerant species may in part result from selection in favour of enhanced facilitation. As stated by Brooker & Callaghan (1998) 'It may be the case that we already have evidence of the evolutionary impact of positive plant-plant interactions, but have never examined it in the light of this possible interpretation'.

The outcome of the selective process will depend not only upon the frequency and relative strength of the interactions, but also upon evolutionary constraints on specific traits - is evolution of an avoidance mechanism more likely than reduced competitive impact? The evolution of life-history traits will play an important role in determining the spatial dynamics of an assemblage, resulting in a continuous interaction between evolutionary dynamics related to the interactions and the spatial ecology of the system. The extension of relatively simple evolutionary models (e.g. Travis & Dytham 1999; Travis 2003), similar to the individual-based models discussed above, could explore the interplay between ecological and evolutionary dynamics that might result from these types of effects. However, given the potential complexity involved, including variability in the two-way nature of facilitation, a simple starting point for evolutionary studies might be to ask whether all facilitative interactions provide common selective forces, from this predicting patterns of traits, and then to explore whether traits vary in the expected manner along environmental gradients in association with quantification of the importance and intensity of interactions. With current rapid technological advances in genetic methodologies capable of looking at quantitative traits (e.g. Barton & Keightley 2002), we should ultimately aim to develop evolutionary models that explicitly incorporate the genetic architectures involved and are able to separate out the heritable from the environmental components of the traits concerned. An improved understanding of their genetic basis would also permit robust estimation of the strength and direction of selection on the suites of traits involved, and would provide insights into correlations and potential pleiotropic effects between these traits.

#### APPLYING OUR UNDERSTANDING

#### Facilitation and ecosystem restoration

In the face of current rapid degradation of terrestrial ecosystems worldwide, there is an increasing need for the development of novel, low-cost and efficient restoration techniques for maintaining ecosystem function and services (e.g. Ormerod *et al.* 2003; Hobbs *et al.* 2006). Because facilitation has been recognized as an important structuring force in natural plant communities, it is being increasingly discussed as an ecological mechanism which could be exploited for developing vegetation restoration tools, particularly for severe and highly disturbed environments (see reviews by Young *et al.* 2005; Padilla & Pugnaire 2006; Halpern *et al.* 2007).

Yet, except for arid environments, the role of positive plant interactions in terrestrial restoration is almost always overlooked. This is despite studies reporting strong facilitative effects during restoration in high mountain environments (Walker & Powell 1999; Aerts *et al.* 2007), tropical forests (Parrotta *et al.* 1997) or highly disturbed sites such as mine spoils (Densmore 2005; Frérot *et al.* 2006), and a range of examples from aquatic and intertidal ecosystems (reviewed by Bruno *et al.* 2003; Halpern *et al.* 2007).

Studies examining plant facilitation in terrestrial ecosystem restoration usually focus on the positive effects of nurse plants. Water-limited ecosystems offer one of the best opportunities for exploring these effects, as well illustrated by the study of Gómez-Aparicio et al. (2004). They investigated the use of naturally occurring shrubs as nurse plants for reforestation in a Mediterranean environment, and found that shrubs had a consistent beneficial effect on tree seedling survival and growth during four consecutive years. Such results directly contradict traditional reforestation management practice, where shrubs are removed prior to tree planting due to their presumed competitive effects on tree seedlings (see also Castro et al. 2004). Unfortunately, most studies addressing the use of nurse plants for restoration in arid systems have usually been conducted over shorter time frames, i.e. fewer than three growing seasons (e.g. Maestre et al. 2001, 2003b, 2004; Barchuk et al. 2005). Given the temporal variability in facilitative interactions in these systems (Tielbörger & Kadmon 2000a; Miriti 2006), this may be an insufficient period over which to assess whether nurse plants have a net beneficial effect, and longer-term studies are clearly needed. Furthermore, these studies commonly plant beneficiary species under existing nurse plants. However, in extremely degraded ecosystems nurse plants may themselves be lost (Gibson & Brown 1991). In such cases management for nurse plant re-establishment may be necessary as a first step, although it is also important to consider whether the restoration of nurse plant cover would be the most effective way of driving beneficiary reestablishment.

Novel techniques currently being developed in agroecosystems and polluted areas demonstrate the wide range of possible uses of facilitative interactions for environmental management. Facilitation can operate via increased pollinator visits, which lead to greater crop yields (Ricketts *et al.* 2004), enhanced water status of crops growing with species capable of hydraulic lift (Pate & Dawson 1999; Sekiya & Yano 2004), the transfer of symbiotically fixed nitrogen from legume to non-legume species (Jensen 1996; see also Hauggaard-Nielsen & Jensen 2005 for a review of facilitative root interactions in agro-ecosystems), or phytostabilization by metallicolous nurse plants in heavy-metal-polluted environments (Frérot *et al.* 2006). Transgressive over-yielding has been reported for multi-species mixtures in intensively managed grassland systems, although the precise mechanism is unclear (Kirwan *et al.* 2007). There may therefore be a wide range of facilitative mechanisms, in addition to the classic nurse plant effect, that could act as the basis for restoration tools.

As strikingly illustrated by the work of Gómez-Aparicio et al. (2004) discussed above, Sutherland et al. (2004) proposed that 'Much of current conservation practice is based upon anecdote and myth'. The acknowledgement of facilitation in studies of restoration, particularly within environments that are difficult to restore, would help to raise facilitation's profile as a tool for natural resource conservation and management. Furthermore, applied facilitation research also has significant unexploited potential for advancing the science of restoration ecology in general. The explicit consideration of facilitation when exploring key topics in this research area, such as stable alternative states (Suding et al. 2004), succession (Walker et al. 2007) or the links between ecosystem structure/function and restoration success (Young et al. 2005; Cortina et al. 2006), are likely further to help managers and practitioners to develop effective tools to achieve the desired restoration targets.

# Facilitation and global change

Climate change, nitrogen deposition, biological invasions and land-use change have been suggested as the current major threats to global biodiversity (Sala *et al.* 2000; CBD 2003), and significant evidence is accumulating that interactions, including facilitative ones, play a role in mediating the impact on natural communities of these environmental change drivers (Brooker 2006; Maestre & Reynolds 2006, 2007). However, the exact nature of that role, and how it will alter in response to environmental change, remains unclear.

Several studies have demonstrated the importance of facilitation for the maintenance of biodiversity in arctic and alpine habitats (e.g. Choler *et al.* 2001; Callaway *et al.* 2002), and these habitats are predicted to be amongst the most sensitive to global warming (Guisan & Theurillat 2000; ACIA 2004). Based on the original SGH, some authors have predicted a general shift in species interactions from more strongly positive to more strongly negative as these environments warm (e.g. Klanderud & Totland 2005; Klanderud 2005). In support, experiments that evaluated the impact of enhanced nitrogen availability in these ecosystems (simulating the expected effect of climate change on nutrient mineralization rates) mostly reported increased competitive interactions (e.g. Chapin *et al.* 1995; Bret-Harte *et al.* 2004; Klanderud & Totland 2005). Similarly, in a North American arctic tundra system, where artificially advanced snowmelt (a phenomenon expected under global warming) created a more severe environment through increased early-season frost events, neighbours increasingly facilitated the survival, growth and reproduction of *Empetrum nigrum* (Wipf *et al.* 2006). However, Shevtsova *et al.* (1997) reported that the positive effects of elevated temperature on the growth and reproduction of *Vaccinium vitis-idaea* and *Empetrum nigrum* in subarctic Finland were amplified by the presence of neighbours – in this instance a reduced level of environmental severity (warming) led to an increased impact of facilitative interactions.

These findings support the argument that the relationship between gradients of environmental severity and the relative role of interactions is more complicated than set out in the original SGH (e.g. Bertness & Ewanchuk 2002). At first glance we might conclude that such a pattern supports the recently proposed humped-back relationship (Michalet et al. 2006). However, it is notable that the increased role of facilitation with decreasing severity found by Shevtsova et al. (1997) occurs within an environment that is apparently less severe than, for example, the open tundra systems studied by Chapin et al. (1995) and Wipf et al. (2006), and in which the opposite pattern was observed. Such apparently contradictory findings indicate that the perception of environmental severity is to some extent species-specific, and that the response of interactions at the individual species level may not reflect the trend of average interactions within a community. Improving our knowledge of the variation in interactions under different environmental conditions between different species, growth-forms or strategies (e.g. competitive vs. stress-tolerant strategies), and how these differ from trends at the community level, will help in the development of general theory and in explaining such apparently contradictory results.

Invasive species are another critical global change driver. In the study of biological invasions, interactions are considered crucial in determining the success of exotics (e.g. Daehler 2003; Simberloff et al. 2003; Vilà et al. 2004). Although most studies have focused on negative interactions as the main drivers of invasive success (e.g. Callaway & Aschehoug 2000; Colautti et al. 2004; Mitchell et al. 2006), a sizable number have also reported that facilitation by other exotics (Simberloff & Von Holle 1999) or by natives (Maron & Connors 1996; Richardson et al. 2000; Lenz & Facelli 2003; Cavieres et al. 2005, 2007) can promote exotic invasion. For instance, Maron & Connors (1996) reported that the nitrogen-fixing native shrub Lupinus arboreus facilitates the establishment of exotic species by ameliorating soil nutrient shortage. Cavieres et al. (2005) found that the cushion plant Azorella monantha facilitates the establishment of the exotic species Taraxacum officinale in the high-alpine zone of the Andes of central Chile by providing microsites with milder microclimatic conditions (see also Cavieres et al. 2007). Reinhart et al. (2006) showed how seedlings of the invasive tree Acer platanoides had higher densities, recruitment and survival, and less photoinhibition and water stress when beneath conspecific canopies than

when growing under adjacent native *Pseudotsuga menziesii* trees; they related these differences to the environmental modification created by the invaders. When exotic species facilitate the establishment of other exotics, accelerated invasion can occur. Simberloff & Von Holle (1999) coined the term 'invasional meltdown' to describe such a process. Invasional meltdown demonstrates that facilitation can be an important driver of plant invasion (Simberloff 2006) but, interestingly, invasion can also be 'resisted' by greater diversity of native species (e.g. Zavaleta & Hulvey 2004; Fargione & Tilman 2005), a mechanism by which facilitation of natives may help limit the influx of invasives.

Improving our understanding of facilitative interactions is therefore of direct relevance to understanding the impacts of environmental change drivers on biodiversity (Callaway 2007). Resolving the debate concerning the SGH (difficult though this may be) may, for example, enable us more accurately to predict changes in the role of interactions in response to environmental change, or the conditions under which interactions might restrict or enhance biodiversity change, for example through the influx of invasives. However, it is not necessarily the case that radical new experiments are needed to explore these issues. As with restoration ecology, simply recognizing the potential role of facilitation, and including it within both experimental designs and the interpretation of ecosystem responses, could provide us with valuable insights into facilitation both as a mediator of global change and as a fundamental ecological process.

# Conclusions

Our coverage cannot hope to be fully comprehensive in an essay review such as this. Other relevant topics which we have touched upon at best only briefly include: the possible impacts of facilitation on ecosystem function (Hector *et al.* 1999); the concept of ecosystem engineering (Jones *et al.* 

1997; Crain & Bertness 2006; Hastings *et al.* 2007); the use of indices in plant interaction studies (as hotly debated with respect to competition indices; Markham & Chanway 1996; Freckleton & Watkinson 1997a,b, 1999; Markham 1997; Weigelt & Jolliffe 2003; Armas *et al.* 2004; Wilson 2007); and the possible contrasting consequences of diffuse and species-specific facilitation (e.g. the general facilitative effects of neighbours compared with the specific one-on-one facilitative impacts of nurse plants). However, we have covered what are widely recognized as some of the central current issues for plant facilitation research, as well as highlighting what we believe to be some novel future directions for this field and a number of testable hypotheses (Table 2).

We should perhaps now answer the questions posed at the outset of this review. First, has the recent substantial research effort taken this field forward? Yes, undoubtedly, both in terms of developing general models and in exploring some of their underlying complexity, and also in raising general awareness of the widespread and important role of facilitative interactions in plant communities.

Secondly, what gaps in our knowledge of facilitative interactions need to be addressed? Clarification of the relationship between interactions and environmental gradients is central for further progress, and necessitates implementation of experiments specifically designed to address this issue. There is also substantial scope for exploring indirect facilitative effects, including their impacts on diversity and evolution, and future studies should attempt to connect the degree of non-transitivity in plant competitive networks to community diversity and facilitative promotion of species coexistence, perhaps exploring how the role of indirect facilitation varies with community productivity and the number of limiting resources. Certain ecological modelling approaches could provide highly useful tools for exploring these fundamental processes, and also clearly lend themselves to studying the evolutionary responses that might result from facilitative

**Table 2.** Some testable hypotheses that might be addressed by future plant facilitation research projects, organized by research theme (as used in this paper)

Research theme	Hypothesis
Facilitation and environmental gradients	Facilitation will be important when environmental severity is high but not extreme. High importance will be indicated by the dependence of a large proportion of species within a community on facilitative interactions.
Indirect facilitation	Indirect facilitative interactions will be more likely when the cause of competition varies between species pairs within a community. Indirect facilitation will be more likely in communities where there are several co-occurring limiting factors. Indirect facilitation will be more frequent in species-rich communities. Indirect facilitation may be the principal mode of facilitation in productive environments.
Ecological modelling	The relationship between environmental severity and positive or negative plant interactions depends upon the factor being influenced by the environment (e.g. reproduction or survival). Facultative positive interactions may not lead to the same evolutionary responses as obligate mutualistic interactions

interactions, including possible re-assessment of the evolution of plant growth forms.

Thirdly, do we know enough about facilitative interactions to understand their role in mediating the impact of environmental change drivers, or to use this knowledge to mitigate such impacts? The answer to this question is probably no. We know enough to recognize that improved understanding of facilitation processes is directly relevant to both ecosystem restoration (and may form the basis of restoration management tools), and to understanding the response of plant species and communities to key environmental change drivers such as invasive alien species and global change. However, attempts to apply our developing ecological knowledge to these fields are at an early stage, and would benefit from explicit recognition of the potential role of facilitative plant– plant interactions in the design and interpretation of studies of restoration and global change ecology.

It is clear therefore that considerable research challenges exist, but that expanding our fundamental understanding of facilitation, applying that knowledge to key ecological problems and attempting to further integrate our developing knowledge of facilitation into mainstream ecological theory will undoubtedly bring an improved understanding of both plant facilitation and community ecology in general.

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# References

- Aarssen, L.W. (1992) Causes and consequences of variation in competitive ability in plant communities. *Journal of Vegetation Science*, 3, 165–174.
- ACIA (2004) Arctic Climate Impact Assessment. Cambridge University Press, Cambridge.
- Aerts, R., Negussie, A., Maes, W., November, E., Hermy, M. & Muys, B. (2007) Restoration of dry Afromontane forest using pioneer shrubs as nurseplants for *Olea europaea ssp. cuspidata. Restoration Ecology*, **15**, 129–138.
- Armas, C., Ordiales, R. & Pugnaire, F.I. (2004) Measuring plant interactions: a new comparative index. *Ecology*, 85, 2682–2686.
- Armas, C. & Pugnaire, F.I. (2005) Plant interactions govern population dynamics in a semi-arid plant community. *Journal of Ecology*, 93, 978– 989.
- Barchuk, A.H., Valiente-Banuet, A. & Díaz, M.P. (2005) Effect of shrubs and seasonal variability of rainfall on the establishment of *Aspidosperma quebracho-blanco* in two edaphically contrasting environments. *Austral Ecology*, **30**, 695–705.

- Barton, N.H. & Keightley, P.D. (2002) Understanding quantitative genetic variation. *Nature Reviews Genetics*, 3, 11–21.
- Bertness, M.D. (1998) Searching for the role of positive interactions in plant communities. *Trends in Ecology and Evolution*, 13, 133–134.
- Bertness, M. & Callaway, R.M. (1994) Positive interactions in communities. Trends in Ecology and Evolution, 9, 191–193.
- Bertness, M. & Ewanchuk, P.J. (2002) Latitudinal and climate-driven variation in the strength and nature of biological interactions in New England salt marshes. *Oecologia*, **132**, 392–401.
- Bret-Harte, M.S., García, E.A., Scaré, V.M., Whorley, J.R., Wagern, J.L., Lipper, S.C. & Chapin, F.S. (2004) Plant and soils responses to neighbour removal and fertilization in Alaskan tussock tundra. *Journal of Ecology*, 92, 635–647.
- Brooker, R.W. (2006) Plant–plant interactions and environmental change. New Phytologist, 171, 271–289.
- Brooker, R.W. & Callaghan, T.V. (1998) The balance between positive and negative plant interactions and its relationship to environmental gradients: a model. *Oikos*, 81, 196–207.
- Brooker, R., Kikvidze, Z., Pugnaire, F.I., Callaway, R.M., Choler, P., Lortie, C.J. & Michalet, R. (2005) The importance of importance. *Oikos*, **109**, 63– 70.
- Brooker, R.W., Scott, D., Palmer, S.C.F. & Swaine, E. (2006) Transient facilitative effects of heather on Scots pine along a grazing disturbance gradient in Scottish moorland. *Journal of Ecology*, 94, 637–645.
- Brooker, R.W., Travis, J.M.J., Clark, E.J. & Dytham, C. (2007) Modelling species' range shifts in a changing climate: the impacts of biotic interactions, dispersal distance and the rate of climate change. *Journal of Theoretical Biology*, 245, 59–65.
- Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003) Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution*, 18, 119–125.
- Callaway, R.M. (1995) Positive interactions among plants. *Botanical Review*, **61**, 306–349.
- Callaway, R.M. (1997) Positive interactions in plant communities and the individualistic-continuum concept. *Oecologia*, 112, 143–149.
- Callaway, R.M. (1998) Are positive interactions species-specific? Oikos, 82, 202–207.
- Callaway, R.M. (2007) Positive Interactions and Interdependence in Plant Communities. Springer, Dordrecht.
- Callaway, R.M. & Aschehoug, E.T. (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science*, 290, 521– 523.
- Callaway, R.M., Brooker, R.W., Choler, P., Kikvidze, Z., Lortie, C.J., Michalet, R., Paolini, L., Pugnaire, F.I., Newingham, B., Aschehoug, E.T., Armas, C., Kikodze, D. & Cook, B.J. (2002) Positive interactions among alpine plants increase with stress. *Nature*, 417, 844–848.
- Callaway, R.M. & Howard, T.G. (2006) Competitive networks, indirect interactions, and allelopathy: a microbial viewpoint on plant communities. *Progress in Botany*, 68, 317–335.
- Callaway, R.M. & Pennings, S.C. (2000) Facilitation may buffer competitive effects: indirect and diffuse interactions among salt marsh plants. *American Naturalist*, **156**, 416–424.
- Callaway, R.M. & Ridenour, W.M. (2004) Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and Evolution*, 2, 436–443.
- Callaway, R.M. & Walker, L.R. (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology*, 78, 1958–1965.
- Castro, J., Zamora, R., Hódar, J.A., Gómez, J.M. & Gómez-Aparicio, L. (2004) Benefits of using shrubs as nurse plants for reforestation in Mediterranean mountains: a 4-year study. *Restoration Ecology*, **12**, 352–358.
- Cavieres, L., Arroyo, M.T.K., Penaloza, A., Molina-Montenegro, M. & Torres, C. (2002) Nurse effect of *Bolax gummifera* cushion plants in the alpine vegetation of the Chilean Patagonian Andes. *Journal of Vegetation Science*, 13, 547–554.
- Cavieres, L., Badano, E.I., Sierra-Almeida, A., Gómez-González, S. & Molina-Montenegro, M.A. (2006) Positive interactions between alpine plant species and the nurse cushion *Laretia acaulis* do not increase with elevation in the Andes of central Chile. *New Phytologist*, **169**, 59–69.
- Cavieres, L.A., Badano, E.I., Sierra-Almeida, A. & Molina-Montenegro, M.A. (2007) Microclimatic modifications of cushion plants and their consequences for seedlings survival of native and non-native plants in the high-Andes of central Chile. *Arctic, Antarctic and Alpine Research*, **39**, 229–236.
- Cavieres, L.A., Quiroz, C.L., Molina-Montenegro, M.A., Muñoz, A.A. & Pauchard, A. (2005) Nurse effect of the native cushion plant *Azorella monantha* on the invasive non-native *Taraxacum officinale* in the high-Andes of central Chile. *Perspectives in Plant Ecology, Evolution and Systematics*, 7, 217–226.
- CBD (2003) Interlinkages Between Biological Diversity and Climate Change. Advice on the Integration of Biodiversity Considerations Into the Implementation of the United Nations Framework Convention on Climate Change and its Kyoto Protocol. Secretariat of the Convention on Biological Diversity, Montreal.
- Chapin, F.S., Shaver, G.R., Giblin, A.E., Nadelhoffer, K.J. & Laundre, J.A. (1995) Response of Arctic tundra to experimental and observed change in climate. *Ecology*, **76**, 694–711.
- Chapin, F.S. III, Walker, L.W., Fastie, C.L. & Sharman, L.C. (1994) Mechanisms of primary succession following degalciation at Glacier Bay, Alaska. *Ecological Monographs*, 64, 149–175.
- Chiariello, N.R., Hickman, J.C. & Mooney, H. (1982) Endomycorrhizal role for interspecific transfer of phosphorus in a community of annual plants. *Science*, 217, 941–943.
- Choler, P., Michalet, R. & Callaway, R.M. (2001) Facilitation and competition on gradients in alpine plant communities. *Ecology*, 82, 3295–3308.
- Clements, F.E. (1916) *Plant Succession*. Carnegie Institute Publication #242. Carnegie Institute, Washington.
- Colautti, R.I., Ricciardi, A., Grigorovich, I.A. & MacIsaac, H.J. (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, 7, 721–7333.
- Connell, J.H. & Slatyer, R.O. (1977) Mechanisms of succession in natural communities and their role in community stability and organization. *American Naturalist*, **111**, 1119–1144.
- Connolly, J., Wayne, P. & Bazzaz, F.A. (2001) Interspecific competition in plants: How well do current methods answer fundamental questions? *American Naturalist*, **157**, 107–125.
- Corcket, E., Callaway, R.M. & Michalet, R. (2003b) Insect herbivory and grass competition in a calcareous grassland: results from a plant removal experiment. Acta Oecologica, 24, 139–146.
- Corcket, E., Liancourt, P., Callaway, R. & Michalet, R. (2003a) The relative importance of competition for two dominant grass species as affected by environmental manipulations in the field. *Ecoscience*, **10**, 186– 194.
- Cortina, J., Maestre, F.T., Vallejo, V.R., Baeza, J., Valdecantos, A. & Pérez-Devesa, M. (2006) Ecosystem function and restoration success: are they related? *Journal for Nature Conservation*, 14, 152–160.
- Crain, C.M. & Bertness, M.D. (2006) Ecosystem engineering across environmental gradients: Implications for conservation and management. *Bioscience*, 56, 211–218.
- Crocker, R.L. & Major, J. (1955) Soil development in relation to vegetation and surface age at Glacier Bay, Alaska. *Journal of Ecology*, 43, 427–448.
- Czaran, T.L., Hoekstra, R.F. & Pagie, L. (2002) Chemical warfare between microbes promotes biodiversity. *Proceedings of the National Academy of Sciences USA*, 99, 786–790.
- Daehler, C.C. (2003) Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. *Annual Review of Ecology and Systematics*, 34, 183–211.
- Densmore, R.V. (2005) Succession on subalpine placer mine spoil: Effects of revegetation with Alnus viridis, Alaska, USA. Arctic, Antarctic and Alpine Research, 37, 297–303.
- Diaz, S., Cabido, M. & Casonoves, F. (1999) Functional implications of trait-environment linkages in plant communities. *Ecological Assembly Rules: Perspectives, Advances, Retreats* (eds E. Weiher & J.P. Keddy), pp. 338–362. Cambridge University Press, Cambridge.
- Dodds, W.K. (1997) Interspecific interactions: constructing a general neutral model for interaction type. *Oikos*, 78, 377–383.
- Dormann, C.F. & Brooker, R.W. (2002) Facilitation and competition in the high Arctic: the importance of the experimental approach. *Acta Oecologica*, 23, 297–301.
- Fargione, J.E. & Tilman, D. (2005) Diversity decreases invasion via both sampling and complementarity effects. *Ecology Letters*, **8**, 604–611.
- Flores, J. & Jurado, E. (2003) Are nurse–protégé interactions more common among plants from arid environments? *Journal of Vegetation Science*, 14, 911–916.
- Freckleton, R.P. & Watkinson, A.R. (1997a) Measuring plant neighbour effects. *Functional Ecology*, 11, 532–534.
- Freckleton, R.P. & Watkinson, A.R. (1997b) Response to Markham. Functional Ecology, 11, 536.
- Freckleton, R.P. & Watkinson, A.R. (1999) The mis-measurement of plant competition. *Functional Ecology*, 13, 285–287.
- Frérot, H., Lefèbvre, C., Gruber, W., Collin, C., Dos Santos, A. & Escarré, J. (2006) Specific interactions between local metallicolous plants improve the phytostabilization of mine soils. *Plant and Soil*, **282**, 53–65.
- García-Moya, E. & McKell, C. (1970) Contribution of shrubs to the nitrogen economy of a desert-wash plant community. *Ecology*, **51**, 81–88.

- Gaucherand, S., Liancourt, P. & Lavorel, S. (2006) Importance and intensity of competition along a fertility gradient and across species. *Journal of Vegetation Science*, 17, 455–464.
- Gibson, C.W.D. & Brown, V.K. (1991) The effects of grazing on local colonisation and extinction during early succession. *Journal of Vegetation Science*, 2, 291–300.
- Gibson, D.J., Connolly, J., Hartnett, D.C. & Weidenhamer, J.D. (1999) Designs for greenhouse studies of interactions between plants. *Journal of Ecology*, 87, 1–16.
- Goldberg, D.E., Rajaniemi, T., Gurevitch, J. & Stewart-Oaten, A. (1999) Empirical approaches to quantifying interaction intensity: competition and facilitation along productivity gradients. *Ecology*, **80**, 1118–1131.
- Gómez-Aparicio, L., Zamora, R., Gómez, J.M., Hódar, J.A., Castro, J. & Baraza, E. (2004) Applying plant positive interactions to reforestation in Mediterranean mountains: a meta-analysis of the use of shrubs as nurse plants. *Ecological Applications*, 14, 1128–1138.
- Grace, J.B. (1991) A clarification of the debate between Grime and Tilman. *Functional Ecology*, 5, 583–587.
- Grace, J.B. (1993) The effects of habitat productivity on competition intensity. *Trends in Ecology and Evolution*, 8, 229–230.
- Greenlee, J.T. & Callaway, R.M. (1996) Abiotic stress and the relative importance of interference and facilitation in montane bunchgrass communities in western Montana. *American Naturalist*, **148**, 386–396.
- Grime, J.P. (1973) Competitive exclusion in herbaceous vegetation. *Nature*, 242, 344–347.
- Grime, J.P. (1974) Vegetation classification by reference to strategy. *Nature*, 250, 26–30.
- Grime, J.P. (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, **111**, 1169–1194.
- Grime, J.P. (1979) Plant Strategies and Vegetation Processes. Wiley, Chichester. Grime, J.P. (1998) Benefits of plant diversity to ecosystems: immediate, filter and founder effects. Journal of Ecology, 86, 902–910.
- Grime, J.P. (2007) Plant strategy theories: a comment on Craine (2005). Journal of Ecology, 95, 227–230.
- Guisan, A. & Theurillat, J.P. (2000) Assessing alpine vulnerability to climate change, a modelling perspective. *Integrated Assessment*, 1, 307–320.
- Hacker, S.D. & Bertness, M.D. (1999) Experimental evidence for factors maintaining species diversity in a New England salt marsh. *Ecology*, 80, 2064–2073.
- Hacker, S.D. & Gaines, S.D. (1997) Some implications of direct positive interactions for community species diversity. *Ecology*, 78, 1990–2003.
- Halpern, B.S., Silliman, B.R., Olden, J.D., Bruno, J.P. & Bertness, M.D. (2007) Incorporating positive interactions in aquatic restoration and conservation. *Frontiers in Ecology and the Environment*, 5, 153–160.
- Hastings, A., Byers, J.E., Crooks, J.A., Cuddington, K., Jones, C.G., Lambrinos, J.G., Talley, T.S. & Wilson, W.G. (2007) Ecosystem engineering in space and time. *Ecology Letters*, 10, 153–164.
- Hauggaard-Nielsen, H. & Jensen, E.S. (2005) Facilitative root interactions in intercrops. *Plant and Soil*, 274, 237–250.
- Hay, M.E. (1986) Associational defenses and the maintenance of species diversity: turning competitors into accomplices. *American Naturalist*, **128**, 617–641.
- Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G., et al. (1999) Plant diversity and productivity experiments in European grasslands. Science, 286, 1123–1127.
- Hobbs, R.J., Arico, S., Aronson, J., Baron, J.S., Bridgewater, P., Cramer, V.A., et al. (2006) Novel ecosystems: theoretical and management aspects of the new ecological world order. *Global Ecology and Biogeography*, 15, 1–7.
- Holmgren, M. (2000) Combined effects of shade and drought on tulip poplar seedlings: trade-off in tolerance of facilitation? *Oikos*, **90**, 67–78.
- Holmgren, M., Scheffer, M. & Huston, M.A. (1997) The interplay of facilitation and competition in plant communities. *Ecology*, 78, 1966–1975.
- Holzapfel, C. & Mahall, B.E. (1999) Bidirectional facilitation and interference between shrubs and annuals in the Mojave Desert. *Ecology*, 80, 1747–1761.
- Holzapfel, C., Tielbörger, K., Parag, H.A., Kigel, J. & Sternberg, M. (2006) Annual plant-shrub interactions along an aridity gradient. *Basic and Applied Ecology*, 7, 268–279.
- Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., et al. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3–35.
- Huisman, J., Johansson, A.M., Folmer, E.O. & Weissing, F.J. (2001) Towards a solution of the plankton paradox: the importance of physiology and life history. *Ecology Letters*, 4, 408–411.
- Huisman, J. & Weissing, F.J. (1999) Biodiversity of plankton by species oscillations and chaos. *Nature*, 402, 407–410.

- Hunter, A.F. & Aarssen, L.W. (1988) Plants helping plants. *Bioscience*, 38, 34– 39.
- Huston, M.A. (1979) A general hypothesis of species diversity. American Naturalist, 113, 81–101.
- Jensen, E.S. (1996) Barley uptake of N deposited in the rhizosphere of associated field pea. *Soil Biology and Biochemistry*, **28**, 159–168.
- Jones, C.G., Lawton, J.H. & Shachak, M. (1997) Positive and negative effects of organisms as physical ecosystem engineers. *Ecology*, 78, 1946–1957.
- Kikvidze, Z., Armas, C. & Pugnaire, F.I. (2006a) The effect of initial biomass in manipulative experiments on plants. *Functional Ecology*, 20, 1–3.
- Kikvidze, Z., Khetsuriani, L., Kikodze, D. & Callaway, R.M. (2006b) Seasonal shifts in competition and facilitation in subalpine plant communities of the central Caucasus. *Journal of Vegetation Science*, **17**, 77–82.
- Kikvidze, Z., Pugnaire, F.I., Brooker, R.W., Choler, P., Lortie, C.J., Michalet, R. & Callaway, R.M. (2005) Linking patterns and processes in alpine plant communities: a global study. *Ecology*, **86**, 1395–1400.
- Kirwan, L., Lüscher, A., Sebastia, M.T., Finn, J.A., Collins, R.P., Porqueddu, C., et al. (2007) Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology*, 95, 530– 539.
- Klanderud, K. (2005) Climate change effects on species interactions in an alpine plant community. *Journal of Ecology*, 93, 127–137.
- Klanderud, K. & Totland, Ø. (2005) The relative importance of neighbours and abiotic environmental conditions for population dynamic parameters of two alpine plant species. *Journal of Ecology*, **93**, 493–501.
- Kunstler, G., Curt, T., Bouchaud, M. & Lepart, J. (2006) Indirect facilitation and competition in tree species colonization of sub-mediterranean grasslands. *Journal of Vegetation Science*, **17**, 379–388.
- Laird, R.A. & Schamp, B.S. (2006) Competitive intransitivity promotes species coexistence. *American Naturalist*, 168, 182–193.
- Lenz, T.I. & Facelli, J.M. (2003) Shade facilitates an invasive stem succulent in a chenopod shrubland in South Australia. *Austral Ecology*, 28, 480– 490.
- Levine, S.H. (1976) Competitive interactions in ecosystems. *American Naturalist*, **110**, 903–910.
- Levine, J. (1999) Indirect facilitation: evidence and predictions from a riparian community. *Ecology*, 80, 1762–1769.
- Liancourt, P., Callaway, R.M. & Michalet, R. (2005) Stress tolerance and competitive-response ability determine the outcome of biotic interactions. *Ecology*, 86, 1611–1618.
- Lortie, C.J., Brooker, R.W., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F.I. & Callaway, R.M. (2004) Rethinking plant community theory. *Oikos*, **107**, 433–438.
- Lortie, C.J. & Callaway, R.M. (2006) Re-analysis of meta-analysis: support for the stress-gradient hypothesis. *Journal of Ecology*, 94, 7–16.
- Maestre, F.T., Bautista, S. & Cortina, J. (2003a) Positive, negative, and net effects in grass–shrub interactions in Mediterranean semiarid grasslands. *Ecology*, **84**, 3186–3197.
- Maestre, F.T., Bautista, S., Cortina, J. & Bellot, J. (2001) Potential for using facilitation by grasses to establish shrubs on a semiarid degraded steppe. *Ecological Applications*, 11, 1641–1655.
- Maestre, F.T. & Cortina, J. (2004) Do positive interactions increase with abiotic stress? A test from a semi-arid steppe. *Proceedings of the Royal Society of London B Supplement*, 271, S331–S333.
- Maestre, F.T., Cortina, J. & Bautista, S. (2004) Mechanisms underlying the interaction between *Pinus halepensis* and the native late-successional shrub *Pistacia lentiscus* in a semi-arid plantation. *Ecography*, 27, 776–786.
- Maestre, F.T., Cortina, J., Bautista, S. & Bellot, J. (2003b) Does *Pinus halepensis* facilitate the establishment of shrubs under semi-arid climate? *Forest Ecology* and Management, **176**, 147–160.
- Maestre, F.T. & Reynolds, J.F. (2006) Nutrient availability and atmospheric CO<sub>2</sub> partial pressure modulate the effects of nutrient heterogeneity on the size structure of populations in grassland species. *Annals of Botany*, **98**, 227–235.
- Maestre, F.T. & Reynolds, J.F. (2007) Biomass responses to elevated CO<sub>2</sub>, soil heterogeneity and diversity: an experimental assessment with grassland assemblages. *Oecologia*, **151**, 512–520.
- Maestre, F.T., Valladares, F. & Reynolds, J.F. (2005) Is the change of plantplant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. *Journal of Ecology*, 93, 748–757.
- Maestre, F.T., Valladares, F. & Reynolds, J.F. (2006) The stress-gradient hypothesis does not fit all relationships between plant–plant interactions and abiotic stress: further insights from arid environments. *Journal of Ecology*, 94, 17–22.
- Markham, J.H. (1997) Measuring and modelling plant neighbour effects: a reply to Freekleton and Watkinson. *Functional Ecology*, **11**, 534–535.

- Markham, J.H. & Chanway, C.P. (1996) Measuring plant neighbor effects. *Functional Ecology*, 10, 548–549.
- Maron, J.L. & Connors, P.G. (1996) A native nitrogen-fixing shrub facilitates weed invasion. *Oecologia*, 105, 302–312.
- Menge, B.A. (1976) Organization of the New England rocky intertidal community: role of predation, competition and environmental heterogeneity. *Ecological Monographs*, 46, 355–369.
- Michalet, R. (2006) Is facilitation in arid environments the result of direct or complex interactions? *New Phytologist*, 169, 3–6.
- Michalet, R. (2007) Highlighting the multiple drivers of change in interactions along stress gradients. *New Phytologist*, **173**, 3–6.
- Michalet, R., Brooker, R.W., Cavieres, L.A., Kikvidze, Z., Lortie, C.J., Pugnaire, F.I., Valiente-Banuet, A. & Callaway, R.M. (2006) Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecology Letters*, 9, 767–773.
- Miller, T.E. (1994) Direct and indirect species interactions in an early old-field plant community. *American Naturalist*, 143, 1007–1025.
- Miriti, M.N. (2006) Ontogenetic shift from facilitation to competition in a desert shrub. *Journal of Ecology*, 94, 973–979.
- Mitchell, C.E., Agrawal, A.A., Bever, J.D., Gilbert, G.S., Hufbauer, R.A., Klironomos, J.N., et al. (2006) Biotic interactions and plant invasions. *Ecology Letters*, 9, 726–740.
- Murrell, D.J. & Law, R. (2003) Heteromyopia and the spatial coexistence of similar competitors. *Ecology Letters*, 6, 48–59.
- Odum, E.P. (1968) Fundamentals of Ecology, 2nd edn. W.B. Saunders, London.
- Ormerod, S.J., Marshall, E.J.P., Kerby, G. & Rushton, S.P. (2003) Meeting the ecological challenges of agricultural change: editors' introduction. *Journal* of Applied Ecology, 40, 939–946.
- Padilla, F.M. & Pugnaire, F.I. (2006) The role of nurse plants in the restoration of degraded environments. *Frontiers in Ecology and the Environment*, 4, 196–202.
- Pagès, J.P. & Michalet, R. (2003) A test of the indirect facilitation model in a temperate hardwood forest of the northern French Alps. *Journal of Ecology*, 91, 932–940.
- Pagès, J.P., Pache, G., Joud, D., Magnan, N. & Michalet, R. (2003) Direct and indirect effects of shade on four forest tree seedlings in the French Alps. *Ecology*, 84, 2741–2750.
- Parrotta, J.A., Turnbull, J.W. & Jones, N. (1997) Introduction Catalyzing native forest regeneration on degraded tropical lands. *Forest Ecology and Management*, 99, 1–7.
- Pate, J.S. & Dawson, T.E. (1999) Assessing the performance of woody plants in uptake and utilisation of carbon, water and nutrients – Implications for designing agricultural mimic systems. *Agroforestry Systems*, 45, 245– 275.
- Pennings, S.C., Seling, E.R., Houser, L.T. & Bertness, M.D. (2003) Geographic variation in positive and negative interactions among salt marsh plants. *Ecology*, 84, 1527–1538.
- Pugnaire, F.I., Haase, P. & Puigdefábregas, J. (1996) Facilitation between higher plant species in a semiarid environment. *Ecology*, 77, 1420– 1426.
- Pugnaire, F.I. & Luque, M.T. (2001) Changes in plant interactions along a gradient of environmental stress. *Oikos*, 93, 42–49.
- Reader, R.J., Wilson, S.D., Belcher, J.W., Wisheu, I., Keddy, P.A., Tilman, D., et al. (1994) Plant competition in relation to neighbor biomass: an intercontinental study with *Poa pratensis. Ecology*, **75**, 1753–1760.
- Reinhart, K.O., Maestre, F.T. & Callaway, R.M. (2006) Facilitation and inhibition of seedlings of an invasive tree (*Acer platanoides*) by different tree species in a mountain ecosystem. *Biological Invasions*, 8, 231–240.
- Richardson, D.M., Allsop, N., D'Antonio, C.M., Milton, S.J. & Rejmánek, M. (2000) Plant invasions-the role of mutualisms. *Biological Reviews*, 75, 65–93.
- Ricketts, T.H., Daily, G.C., Ehrlich, P.R. & Michener, C.D. (2004) Economic value of tropical forest to coffee production. *Proceedings of the National Academy of Sciences USA*, 101, 12579–12582.
- Root, R.B. (1973) Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*, 43, 95–124.
- Rousset, O. & Lepart, J. (2000) Positive and negative interactions at different life stages of a colonizing species (*Quercus humilis*). *Journal of Ecology*, 88, 401–412.
- Sala, O.E., Chapin, F.S. III, Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., et al. (2000) Global biodiversity scenarios for the year 2100. Science, 287, 1770–1774.
- Scheffer, M. & van Nes, E.H. (2006) Self-organized similarity, the evolutionary emergence of groups of similar species. *Proceedings of the National Academy* of Sciences USA, 103, 6230–6235.
- Schiffers, K. & Tielbörger, K. (2006) Ontogenetic shifts in interactions among annual plants. *Journal of Ecology*, 94, 336–341.
- © 2007 The Authors. Journal compilation © 2007 British Ecological Society, Journal of Ecology, 96, 18-34

- Seifan, M. & Kadmon, R. (2006) Indirect effects of cattle grazing on shrub spatial pattern in a mediterranean scrub community. *Basic and Applied Ecology*, 7, 496–506.
- Sekiya, N. & Yano, K. (2004) Do pigeon pea and sesbania supply groundwater to intercropped maize through hydraulic lift? Hydrogen stable isotope investigation of xylem waters. *Field Crops Research*, 86, 167–173.
- Shevtsova, A., Haukioja, E. & Ojala, A. (1997) Growth response of subarctic dwarf shrubs, *Empetrum nigrum* and *Vaccinium vitis-idaea*, to manipulated environmental conditions and species renoval. *Oikos*, 78, 440–458.
- Siemann, E. & Rogers, W.E. (2003) Changes in light and nitrogen avaibility under pioneer trees may indirectly facilitate tree invasions of grasslands. *Journal of Ecology*, **91**, 923–931.
- Simberloff, D. (2006) Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? *Ecology Letters*, 9, 912–919.
- Simberloff, D., Relva, M.A. & Nuñez, M. (2003) Introduced species and management of a *Nothofagus/Austrocedrus* forest. *Environmental Management*, 31, 263–275.
- Simberloff, D. & Von Holle, B. (1999) Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions*, **1**, 21–32.
- Stoll, P. & Weiner, J. (2000) A neighborhood view of interactions among individual plants. *The Geometry of Ecological Interactions: Simplifying Spatial Complexity* (eds U. Dieckmann, R. Law & J.A.J. Metz), pp. 11–27. Cambridge University Press, Cambridge.
- Suding, K.N., Gross, K.L. & Houseman, G.R. (2004) Alternative states and positive feedbacks in restoration ecology. *Trends in Ecology and Evolution*, 19, 46–53.
- Sutherland, W.J., Pullin, A.S., Dolman, P.M. & Knight, T.M. (2004) The need for evidence-based conservation. *Trends in Ecology and Evolution*, 19, 305–308.
- Thomson, J.D. (1978) Effects of stand composition on insect visitation in twospecies mixtures of *Hieracium*. American Midland Naturalist, 100, 431–440.
- Tielbörger, K. & Kadmon, R. (1995) Effect of shrubs on emergence, survival and fecundity of four coexisting annual species in a sandy desert ecosystem. *Ecoscience*, **2**, 141–147.
- Tielbörger, K. & Kadmon, R. (2000a) Temporal environmental variation tips the balance between facilitation and interference in desert plants. *Ecology*, 81, 1544–1553.
- Tielbörger, K. & Kadmon, R. (2000b) Indirect effects in a desert plant community: is competition among annuals more intense under shrub canopies? *Plant Ecology*, **150**, 53–63.
- Tilman, D. (1988) *Plant Strategies and the Dynamics and Structure of Plant Communities*. Princeton University Press, Princeton, NJ.
- Tirado, R. & Pugnaire, F.I. (2003) Shrub spatial aggregation and consequences for reproductive success. *Oecologia*, **136**, 296–301.
- Travis, J.M.J. (2003) Climate change and habitat destruction: a deadly anthropogenic cocktail. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 270, 467–473.
- Travis, J.M.J., Brooker, R.W., Clark, E.J. & Dytham, C. (2006) The distribution of positive and negative species interactions across environmental gradients on a dual-lattice model. *Journal of Theoretical Biology*, 241, 896–902.
- Travis, J.M.J., Brooker, R.W. & Dytham, C. (2005) The interplay of positive and negative interactions across an environmental gradient: insights from an individual-based simulation model. *Biology Letters*, 1, 5–8.
- Travis, J.M.J. & Dytham, C. (1999) Habitat persistence, habitat availability and the evolution of dispersal. *Proceedings of the Royal Society of London Series* B, 266, 723–728.
- Valiente-Banuet, A., Rumebe, A.V., Verdú, M. & Callaway, R.M. (2006) Quaternary Plant lineages sustain global diversity by facilitating Tertiary lineages. *Proceedings of the National Academy of Sciences USA*, **103**, 16812– 16817.
- Vilà. M., Williamson, M. & Lonsdale, M. (2004) Competition experiments on alien weeds with crops: lessons for measuring plant invasion impact? *Biological Invasions*, 6, 59–69.

- Walker, L.R. & del Moral, R. (2003) Primary Succession and Ecosystem Rehabilitation. Cambridge University Press, Cambridge.
- Walker, L.R. & Powell, E.A. (1999) Regeneration of the Mauna Kea silversword Argyroxiphium sandwicense (Asteraceae) in Hawaii. *Biological Conservation*, 89, 61–70.
- Walker, L.R., Walker, J. & Hobbs, R.J. (2007) Linking Restoration and Ecological Succession. Springer, New York.
- Weigelt, A. & Jolliffe, P. (2003) Indices of plant competition. *Journal of Ecology*, 91, 707–720.
- Weigelt, A., Schumacher, J., Walther, T., Bartelheimer, M., Steinlein, T. & Beyschlag, W. (2007) Identifying mechanisms of competition in multi-species communities. *Journal of Ecology*, 95, 53–64.
- Welden, C.W. & Slauson, W.L. (1986) The intensity of competition versus its importance: an overlooked distinction and some implications. *Quarterly Review of Biology*, 61, 23–44.
- Went, F.W. (1942) The dependence of certain annual plants on shrubs in southern California deserts. Bulletin of the Torrey Botanical Club, 69, 100–114.
- Wilson, M.V. (2007) Measuring the components of competition along productivity gradients. *Journal of Ecology*, 95, 301–308.
- Wipf, S., Rixen, C. & Mulder, C.P.H. (2006) Advanced snowmelt causes shift toward positive neighbour interactions in a subarctic tundra community. *Global Change Biology*, **12**, 1–11.
- Wooton, J.T. (1994) The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology and Systematics, 25, 443–466.
- Yamamura, N., Higashi, M., Behera, N. & Wakano, J.Y. (2004) Evolution of mutualism through spatial effects. *Journal of Theoretical Biology*, 226, 421– 428.
- Young, T.P., Petersen, D.A. & Clary, J.J. (2005) The ecology of restoration: historical links, emerging issues and unexplored realms. *Ecology Letters*, **8**, 662–673.
- Zavaleta, E.S. & Hulvey, K.B. (2004) Realistic species losses disproportionately reduce grassland resistance to biological invaders. *Science*, **306**, 1175– 1177.

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#### Supplementary material

The following supplementary material is available for this article:

**Table S1.** Survey of recent (1996–present) publications exploring positive interactions between vascular plants

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## **Reintroduction of** *Nassella pulchra* **to California coastal grasslands:** Effects of topsoil removal, plant neighbour removal and grazing

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#### Abstract

**Question:** What is the most appropriate combination of treatments to reintroduce *Nassella pulchra*, a perennial bunchgrass, into degraded mediterranean coastal grasslands?

Location: Central coast of California, USA.

**Methods:** *N. pulchra* was sown from seeds and transplanted into a degraded grassland in a multi-factorial experiment testing the effects of (1) two grazing intensities (lightly grazed by native mammal species or ungrazed); (2) topsoil removal and (3) reduction of plant neighbours. The experiment was carried out on two types of surrounding vegetation (exotic annual grasses and exotic forbs).

**Results:** Topsoil removal greatly enhanced establishment from seeds and transplant survival, mainly because it reduced the exotic vegetation and thus reduced competition. While removing neighbours was essential when topsoil was left intact, it had a negative effect on *N. pulchra* when surrounding species included exotic forbs (*Brassica* spec. and *Asteraceae*) at low density (after topsoil removal). Moderate grazing by native mammals (deer, rabbits and gophers) did not affect *N. pulchra*.

**Conclusion:** Our results suggest that seeding after topsoil has been removed is a promising method to reintroduce *N*. *pulchra* to highly degraded sites where there is little to no native seed bank.

**Keywords**: Competition; Exotic annual species; Native perennial bunchgrass; Nitrogen reduction; Weeding.

Nomenclature: Hickman 1993.

#### Introduction

Natural and semi-natural grasslands were once widespread, species-rich ecosystems worldwide and are now threatened by development, agricultural intensification and altered disturbance regimes (e.g. Heady et al. 1988; Jacobs et al. 1999; Hoekstra et al. 2005). In California, grasslands evolved with a number of disturbances, including intentional high frequency burning by native Americans (Anderson 2006), seasonal grazing by native ungulates, soil disturbance and grazing by burrowing mammals and periodic drought stress (Heady et al. 1988). They are important habitats for wildlife and are hot-spots of plant species diversity (Stromberg et al. 2001). In the late 18th century, Europeans limited fires and introduced year round, intensive cattle grazing, along with numerous exotic forage grasses. These grasslands are now dominated by annual exotic grasses and forbs of mediterranean origin (Heady et al. 1988; Stromberg et al. 2001; Hayes & Holl 2003a, b).

While restoration can sometimes be easily promoted by re-establishing historical abiotic conditions, it can often be much harder to achieve when feedbacks have developed, in which case a severely degraded ecosystem may have shifted to an alternative state (Suding et al. 2004). When grasslands have been severely degraded, recovery is unlikely (Stromberg & Griffin 1996; Hamilton et al. 2002) and single treatment restoration strategies have not been successful (for review see Corbin et al. 2004).

In these conditions, restoration can be initiated by reintroducing some perennial species to improve habitats by enhancing soil and micro-environmental conditions

(Whisenant et al. 1995). To enhance environmental conditions in degraded grasslands for native species to establish, the influence of exotic plant species has to be reduced. Various exotic plant reduction techniques have been tested (for review see Corbin et al. 2004), including lowering plant available nitrogen to reduce all plant growth during the establishment phase and thus favour slow growing native species (Huenneke et al. 1990; Corbin et al. 2004). Whereas biomass removal (Maron & Jefferies 2001) and carbon addition (Wilson & Gerry 1995; Reever Morghan & Seastedt 1999; Alpert & Maron 2000; Török et al. 2000; Corbin & D'Antonio 2004) have had limited positive effects on native species (Wilson 2002; Corbin et al. 2004), topsoil removal has shown promising results in reducing nitrogen concentrations in grasslands in Europe and in the midwestern US (e.g. Peeters & Janssens 1998; Marrs 2002; Wilson 2002), but has not been tested in Californian grasslands.

Topsoil removal can simultaneously address two obstacles to recovery of native grasses in exotic dominated grasslands. First, it may serve to reduce the abundant exotic plant species seed bank (Marrs 2002; Wilson 2002). Second, it can serve to reduce N availability (Aerts et al. 1995; Walker et al. 2007) which tends to favor exotic species.

Two additional techniques to reduce the influence of exotic plant species on native grasses and provide native seeds with suitable safe sites to germinate are neighbour removal and grazing. Neighbour removal of mainly exotic species will further reduce exotic plant cover and may be beneficial for native species (Corbin et al. 2004), but should be tested in combination with other treatments. Since the effect of cattle and sheep (Dyer et al. 1996; Dyer & Rice 1997; Dyer 2003; Fehmi & Bartolome 2003; Bartolome et al. 2004; Corbin et al. 2004; Marty et al. 2005) has already been widely studied and results have shown mixed effects on native coastal prairie vegetation (Hatch et al. 1999; Hayes & Holl 2003a; Corbin et al. 2004), we tested the effect of native fauna grazing (deer, ground-squirrels and other small mammals) on the establishment of a native perennial species. We tested these effects in a factorial design as they may interact with one another.

The aim of this experiment was to test the efficacy of various combinations of grazing (moderately grazed by native mammals/ungrazed), topsoil removal or intact topsoil and two neighbour removal intensities (neighbour removal/neighbour intact) to enhance establishment from seeds and survival and growth of planted seedlings (transplants) of a native perennial species. We tested all these combinations of treatments in areas dominated by two types of exotic vegetation (exotic annual grasses or exotic forbs). Specifically, we hypothesized that: (1) moderate grazing by native mammals would benefit native perennial species establishment by reducing exotic plant biomass without inducing too much trampling; (2) topsoil removal would benefit native perennial species establishment, as topsoil removal generally reduces soil nitrogen and the exotic plant seed bank and (3) neighbour removal would benefit native perennial species establishment by reducing exotic plant competition.

#### Methods

#### Site description

We conducted experiments on a coastal grassland of the Stanford foothills, located 25 km from the ocean (Stanford University campus, Palo Alto, California, 37°18'N, 122°9'W). The site is a small hill (max. 3% slope) with a few large Quercus trees and herbaceous vegetation comprised of large patches of annual exotic grasses (Avena barbata, A. fatua, Brachypodium distachyon, Bromus diandrus, B. hordeaceus, B. madritensis, Lolium multiflorum and Vulpia microstachys) and large patches of exotic forbs (Brassica nigra and various Asteraceae such as Carduus pycnocephalus, Picris echioides, Silybum marianum and Sonchus asper). N. pulchra represents < 0.5% springtime biomass and <0.02% of the seeds in the seed bank (S. Anderson unpubl. data). The area was moderately grazed by cattle from the early 19th century until 1984 and appears to have been a popular cattle resting area. As with other such landscapes in California where Quercus cover is low or discontinuous (due either to natural distribution or ranchers reducing tree abundance to boost grass productivity), cattle typically take refuge in the warm spring and summer months under focal shade trees. The resulting cattle trampling around this hill apex likely represented a significant disturbance independent of the treatments. The extremely clay-rich soils of this site are not subject to extensive compression from trampling (as more loamy soils would be), but such continual disturbance tends to shift the surrounding herbaceous community away from grass to a more forb-dominated community (especially favouring Asteraceae and Brassicaceae). In summary, the area is moderately disturbed compared to most remnant coastal California grasslands, but heavily transformed from the native grass and native forb-dominated coastal grasslands that were present prior to European colonization.

We compiled precipitation and air temperature data from the closest weather station (Anon. 2005a). The site received 515 mm of rainfall in 2002, which is similar to the mean over the 50 previous years (516 mm), whereas rainfall was lower in 2003 (420 mm). Mean air temperatures in 2002 (15.2°C) and 2003 (15.7°C) were comparable to the 50 previous years (15.1°C).

In 2004 we collected ten soil samples in areas where the topsoil had not been removed in order to confirm that the surface soil had more total N than at the beginning. We randomly collected five samples from the soil surface and five samples from <10 cm deep, and analysed them for Kjeldahl total N.

#### Experimental design

Initially, we set out 24 experimental units, each composed of two 3 m × 1.5 m plots, in order to encompass an equal number of units on north and south facing slopes. As aspect did not appear to strongly influence results, we did not consider it any further. However, the 24 units were also arranged to encompass an equal number of units on exotic forb patches and exotic grass patches and this factor appeared to influence the outcome of treatment effects. In each experimental unit, we experimentally manipulated grazing, topsoil and plant interactions in a split-split plot design: each plot (grazed or ungrazed) was split into two 1.5 m × 1.5 m plots, allocated to intact topsoil or topsoil removal. Each subplot was further split into two 0.75 m × 1.5 m split plots and allocated to intact neighbour or neighbour removal.

The ungrazed treatment consisted of installing a cage around one of each pair of plots during the winter of 2002-2003 to exclude mammalian grazers. During the experiment, the uncaged plots were grazed by deer, ground squirrels and other small mammals.

We removed the topsoil layer (0 - 10 cm: litter layer and part of the A horizon) in one  $1.5 \text{ m} \times 1.5 \text{ m}$  split plot in each plot in August 2002 by tilling and then scraping. Topsoil removal also eliminated neighbours and the top layer of the seed bank (Marrs 2002). In January 2003, at transplanting, 25-35% of the ground cover was recolonized by plant species and in March 2003 ground cover was > 70% on split plots with topsoil removed vs. 90-100% on plots with intact topsoil.

To test the effect of plant neighbour interactions on seedlings and transplants, we allocated one half of each split plot to neighbour removal and we left neighbours intact in the other half. Neighbour removal was performed by hand-pulling all small seedlings (native and exotic) and clipping all larger plants to the ground within a 25 cm diameter area surrounding the target plants (see below); we chose a 25 cm diameter as Davies et al. (1999) showed that some grassland plants responded to neighbour removal in areas greater than 15 cm diameter. We removed neighbours before sowing and transplanting, twice in spring 2003 and twice in winter 2003-2004.

#### Seeding and transplanting

The selected perennial species was *Nassella pulchra* as it is a widely available native bunchgrass, it has a broad habitat tolerance and it can create ecological resistance against various weeds (Dukes 2001; Reever-Morghan & Rice 2005). Once established it can live a long time (Hamilton et al. 1999), it was propbably an abundant species in portions of the coastal range grasslands and it is present around the study site.

In each 0.75 m × 1.5 m split-split plot, we allocated a 0.75 m × 0.75 area to a seeding experiment and a 0.75 m × 0.75 m area to a transplanting experiment. We sowed 25 *N. pulchra* seeds in each of the 24 experimental units for each grazing × topsoil × neighbour treatment at the end of October 2002, before the first autumn rain, which occurred on 31 October 2002 (total of 4800 seeds). Seeds were sown 6.25 cm apart in 5 × 5 seed grids and pushed into the soil to a depth approximately equal to their length. *N. pulchra* seeds were collected the previous spring from Russian Ridge Open Space Preserve, ca. 10 km from the experimental location but within the same watershed. Seed germination was also measured in the laboratory at 20°C, 16 h day light: 8 h darkness.

In January 2003, we transplanted four *N. pulchra* plants into each of the 24 experimental units for each grazing  $\times$  topsoil  $\times$  neighbour treatment. These plants had been grown outdoors in individual containers for two months, watered as required and not fertilized. The four transplants were planted 50 cm apart to minimize interactions and were watered once when planted out. Transplants that died within the first month were replaced.

#### Data collection

We recorded the number of seedlings that germinated and survived until 17 weeks after the first rain on 05/06.03.2003. All seedlings (out of 25 seeds) were summed in each split-split plot to carry out statistical analyses – hereafter this value is referred to as 'percent establishment from seed'.

We monitored transplanted *N. pulchra* survival and growth over 1.5 years. We measured survival in March 2003, June 2003, December 2003 and to June 2004, after which we collected the above- and below-ground biomass of all the transplants by sampling the same volume of soil around each plant (500 cm<sup>3</sup>), reaching the end of most major roots which comprise most of the biomass. We washed the roots and dried them at 70 °C to a constant mass and weighed the above- and below-ground biomass, and calculated a mean biomass for each set of four transplants.

#### Statistical analyses

Soil sample data were analysed with a *t*-test. We conducted ANOVA for split-split plot design on all data (emergence from seeds, transplant survival and biomass) using two separate models: one for each of the surrounding vegetation (forb-dominated and grass-dominated patches) (Anon. 2005b). Grazing, topsoil removal and neighbour removal were treated as categorical explanatory variables with grazing as the whole plot treatment, topsoil removal as the first split plot and neighbour removal as the second split plot, p < 0.05 was considered significant and p < 0.10 marginally significant. For transplant survival and biomass, only results 1.5 years after planting (June 2004) are presented here as analyses from other sampling dates were similar. Arcsine square-root transformations were applied to emergence and survival data and log transformations to biomass measurements (Sokal & Rohlf 1998). A few cages were destroyed during the experiment resulting in the loss of those plots, so the degrees of freedom varied slightly for some analyses.

#### Results

#### Soil nitrogen

Soil samples taken in the top 10 cm of unscraped areas had higher N (0.34 mg-N.g<sup>-1</sup>) than samples taken > 10 cm deep (0.20 mg-N.g<sup>-1</sup>, F = 45.7, p < 0.001); topsoil removal thus removed most of the N-rich topsoil layer.

#### Establishment from seed

Percent establishment from seed in the field was 37  $\pm 2\%$  (1793 seeds germinated and survived to 17 weeks out of 4800 planted) whereas the value in the laboratory reached  $74 \pm 3\%$ . Overall, establishment from seed was  $40 \pm 2\%$  in exotic forb patches and  $35 \pm 2\%$  in exotic grass patches. In both types of vegetation, topsoil removal substantially increased establishment from seed (up to 55 %) compared with intact topsoil (up to 35% only; Table 1; Fig. 1). In exotic forb patches, neighbour removal resulted in a marginal increase in establishment from seed (neighbour removal 42% versus intact neighbours 37%; Table 1, Fig. 1). In exotic grass patches, there was a significant topsoil removal × neighbour removal interaction term as neighbour removal also increased establishment; establishment was by far the lowest (12%) in plots with both intact neighbours and intact topsoil (Table 1, Fig. 1).



Fig. 1. Establishment of *Nassella pulchra* from seeds after 17 weeks. See Table 1 for ANOVA results. Ti = Intact topsoil; Tr = Topsoil removal; Dark bars = Neighbours intact; White bars = Neighbour removal. Values are means  $\pm$  SE.

Surrounding vegetation	Ex	otic grass pat	ches	Ex	otic forb pate	hes	
	df	F	р	df	F	р	
Replication	10			12			
Grazing	1	1.1	0.320	1	2.5	0.142	
Error (whole plot)	10			12			
Topsoil removal	1	46.1	< 0.001	1	24.9	< 0.001	
Grazing × topsoil	1	0.2	0.627	1	1.9	0.182	
Error (subplot)	20			24			
Neighbour removal	1	47.2	< 0.001	1	<u>3.0</u>	0.091	
Grazing × neighbour	1	< 0.1	0.992	1	0.2	0.663	
Topsoil × neighbour	1	25.2	< 0.001	1	1.0	0.317	
Grazing × topsoil × neighbour	1	1.9	0.174	1	0.1	0.830	
Error (split-plot)	40			48			

**Table 1.** ANOVA for treatment effects on establishment of *Nassella pulchra* from seed 17 weeks after seeding in the two types of surrounding vegetation. Values in bold are significant (p < 0.05), underlined values are marginally significant (p < 0.10).

**Table 2.** ANOVA for treatment effects on survival of *Nassella pulchra* transplants 1.5 years after transplanting in the two types of surrounding vegetation. Values in bold are significant (p < 0.05), underlined values are marginally significant (p < 0.10).

Surrounding vegetation	Exe	otic grass pat	ches	Ex	totic forb pate		
	df	F	р	df	F	Р	
Replication	9			9			
Grazing	1	0.2	0.693	1	0.8	0.406	
Error (whole plot)	9			9			
Topsoil removal	1	12.8	0.002	1	0.9	0.354	
Grazing × topsoil	1	< 0.1	0.845	1	2.2	0.159	
Error (subplot)	18			18			
Neighbour removal	<u>1</u>	<u>3.4</u>	0.075	1	1.8	0.191	
Grazing × neighbour	1	0.3	0.586	1	< 0.1	0.930	
Topsoil × neighbour	1	0.8	0.365	1	5.8	0.022	
$Grazing \times topsoil \times neighbour$	1	0.5	0.468	1	0.4	0.538	
Error (split-plot)	36			36			

#### Transplant survival and biomass 1.5 years after planting

At the end of the experiment, transplant survival was similar in both surrounding vegetation types (exotic grasses:  $15.0 \pm 0.2\%$ ; exotic forbs:  $15.3 \pm 0.2\%$ ). In exotic grass patches, topsoil removal and neighbour removal as separate treatments increased transplant survival with topsoil removal having a stronger effect (Table 2, Fig. 2). In exotic forb patches, topsoil removal × intact

neighbours maximized transplant survival (Table 2): 28% vs 11% in other treatments (Fig. 2).

In exotic grass patches, neighbour removal had a significant positive effect on transplant biomass (neighbour removal 1.24 g vs. intact neighbours 0.30 g; Table 3, Fig. 3). In exotic forb patches, topsoil removal × intact neighbours resulted in a marginal increase in transplant biomass (0.9 g vs. 0.2 g for other treatments; Table 3; Fig. 3).



Fig. 2. Survival of transplanted *Nassella pulchra* seedlings after 1.5 years. See Table 2 for ANOVA results. Ti = intact Topsoil. Tr = Topsoil removal; dark bars = Neighbours intact. white bars = Neighbour removal. Values are means  $\pm$  SE.

Surrounding vegetation	Ex	otic grass pat	ches	Ex	Exotic forb patches				
	df	F	р	df	F	р			
Replication	9			9					
Grazing	1	< 0.1	0.907	1	2.1	0.186			
Error (whole plot)	9			9					
Topsoil removal	1	1.2	0.286	1	1.5	0.234			
Grazing × topsoil	1	< 0.1	0.872	1	0.2	0.658			
Error (subplot)	18			18					
Neighbour removal	1	14.0	< 0.001	1	0.7	0.394			
Grazing × neighbour	1	< 0.1	0.971	1	0.7	0.414			
Topsoil × neighbour	1	0.4	0.542	<u>1</u>	<u>3.3</u>	<u>0.078</u>			
Grazing × topsoil × neighbour	1	0.1	0.767	1	1.5	0.225			
Error (split-plot)	36			36					

**Table 3.** ANOVA for treatment effects on the biomass of *Nassella pulchra* transplants 1.5 years after transplanting in the two types of surrounding vegetation. Values in bold are significant (p < 0.05), underlined values are marginally significant (p < 0.10).

#### Discussion

This study showed that topsoil removal maximized Nassella pulchra establishment from seed and transplant survival. This is consistent with another study on the effect of topsoil removal on a sown native perennial grass in Australia (Cole et al. 2005). Topsoil removal changes both available nutrients (this study; Aerts et al. 1995; Buisson et al. 2006), can reduce the exotic seed bank (Marrs 2002; Wilson 2002) and reduces cover of the surrounding vegetation (Buisson et al. 2006). Only ca. 30% of the ground was covered with exotics in January 2003 and ca. 70% in March 2003 vs 90% - 100% at all times on plots with intact topsoil. It appears that this reduced cover contributed to N. pulchra establishment by decreasing competition. Previous studies have shown that perennial species suffer from competition of exotic species (Lenz et al. 2003; Cole & Lunt 2005; Prober & Thiele 2005) because exotics germinate earlier (Bartolome & Gemmill 1981; Chiariello 1989), even when they

provide germination cues for N. pulchra to reduce its time of emergence (Dyer et al. 2000). Exotic annual grasses also reduce water availability (Eliason & Allen 1997; Holmes & Rice 1997; Dyer & Rice 1999; Hamilton et al. 1999; Clarke & Davison 2004). The competition of exotic annual grasses at the emergence stage was slightly greater than that of exotic forbs. While these plants remained low growing rosettes in early stages, annual exotic grasses emerged quickly which can greatly reduce the amount of light reaching the ground and decrease water availability through high transpiration (Moyes et al. 2005). Annual exotic grasses and the small seedlings of our experiment have the same requirements: they have similar root depth and leaf height, and they compete for the same resources. Whatever the surrounding vegetation, topsoil removal led to the highest N. pulchra emergence and additional exotic species removal was not necessary.

*N. pulchra* transplants on subplots with intact topsoil and neighbours survived six months after planting (data not shown), but they grew little above-ground or



**Fig. 3.** Biomass of transplanted *N. pulchra* seedlings after 1.5 years. See Table 3 for ANOVA results. Ti = Intact topsoil; Tr = Topsoil removal; Dark bars = Neighbours intact; White bars = Neighbour removal. Values are means  $\pm$  SE.

below-ground biomass before summer and may not have been able to withstand summer water stress. Past research concurs that perennial grasses cannot survive dry conditions when grown with exotic annual grasses because annuals out compete young perennials for light and perennials are thus unable to develop sufficient root biomass before summer to use deep soil moisture (Bartolome 1981; Dyer & Rice 1997, 1999; Hamilton et al. 1999; Buisson et al. 2006). Here we show that when competition was not reduced by topsoil removal, neighbour removal had a positive effect on N. pulchra survival. We showed that this was also true in dense exotic forb patches. This is consistent with studies carried out on Centaurea solstitialis (Enloe et al. 2004) and Brassica nigra (Moyes et al. 2005), which showed that these two species depleted soil moisture.

However, at low density, in the treatment combining topsoil removal and intact neighbours, exotic forbs had a positive effect on N. pulchra survival and biomass. This may be because a low density of forbs shaded N. puchra compared to the otherwise harsh condition plots: either bare and dry (topsoil and neighbour removal) or highly competitive (intact topsoil). Another explanation may be that, contrary to exotic annual grasses, the forbs had taproots and, at that stage, N. pulchra seedlings had short roots. These species may therefore not be in competition for water, and N. pulchra may even benefit from hydraulic lift of the forbs (Callaway et al. 2003), although this is speculative as we do not have soil moisture data from this system. The functional type of plant neighbours therefore determines the type of plant interactions, as grass neighbours showed greater competitive effects than forbs. This means that N. pulchra is affected by competitive interactions by species with similar functional traits (grasses), whereas plants with different functional traits may help species to persist (complementarity hypothesis among functional groups; Loreau et al. 2001).

While native mammal grazing did not have any detrimental effect on the reintroduced perennial native species, it did not have any direct or indirect positive effects either. While excluding grazing by native mammals did not indirectly affect N. pulchra through the reduction of the surrounding exotic plant biomass, the direct effect of exclosures on these exotic species was not measured. The low impact of grazing in our experiment compared with other similar experiments (Buisson et al. 2006) may be explained by the nature of the herbivory; domestic mammals in Buisson et al. (2006) and nondomestic native mammals in this study. Cattle and native grazers may have very different impacts on vegetation: domestic grazing is usually a disturbance relatively more intensive and frequent than wild herbivory which is much more stochastic and extensive, following animal demographic oscillations. Even if a large amount of evidence suggests an impact of wild herbivores on vegetation in a given ecosystem; for a given stocking rate, the direct or indirect consequences of wild herbivory on plant performances are questionable. This is the case in this study, since direct observations of native mammals and of their grazing impacts on vegetation are common on the Stanford foothills: the primary grazers are deer and small mammals (in particular rabbits); and while no quantitative grazer counts were conducted, rabbits were seen in and around the site nearly every visit and deer were often seen in the vicinity.

This result is not consistent with another study focusing on non-herbaceous targets in highly degraded riparian habitats (Opperman & Merenlender 2000), where deer grazing substantially reduces the establishment of saplings. However, it is consistent with a study on another native browser, the tule elk (*Cervus elaphus nannodes*) (Johnson & Cushman 2007) and on cattle (Hayes & Holl 2003a) which showed that these large herbivores had no effect on the abundance or cover of native perennial grasses. The impact of grazing is therefore most probably linked with native mammal density, cattle grazing rate and rotation and the type of plant targets. Further studies should measure grazing intensity of native mammals in order to compare these results to other grazing systems (other species, seasonal grazing, stocking rates etc.).

#### Conclusions

Our study suggests that, at the highly degraded California coastal grassland site studied, the best treatment to restore N. pulchra is to remove topsoil to provide a suitable establishment environment. Topsoil removal is a resource intensive restoration treatment (Buisson et al. 2006) that has already been carried out on large areas (Patzelt et al. 2001; Verhagen et al. 2001; Grootjans et al. 2002). Once removed, topsoil can be sold and this income used toward restoration (Marrs 1985, 2002). Topsoil removal is an intensive restoration technique which should be considered carefully, (1) as it may alter other soil physical and chemical characteristics (e.g. moisture, exchangeable magnesium) and microbial community composition (Steenwerth et al. 2002); (2) as it may change nutrient availability and mycorrhizae composition and (3) only in highly degraded grasslands and only after testing the seed bank for native seeds (Grootjans et al. 2002; Marrs 2002).

Our results suggest that removing exotic annual grass neighbours is not necessary if topsoil is removed but the two techniques combined will increase early survival and ensure maximum growth, but this is dependent on the composition of the surrounding vegetation. Later growth of *N. pulchra* should not be a problem as large *N. pulchra* have been shown to outcompete *Centaurea solstitialis*, a deep-rooted invasive thistle (Reever Morghan & Rice 2005).

At our site, moderate grazing by native mammals did not influence *N. pulchra* establishment. Fencing is therefore unnecessary in similar conditions.

Our and other studies (Corbin & D'Antonio 2004) suggest that *N. pulchra* can be successfully sown from seeds into exotic grasslands particularly if other treatments are applied (e.g. topsoil removal in this study; ploughing in Seabloom et al. 2003). Even with low establishment, *N. pulchra* can be seeded in large quantity because seeds are affordable (Pacific Coast Seed, Livermore, CA, US, 2005), to produce densities of surviving grasses similar to those achieved by more costly seedling transplanting.

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#### References

- Anon. 2005a. Weather data and products. California Department of Water Resources. url: http://www.ipm.ucdavis. edu/WEATHER/wxretrieve.html [September 2006; last checked May 2007].
- Anon. 2005b. The R Project for Statistical Computing. [html pages]. <u>http://www.r-project.org/</u> [April 2005; last checked May 2007].
- Aerts, R., Huiszoon, A., van Oostrum, J.H.A., van De Vijver, C.A.D.M. & Willems, J.H. 1995. The potential for heathland restoration on formerly arable land at a site in Drenthe, The Netherlands. *Journal of Applied Ecology* 32: 827-835.
- Alpert, P. & Maron, J.L. 2000. Carbon addition as a countermeasure against biological invasion by plants. *Biological Invasions* 2: 33-40
- Anderson, M.K. 2006. Tending the wild: Native American knowledge and the management of California's natural resources. University of California Press, Berkeley, CA, US.
- Bartolome, J.W. 1981. *Stipa pulchra*, a survivor from the pristine past. *Fremontia* 9: 3-6.
- Bartolome, J. & Gemmill, B. 1981. The ecological status of *Stipa pulchra* (Poaceae) in California. *Madroño* 28: 172-184.
- Bartolome, J.W., Fehmi, J.S., Jackson, R.D. & Allen-Diaz, B. 2004. Response of a native perennial grass stand to disturbance in California's coast range grassland. *Restoration Ecology* 12: 279-289.
- Buisson, E., Holl, K.D., Anderson, S., Corcket, E., Hayes,

G.F., Torre, F., Peteers, A. & Dutoit, T. 2006. Effect of seed source, topsoil removal, and plant neighbor removal on restoring California coastal prairies. *Restoration Ecology* 14: 569-577.

- Callaway, R.M., Pennings, S.C. & Richards, C.L. 2003. Phenotypic plasticity and interaction among plants. *Ecology* 84: 1115-1128.
- Chiariello, N.R. 1989. Phenology of California grasslands. In: Huenneke, L.F. & Mooney, H. (eds.) Grassland structure and function: California annual grassland, pp. 47-58. Kluwer Academic, Dordrecht, NL.
- Clarke, P.J. & Davison, E.A. 2004. Emergence and survival of herbaceous seedlings in temperate grassy woodlands: recruitment limitations and regeneration niche. *Austral Ecology* 29: 320-331
- Cole, I. & Lunt, I.D. 2005. Restoring Kangaroo Grass (*Themeda triandra*) to grassland and woodland understoreys: a review of establishment requirements and restoration exercises in south-east Australia. *Ecological Management and Restoration* 6: 28-33.
- Cole, I., Lunt, I.D. & Koen, T. 2005. Effects of sowing treatment and landscape position on establishment of the perennial tussock grass *Themeda triandra* (Poaceae) in degraded *Eucalyptus* woodlands in Southeastern Australia. *Restoration Ecology* 13: 552-561.
- Corbin, J.D. & D'Antonio, C.M. 2004. Competition between native perennial and exotic annual grasses: implications for an historical invasion. *Ecology* 85: 1273-1283.
- Corbin, J.D., D'Antonio, C.M. & Bainbridge, S. 2004. Tipping the balance in the restoration of native plants: Experimental approaches to changing the exotic:native ratio in California grassland. In: Gordon, M. & Bartol, S. (eds.) *Experimental approaches to conservation biology*, pp. 154-179. University of California Press, Berkeley, CA, US.
- Davies, A., Dunnett, N.P. & Kendel, T. 1999. The importance of transplant size and gap with in the botanical enrichment of species-poor grasslands in Britain. *Restoration Ecology* 7: 271-280.
- Dukes, J.S. 2001. Biodiversity and invasibility in grassland microcosms. *Oecologia* 126: 563-568.
- Dyer, A.R. 2003. Burning and grazing management in a California grassland: growth, mortality, and recruitment of *Nassella pulchra. Restoration Ecology* 11: 291-296.
- Dyer, A.R. & Rice, J.R. 1997. Intraspecific and diffuse competition: the response of *Nassella pulchra* in a California grassland. *Ecological Applications* 7: 484-492.
- Dyer, A.R. & Rice, J.R. 1999. Effects of competition on resource availability and growth of a California bunchgrass. *Ecology* 80: 2697-2716.
- Dyer, A.R., Fossum, H.C. & Menke, J.W. 1996. Emergence and survival of *Nassella pulchra* in a California grassland. *Madroño* 43: 316-333.
- Dyer, A.R., Fenech, A. & Rice, J.R. 2000. Accelerated seedling emergence in interspecific competitive neighbourhoods. *Ecology Letters* 3: 523-529.
- Eliason, S.A. & Allen, E.B. 1997. Exotic grass competition in suppressing native shrubland re-establishment. *Restoration Ecology* 5: 245–255.
- Enloe, S.F., DiTomaso, J.M., Orloff, S.B. & Drake, D.J. 2004.

Soil water dynamics differ among rangeland plant communities by yellow starthistle (*Centaurea solstitialis*), annual grasses, or perennial grasses. *Weed Science* 52: 248-254.

- Fehmi, J.S. &. Bartolome, J.W. 2003. Impacts of livestock and burning on the spatial patterns of the grass *Nassella pulchra* (Poaceae). *Madroño* 50: 8-14.
- Grootjans, A.P., Bakker, J.P., Jansen, A.J.M. & Kemmers, R.H. 2002. Restoration of brook valley meadows in the Netherlands. *Hydrobiologia* 478: 149-170.
- Hamilton, J.G., Holzapfel, C. & Mahall, B.E. 1999. Coexistence and interference between a native perennial grass and non-native annual grasses in California. *Oecologia* 121: 518-526.
- Hamilton, J.G., Griffin, J.R. & Stromberg, M.R. 2002. Longterm population dynamics of native *Nassella* (Poaceae) bunchgrasses in Central California. *Madroño* 49: 274-284.
- Hatch, D.A., Bartolome, J.W., Fehmi, J.S. & Hillyard, D.S. 1999. Effects of burning and grazing on a coastal California grassland. *Restoration Ecology* 7: 376-381.
- Hayes, G.F. & Holl, K.D. 2003a. Cattle grazing impacts on annual forbs and vegetation composition of mesic grasslands in California. *Conservation Biology* 17: 1694-1702.
- Hayes, G.F. & Holl, K.D. 2003b. Site-specific responses of native and exotic species to clipping frequency, litter accumulation and soil disturbance in a mesic grassland community. *Applied Vegetation Science* 6: 235-244.
- Heady, J.H., Foin, T.C., Hektner, M.M., Barbour, M.G., Taylor, D.W. & Barry, W.J. 1988. Coastal prairies and northern coastal scrub. In: Barbour, M.G. & Major, J. (eds.) *Terrestrial vegetation of California*, pp. 733-760. Wiley, New York, NY, US.
- Hickman, J. (ed.) 1993. The Jepson Manual: Higher plants of California. University of California, Berkeley, CA, US.
- Hoekstra, J.M., Boucher, T.M., Ricketts, T. H. & Roberts, C. 2005. Confronting a biome crisis: global disparities of habitat loss and protection. *Ecology Letters* 8: 23-29.
- Holmes, T.H. & Rice, K.J. 1996. Patterns of growth and soil-water utilization in some exotic annuals and native perennial bunchgrasses of California. *Annals of Botany* 78: 233-243.
- Huenneke, L.F., Hamburg, S.P., Koide, R., Mooney, H.A. & Vitousek, P.M. 1990. Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. *Ecology* 71: 478-491.
- Jacobs, B.F., Kingston, J. & Jacobs, L.L. 1999. Origin of grassdominated ecosystems. Annals of the Missouri Botanical Garden 86: 590-643.
- Johnson, B.E. & Cushman, J.H. 2007. Influence of a large herbivore reintroduction on plant invasions and community composition in a California grassland. *Conservation Biology* 21: 515-526.
- Lenz, T.I., Moyle-Croft, J.L. & Facelli, J.M. 2003. Direct and indirect effects of exotic annual grasses on species composition of South Australian grassland. *Austral Ecol*ogy 28: 23-32.
- Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D. & Wardle, D.A. 2001. Biodiversity

and ecosystem functioning: current knowledge and future challenges. *Science* 294: 804-808.

- Maron, J.L. & Jefferies, R.L. 2001. Restoring enriched grasslands: Effects of mowing on species richness, productivity, and nitrogen retention. *Ecological Applications* 11: 1088-1100
- Marrs, R.H. 1985. Techniques for reducing soil fertility for nature conservation purposes: A review in relation to research at Roper's Heath, Suffolk, England. *Biological Conservation* 34: 307-332.
- Marrs, R.H. 2002. Manipulating the chemical environment of the soil. In: Perrow, M.R. & Davy, A.J. (eds.) *Handbook* of restoration ecology: 2. Restoration in practice, pp. 155-183. Cambridge University Press, Cambridge, UK.
- Marty, J.T., Collinge, S.K. & Rice, K.J. 2005. Responses of a remnant California native bunchgrass population to grazing, burning and climatic variation. *Plant Ecology* 181: 101-112.
- Moyes, A.B., Witter, M.S. & Gamon, J.A. 2005. Restoration of native perennials in a California annual grassland after prescribed spring burning and solarization. *Restoration Ecology* 13: 659-666.
- Opperman, J.J. & Merenlender, A.M. 2000. Deer herbivory as an ecological constraint to restoration of degraded riparian corridors. *Restoration Ecology* 8: 41-47.
- Pakeman, R.J. & Small, J.L. 2005. The role of the seed bank, seed rain and the timing of disturbance in gap regeneration. *Journal of Vegetation Science* 16: 121-130.
- Patzelt, A., Wild, U. & Pfadenhauer, J. 2001. Restoration of wet fen meadow by topsoil removal: vegetation development and germination biology of fen species. *Restoration Ecology* 9: 127-136.
- Peeters, A. & Janssens, F. 1998. Species-rich grasslands: diagnostic, restoration and use in intensive livestock production systems. *Grassland Science in Europe* 3: 375-393.
- Prober, S.M. & Thiele, K.R. 2005. Restoring Australia's temperate grasslands and grassy woodlands: integrating function and diversity. *Ecological Management & Restoration* 6: 16-27.
- Reever Morghan, K.J., & Rice, K.J.. 2005. *Centaurea solstitialis* invasion success is influenced by *Nassella pulchra* size. *Restoration Ecology* 13: 524-528.
- Reever Morghan, K.J. & Seastedt, T.R. 1999. Effects of soil nitrogen reduction on nonnative plants in restored grasslands. *Restoration Ecology* 7: 51–55.
- Seabloom, E.W., Harpole, W.S., Reichman, O.J. & Tilman, D. 2003. Invasion, competitive dominance, and resource use by exotic and native California grassland species. *Proceedings of the National Academy of Sciences of the* United States of America 100: 13384-13389.
- Sokal, R.R. & Rohlf, F.J. 1998. Biometry: the principles and practice of statistics in biological research. 3rd. ed. Freeman, New York, NY, US.
- Steenwerth, K.L., Jackson, L.E., Calderon, F.J., Stromberg, M.R. & Scow, K.M. 2002. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. *Soil Biology and Biochemistry* 34: 1599-1611.
- Stromberg, M.R. & Griffin, J.R. 1996. Long-term patterns

in coastal California grasslands in relation to cultivation, gophers, and grazing. *Ecological Applications* 6: 1189-1211.

- Stromberg, M.R., Kephart, P. & Yadon, V. 2001. Composition, invasibility, and diversity in coastal California grasslands. *Madroño* 48: 236-252.
- Suding, K.N., Gross, K.L. & Houseman, G.R. 2004. Alternative states and positive feedbacks in restoration ecology. *Trends in Ecology and Evolution* 19: 46-53.
- Török, K., Szili-Kovàcs, T., Halassy, M., Toth, T., Hayek, Zs. & Paschke, M.K. 2000. Immobilization of soil nitrogen as a possible method for the restoration of sandy grassland. *Applied Vegetation Science* 3: 7-14.
- Verhagen, R., Klooker, J., Bakker, J.P. & van Diggelen, R. 2001. Restoration success of low-production plant communities

on former agricultural soils after topsoil removal. *Applied Vegetation Science* 4: 75-82.

- Walker, K.J., Warman, E.A., Bhogal, A., Cross, R.B., Pywell, R.F., Meek, W.R., Chambers, B.J. & Pakeman, R.J. 2007. Recreation of lowland heathland on ex-arable land: assessing the limiting processes on two sites with contrasting soil fertility and pH. *Journal of Applied Ecology* 44: 573-582.
- Whisenant, S.G., Thurow, T.L. & Maranz, S.J. 1995. Initiating autogenic restoration on shallow semiarid sites. *Restoration Ecology* 3: 61-67.
- Wilson, S.D. 2002. Prairies. In: Perrow, M.R. & Davy, A.J. (eds.) Handbook of restoration ecology: 2. Restoration in practice, pp. 443-465. Cambridge University Press, Cambridge, UK.
- Wilson, S.D. & Gerry, A.K. 1995. Strategies for mixed-grass prairie restoration: herbicide, tilling, and nitrogen manipulation. *Restoration Ecology* 3: 290-298.

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### RESEARCH ARTICLE

# Limiting processes for perennial plant reintroduction to restore dry grasslands

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In restored grasslands of southern Europe, perennial plants remain highly underrepresented compared with the reference ecosystems. We tested various treatments to reintroduce common perennial plant species (*Brachypodium retusum*, Poaceae, and *Thymus vulgaris*, Lamiaceae), which are usually not or poorly reintroduced via soil and hay transfer. Treatments included microenvironmental manipulations (rock cover and plant interactions) and two grazing intensities. Target perennial species were transplanted in 2002 in the reference grassland ecosystem (intact grassland area used as a control) and in two abandoned fields. Survival was assessed in June 2003 and June 2004. Target species shoot and root biomass were measured in June 2004. Grazing greatly reduced the survival and biomass of both target species and its effects were reinforced by summer drought: plants that did not establish well enough during the autumn and spring did not survive summer. The restored rock cover had a mild positive effect, particularly on *B. retusum*. There were no negative or positive plant neighbor interactions in the steppe, while there was competition in both abandoned fields. Competition was particularly intense in the abandoned melon field, composed of a dense sward of annual grasses (*Bromus* sp.). In order to reintroduce perennial species to dry grasslands, the ideal combination of treatments is to exclude or reduce grazing during the first year to allow seedlings to establish and to recreate adequate microenvironmental conditions. Reducing competition from arable weeds may help but is not essential in such dry grasslands.

Key words: Brachypodium retusum, competition, grazing, Mediterranean steppe, plant interactions, rock, stone, Thymus vulgaris

#### **Implications for Practice**

- In order to reintroduce perennial species to Mediterranean dry grasslands, grazing should be excluded or reduced the first growing season after outplanting to allow seedlings to establish better. This was especially true for the studied chamaephyte *Thymus vulgaris*.
- Dense arable weed swards (*Bromus* sp.) significantly reduced the survival and growth of perennial species; the cost of removing arable weeds may however be prohibitive.
- In the Mediterranean climate, being well known for its strong interannual variations, improving microenvironmental conditions, such as restoring rock cover, may help to increase *Brachypodium retusum* (Poaceae) survival.

#### Introduction

Since the beginning of the twentieth century, both globally and in Europe, many pristine natural and seminatural grasslands have been lost or degraded by agricultural intensification: plowing, fertilization, drainage, liming, and overgrazing (White et al. 2000). Low resilience of grassland ecosystems after such disturbances can be due to (1) the lack of propagules in the seed bank and in the landscape (Perrow & Davy 2002; Römermann et al. 2005); (2) limited long-distance seed dispersal of herbaceous species (Perrow & Davy 2002; Buisson et al. 2006*a*); (3) degraded habitat quality, such as eutrophication (Fenn et al. 2010); and/or (4) competition through the presence of nontarget species like arable weeds or exotic species (D'Antonio & Meyerson 2002). Grasslands around the Mediterranean Basin suffer the same types of degradation and their resilience is particularly low (Römermann et al. 2005; Buisson et al. 2006*a*; Coiffait-Gombault et al. 2012*a*). The restoration of such grasslands is of increasing interest, particularly in southeastern France where several large-scale projects have been implemented (Coiffait-Gombault et al. 2012*b*; Dutoit et al. 2013*a*, 2013*b*; Bulot et al. 2014; Jaunatre et al. 2014*a*, 2014*b*). In these projects, soil and hay transfer have led to positive results on plant species richness and composition, but some perennial species remain highly underrepresented in restored plots as

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compared to the reference ecosystem (Coiffait-Gombault et al. 2012*b*; Jaunatre et al. 2014*a*, 2014*b*).

The aim of this study was to reintroduce two perennial plant species, Thymus vulgaris (Lamiaceae, chamaephyte) and Brachypodium retusum (Poaceae, hemicryptophyte) to degraded abandoned fields as a first step toward the restoration of Mediterranean grasslands. We chose these two perennial species because (1) they are common species in the western Mediterranean Basin, (2) they are dominant in the reference ecosystem (Buisson & Dutoit 2006), (3) they may improve habitat quality for other species (Buisson & Dutoit 2004; Coiffait-Gombault et al. 2012b) and (4) they are usually not reintroduced via soil and hay transfer (Coiffait-Gombault et al. 2011; Jaunatre et al. 2014a, 2014b). The main objective of this study was therefore to test the main limiting factors to reintroduce these perennial species by manipulating the microenvironment (rock cover) and biotic interactions (directly or via grazing).

Concerning the microenvironment treatment, we aimed to restore the original rock cover in abandoned fields to what it was in the reference grassland ecosystem before its cultivation. Rocks (>15 to 30 cm diameter) cover about 50% of the ground in the reference grassland ecosystem and had been removed for cultivation (Buisson & Dutoit 2006). Such rocks can provide physical nurse effects to plants, such as (1) enhanced soil moisture by increasing shade and thus reducing concomitant evaporation (Fowler 1988; Noy-Meir 2001; Elmarsdottir et al. 2003) or by increasing water condensation (Lahav & Steinberger 2001; Peters et al. 2008); (2) increased microbial activity under rocks due to increased soil moisture (Lahav & Steinberger 2001); (3) root protection from the heat, thus favoring perennials over annuals (Blumler 1992); (4) source of nutrients (Ugolini et al. 2001; Lopez et al. 2012); (5) wind sheltering, and (6) trapping fine sediments (Haussmann et al. 2010).

Concerning the biotic interactions, two treatments should be compared in our study: direct removal of plant neighbors and grazing. When competitive invasive or weedy plant species are found in a restoration site, their vigor and/or density has to be reduced because they may have a competitive advantage over target species (Perrow & Davy 2002; Gómez-Aparicio 2009). Some grassland restoration projects have used grazing in an effort to reduce competition of annual plant species over establishing perennials (Stahlheber & D'Antonio 2013), but this review was inconclusive overall because of site specificities. In this study, we tested whether grazing improved perennial species establishment in abandoned fields because our reference grassland ecosystem is traditionally grazed by sheep from February to June every year (Buisson & Dutoit 2006). In an effort to reduce competition of nontarget plant species, removal (cutting, mowing, herbicide application) of the aboveground biomass of standing vegetation is also used in restoration (Perrow & Davy 2002; Buisson et al. 2006b; Buisson et al. 2008). We therefore also tested the effects of hand removal of all plant neighbors in the vicinity of reintroduced perennials.

Plant interaction intensity can change with the level of abiotic constraint (Corcket et al. 2003) and plant strategies (Liancourt et al. 2005) in dry grasslands. Positive interactions between

plants are expected in harsh environments (Bertness & Callaway 1994) and may be an ecological force for ecosystem restoration (Brooker et al. 2008). In order to determine whether site agricultural history and soil fertility had an influence on perennial species establishment, we used three sites with various levels of nutrient availability: the reference grassland ecosystem (an intact grassland area, with low nutrient availability, considered as a control) and two abandoned fields (with higher fertility).

Altogether, the goal of this experiment was thus to test the effect of manipulating grazing (grazed or ungrazed), the microenvironment (rock cover), and plant neighbor interactions (neighbor removal or intact neighbors) on the survival and growth of two common Mediterranean perennial grassland species *T. vulgaris* and *B. retusum* in the reference grassland ecosystem (intact grassland area used as a control) and two abandoned fields.

#### Methods

#### Site Description

The studied area is located in the plain of La Crau, in southeastern France (43°33'E 4°50'N; 10 m a.s.l.). The reference grassland ecosystem composed of intact dry grasslands or pseudo-steppe of La Crau (phytosociological association: Asphodeletum fistulosi Molin. et Tallon-class: Stipo-Trachynietea distachyae Brullo), hereafter referred to as steppe, is species-rich with a unique set of common Mediterranean species that evolved with grazing as well as edaphic and climatic aridity (Buisson & Dutoit 2006). Parts of this area have been degraded through cultivation. The abiotic conditions of the fields are still altered 20-40 years after abandonment. In addition, the plant community is composed of arable weeds instead of steppe species although the traditional disturbance regime, grazing, was reestablished just after cultivation abandonment (Römermann et al. 2005; Sauguet 2013). Moreover, remnant intact patches of original steppe habitat have a limited role in supplying propagules to abandoned fields due to limited seed dispersal (Buisson & Dutoit 2004; Buisson et al. 2006a).

Soils (Haplic Cambisols) are less than 40 cm deep and are composed of 50% of 15–30 cm rocks (Buisson & Dutoit 2006). Typical for the Mediterranean climate, rainfall occurs mainly in autumn. Mean precipitation is 541 mm/year, varying between 350 and 800 mm depending on the year, and mean annual temperature is  $15.3^{\circ}$ C (data from 1997 to 2006; Wolff et al. 2013). Mean temperatures in 2003 were only slightly higher than during the previous year ( $15.7 \pm 0.4^{\circ}$ C in 2003 vs.  $15.4 \pm 0.3^{\circ}$ C in 2002). However, June temperatures were much higher in 2003 ( $T_{\text{mean}}$  in 2003:  $25.5 \pm 0.4^{\circ}$ C vs.  $22.5 \pm 0.5^{\circ}$ C from 2002 to 2004). The area received 683 mm of rainfall in 2003, which was less than that in 2002 (763 mm) (Istres weather station, 14 km from the study area). Shifting sheep flocks have been grazing the steppe and the cultivated sites before and after cultivation, from February to June (stocking rate of 4.6 animals ha yr<sup>-1</sup>).

The first study site was a remnant patch of the reference grassland ecosystem or intact steppe (35 ha), representative of the remaining 10,500 ha, used as a control. The composition

of the intact steppe plant community was assessed by visually estimating all species % cover in forty-eight  $40 \text{ cm} \times 40 \text{ cm}$  quadrats. It was composed of 44 species and was dominated by the perennial grass *Brachypodium retusum* (20% cover) as well as *Dactylis glomerata* (6% cover), *Aegilops ovata* (5% cover), *Bellis sylvestris* (5% cover), and *Euphorbia cyparissias* (5% cover) (Buisson 2005; Table S1, Supporting Information). Plant community biomass and mean plant height were 179.6 g/m<sup>2</sup> and 3 cm, respectively.

The second site (a 12 ha abandoned cereal field) was intact steppe until 1960, cultivated with cereals between 1960 and 1966 and then abandoned. The disturbance involved chisel plowing, and some rock removal. The plant community (assessed in forty-eight 40 cm × 40 cm quadrats) at the time of this experiment, 38 years after cultivation abandonment, was composed of 49 species and was dominated by the annual grass *A. ovata* (29%) and the annual forb *Trifolium subterraneum* (11%) (Buisson 2005; Table S1). Plant community biomass and mean plant height were 178.8 g/m<sup>2</sup> and 2 cm, respectively. Soil samples taken in this site had significantly higher total N (0.22 mg/g of soil) than the two other sites (0.17 mg/g of soil) (Buisson 2005; Table S2).

The third site (a 5 ha abandoned melon field) was intact steppe until 1971, cultivated with melons between 1971 and 1975 and then abandoned. The disturbance involved moldboard plowing, watering, fertilizing, and rock removal (see Römermann et al. 2005 and Buisson & Dutoit 2004 for site history). The plant community (assessed in forty-eight  $40 \text{ cm} \times 40 \text{ cm}$ quadrats) at the time of this experiment, 29 years after cultivation abandonment was composed of 25 species, mainly annual grasses, such as Bromus sp. (26% cover), Avena barbata (10% cover), and Vulpia ciliata (6% cover) (Buisson 2005; Table S1). Plant community biomass and mean plant height were 112.8 g/m<sup>2</sup> and 4.8 cm, respectively. Soil concentrations of  $P_2O_5$ and  $K_2O$  were significantly higher in this site (2.3 mg/100 g of soil and 17.3 mg/100 g of soil, respectively) than in the cereal field (1.3 mg/100 g of soil and 13.9 mg/100 g of soil, respectively) and the steppe (0.6 mg/100 g of soil and 9.5 mg/100 g of soil, respectively) (Buisson 2005; Table S2).

#### Experimental Design

*Brachypodium retusum* seeds were collected in June 2001 and *Thymus vulgaris* seeds were purchased locally. Seedlings were grown outdoors, in individual containers for 9 months, in 2/3 sand—1/3 soil from each site (steppe, abandoned cereal field, or abandoned melon field depending on where seedling were to be planted), watered as needed and not fertilized.

We used a split–split plot design where we manipulated grazing, rock cover, and competition (Fig. 1). At each site, we randomly set 12 experimental units in autumn 2002. Each unit was composed of two  $3 \text{ m} \times 1.5 \text{ m}$  plots. These two plots were located 3 m away from each other to avoid edge effects: one plot was fenced (ungrazed treatment) and the other was not fenced (grazed treatment). In the steppe, the effect of the presence of rocks was tested by removing rocks (>15 cm diameter) from



Figure 1. Experimental design layout. Each site had 12 experimental units composed of two 3 m  $\times$  1.5 m plots, one fenced plot (ungrazed treatment), and one nonfenced plot (grazed treatment). Each plot was split in to two 1.5 m  $\times$  1.5 m subplots allocated to low rock cover (white) or 50% rock cover (shaded). Two *Thymus vulgaris* (stars) and two *Brachypodium retusum* (triangles) were planted in each subplot. Neighbor removal (dashed circle) was applied around one randomly chosen individual of each species.

one of each of the 1.5 m × 1.5 m subplots within a grazed treatment as well as within the ungrazed treatment. Conversely, in the abandoned fields, the effect of the presence of rocks was tested by restoring the 50% rock cover in one of each of the  $1.5 \,\mathrm{m} \times 1.5 \,\mathrm{m}$  subplots within a grazed treatment as well as within the ungrazed treatment. In October 2002, we transplanted two T. vulgaris and two B. retusum seedlings (randomly chosen among the seedlings grown on the soil corresponding to each planting site) in each subplot. The seedlings were watered only once when outplanted as plugs. To test the effect of the plant community (neighbors) on planted seedling, we hand-pulled all small seedlings and clipped all larger plants to the ground within a 25-cm diameter area surrounding one randomly chosen individual of each plant species in a subplot (n = 12 individuals/species/treatment combination × 3 sites; total number of plants of each species = 288). We removed neighbors in fall 2002, in spring 2003 and winter 2003-2004. Only seedlings that died within the first month were replaced.

#### Monitoring

Plant survival monitoring was carried out over a few contiguous days (1) in June 2003 and (2) in June 2004 (Fig. 2). In June 2004, we also collected shoot and root biomass of all outplanted *T. vulgaris* and *B. retusum* plants by sampling the same volume of soil (approximately 2 L) around each plant. Shoot and root were washed, dried (70°C) to a constant mass, and weighted.

#### **Statistical Analyses**

Analyses were performed using the program R (R Core Team 2015) and made use of the "coin" R package (Hothorn et al.



Figure 2. Plant survival monitoring was carried out over a few contiguous days (1) in June 2003, after one growing season and one grazing cycle, and before the 2003 summer drought; and (2) in June 2004, after two growing seasons, two grazing cycles and the particularly dry summer 2003 (Gobron et al. 2005). Planting occurred in October 2002.

2008). Permutation tests were used to analyze both survival and growth data. These tests (which are also called exact tests) are a modern and powerful alternative to tests based on statistics tables for which the assumptions are rarely verified in ecology (e.g. Mestre et al. 2013). Permutation tests are a type of statistical significance tests in which the population distribution is obtained by calculating the sample statistics under every possible permutation of the observed data points, and such tests are appropriate for small sample sizes.

For each species and for each site, the effects of the three treatments (starting by the interactions of the three treatments, then, if not significant by interactions of two treatments, and if not significant by treatments separately) on survival rate in 2003, survival rate in 2004, and shoot and root biomass were analyzed using permutation tests (Hothorn et al. 2008). When permutation tests were significant, multiple comparison tests were run and *p*-values recalculated with the Benjamini–Hochberg adjustment (Benjamini & Hochberg, 1995). The biomass data were unbalanced due to mortality, but the "coin" package does not require balanced designs (Hothorn et al. 2008).

#### Results

#### Transplant Survival in 2003

In 2003, survival of both species was relatively high, although that of *Brachypodium retusum* (84.7%) was lower than that of *Thymus vulgaris* (92.7%). Considered together, both species survival was significantly higher in the steppe (93.2%) than in the two other sites (melon field = 85.9%, cereal field = 87.0%).

The two species showed different survival patterns in the steppe: while treatments did not influence *B. retusum* (overall survival 92.7%), *T. vulgaris* had a higher survival rate either when not grazed or if grazed when neighbors were left intact (98.6 vs. 79.2%; Table 1).

The two species also showed different survival patterns in the former cultivated fields. In the melon field, treatments did not influence *B. retusum* (overall survival 83.3%), whereas grazing with restored rock cover reduced *T. vulgaris* survival (79.2 vs. 88.2%; Table 1). In the cereal field, treatment did not influence *T. vulgaris* (overall survival 95.8%), whereas grazing reduced *B. retusum* survival (68.8 vs. 87.5%; Table 1).

	B. retusum	T. vulgaris
June 2003		
Steppe	n.s.	Grazing × Stones × Neighbors
		$\max T = 4.1, p = 0.017$
Cereal field	Grazing	n.s.
	z = -2.2, p = 0.046	
Melon field	n.s.	Grazing × Stones
		$\max T = 3.1, n = 0.015$
		inali en,p elete
June 2004		
Steppe	n.s.	Grazing
~FF-		z = -24 $p = 0.032$
Cereal field	Grazing × Stones ×	z = 2.1, p = 0.052
Cerear neia	Naighborg	Naighborg
	T = 2 (m = 0.011)	$T = \frac{1}{2} \sum_{i=1}^{n} $
	$\max I = 3.6, p = 0.011$	$\max I = 5.5, p < 0.001$
Melon field	Grazing × Stones	$Grazing \times Stones \times$
	$\max T = 2.6, p = 0.042$	Neighbors
	Grazing × Neighbors	$\max T = 3.2, p = 0.008$
	$\max T = 2.6, p = 0.049$	
	Stones × Neighbors	
	max T = 3.1 n = 0.016	
	max r = 5.1, p = 0.010	

#### **Transplant Survival in 2004**

In 2004, both species survival was low, although that of *B. retusum* (22.6%) was slightly lower than that of *T. vulgaris* (28.1%). Considered together, both species survival was significantly higher in the melon field (48.4%), intermediate in the cereal field (18.8%), and lower in the steppe (8.9%).

The two species showed different survival patterns in the steppe: while treatments did not influence *B. retusum* (overall survival 8.3%), grazing had a tremendous negative impact on *T. vulgaris* (2.1% vs. 16.7%; Table 1, Fig. 3).

Although the two species showed different survival patterns in the fields, they both were negatively impacted by grazing (e.g. in the melon field 25% of grazed *T. vulgaris* survived vs. 89.6% when not grazed). While rock cover restoration had significantly positive effects on *B. retusum* survival whatever the fields or the grazing treatment (e.g. in the cereal field 35.4% with rocks vs. 4.2% without rock; Fig. 3), it only had significantly positive effects on *T. vulgaris* survival when grazing was applied (in the melon field 33.3 vs. 16.1%; Table 1, Fig. 3). Plant neighbors mainly had a significant negative effect on *B. retusum* survival in the melon field (29.2% survived with neighbors vs. 50% without neighbors). In the melon field, rocks and neighbors significantly increased *T. vulgaris* survival when grazed, whereas they significantly decreased it when not grazed (Table 1, Fig. 3).

#### **Transplant Biomass**

Overall, *B. retusum* root biomass was much higher than that of *T.* vulgaris (*B. retusum* = 533.4 g and *T. vulgaris* = 2.1 g), whereas *T. vulgaris* shoot biomass was higher than that of *B. retusum* (*B. retusum* = 1.5 g and *T. vulgaris* = 11.0 g). Root and shoot



Figure 3. Mean (+SE) percent survival of transplanted *Brachypodium retusum* and *Thymus vulgaris* at each site in June 2004. Low R, rocks removed in the steppe or rocks intact in the fields; R, rocks intact in the steppe or rock cover restored in the fields; Ni, neighbors intact; Nr, neighbor removal. No bar for a particular treatment (e.g. *B. retusum*, in the steppe, grazed, low rock cover, with plant neighbors) means that all plants were dead 1.5 years after planting (0% survival; see Table 1 for all permutation test results). As we could not show the results of multiple comparison tests for all permutation tests, the tests selected to be presented are written above each of the six graphs (G, grazing; N, neighbors; n.s. = nonsignificant permutation tests; R, rocks). Within each of the graphs, bars showing similar letters did not differ significantly according to multiple comparison tests with Benjamini–Hochberg *p* adjustment. Treatments tested together were linked to common letters by plain lines.

biomass of both species were lower in the steppe than in the two fields. The potential effects of treatments on plant growth could not be analyzed because of low survival: for both species in the steppe and for *T. vulgaris* in the cereal field (Table 2).

Grazing had a negative effect on both species growth at all sites, but *T. vulgaris* was more sensitive to grazing than *B. retusum* (Fig. 4). Grazing significantly reduced *B. retusum* shoot and root biomass in the cereal field (e.g. root biomass = 370.7 g when grazed vs. 766.0 g when not grazed), whereas it significantly reduced *T. vulgaris* shoot and root biomass in the melon field (e.g. shoot biomass = 0.7 g when grazed vs. 13.0 g when not grazed) (Table 2; Fig. 4).

High rock cover had a positive effect on *B. retusum* growth only, stimulating both root and shoot biomass in the cereal field (e.g. root biomass = 327.4 g with high rock cover vs. 70.8 g with low rock cover) (Table 2; Fig. 4).

When significant, the effects of neighbors were negative suggesting the occurrence of competition. *Brachypodium retusum* shoot and root biomass were stimulated when plant neighbors were removed in cereal field, whereas both *B. retusum* (shoot biomass) and *T. vulgaris* (shoot and root biomass) were stimulated when plant neighbor were removed in the melon field (Table 2). **Table 2.** Results of the permutation tests showing the effects of the treatments on the shoot and root biomass of transplanted *Brachypodium retusum* and *Thymus vulgaris* at each site in June 2004. For abbreviations and further explanation, see caption of Table 1.

	B. retusum	T. vulgaris
Shoot Biomass		
Steppe	n.s.	n.s.
Cereal field	Grazing $\times$ Stones $\times$ Neighbors max $T = 2.84$ , $p = 0.03$	n.s.
Melon field	Neighbors $z = -2.04, p = 0.032$	Grazing × Stones max $T = 2.79$ , $p = 0.023$ Grazing × Neighbors max $T = 3.69$ , $p < 0.001$
Root biomass		· 1
Steppe	n.s.	n.s.
Cereal field	Grazing $\times$ Stones $\times$ Neighbors max $T = 2.96$ , $p = 0.027$	n.s.
Melon field	n.s.	Grazing $\times$ Stones max $T = 3.06$ , $p = 0.005$ Grazing $\times$ Neighbors max $T = 3.63$ , $p < 0.001$

#### Discussion

Establishment and survival in Mediterranean dry grasslands is a challenge even for stress-tolerant species: during the 2003



Figure 4. Mean (+SE) shoot biomass of transplanted *Brachypodium retusum* and *Thymus vulgaris* in abandoned fields in June 2004. low R, low rock cover; R, rock cover ~50%; Ni, neighbors intact; Nr, neighbor removal. No bar for a particular treatment (e.g. *T. vulgaris*, in the cereal field, grazed) means that all plants were dead 1.5 years after planting and no biomass could be weighed (see Table 2 for all permutation test results). For abbreviations and further explanation, see caption of Fig. 3.

summer drought, even some large greater than 10-year-old well-established *Thymus vulgaris* did not survive (E. Buisson 2003, IMBE—Université d'Avignon, personal observation). The Mediterranean climate is well known for its strong interannual variations and recruitment of perennial species likely occurs only under periodically favorable climatic conditions (i.e. in wetter/cooler years). The fact that this study was carried out over the particularly dry summer 2003 (Gobron et al. 2005) explains why so many plants died. Hence our plant biomass data can only be interpreted in the light of these high levels of mortality.

#### **Differences Between Sites**

In 2003, after the first grazing cycle and before the first summer drought, plant survival was slightly higher in the intact steppe than in the two other sites. This may be due to the fact that the intact steppe is composed at 20% by the perennial grass *Brachypodium retusum*, which is less palatable than the annual grasses and forbs of both abandoned fields. The sheep might actually eat less in the intact steppe although it was grazed by the same flock at the same pace. In 2004, after two growing seasons, two grazing cycles, and a particularly hot and dry 2003 summer (Gobron et al. 2005), root and shoot biomasses were lower in the steppe as a consequence of a poorer establishment probably due to lower soil nutrient availability (Römermann et al. 2005). This led to a final lower survival at this site and a higher survival in the fields.

#### Effects of Treatments in the Two Target Species

Grazing had a negative effect on survival and biomass of both species. This negative effect was lower on *B. retusum* 

than on *T. vulgaris*. While the latter produces unpleasant secondary metabolites, *B. retusum* combines low palatability and digestibility (Cingolani et al. 2005), with high root production and tolerance to defoliation (i.e. hemicryptophyte with meristems at the soil surface; Briske 1996) so that plants of *B. retusum* were probably not harmed as much by the animals as plants of *T. vulgaris*. In the melon field, grazing was shown to have a negative effect on survival and on both shoot and root biomass of *T. vulgaris*. In this particular case, rocks and neighbors increased survival probably because (1) rocks partly protected plants against livestock grazing and (2) because the cover of the plant community in the melon field was composed of more than 40% of highly palatable annual grasses, which may have diluted the uptake on *T. vulgaris*.

In this study, in most cases, rocks had a positive effect on B. retusum survival and growth. Although we did not measure environmental parameters around and under rocks, Bourrelly (1984) showed that rocks create a microclimate and allow root biomass development in the steppe of La Crau. We can hypothesize that rocks allow B. retusum to protect roots from extreme heat during summer or/and to produce more roots (Ingelmo et al. 1994), which usually allow species to better cope with summer drought (Julander 1945). This would explain why rock nurse effect on survival of B. retusum was observed only after the first summer drought. Better root growth can be due to increased soil moisture (Fowler 1988; Lahav & Steinberger 2001; Noy-Meir 2001; Elmarsdottir et al. 2003; Peters et al. 2008). In addition, rocks might protect seedlings from total predation. Although sheep can turn pebbles over by kicking them to better reach their favorite plant food, they cannot displace larger rocks (E. Buisson 2003, IMBE-Université d'Avignon,

personal observation); this type of feeding behavior is common and has been observed in other rangelands (M. Meuret 2015, INRA, personal communication).

In the steppe, neighboring plants actually had a positive effect on *T. vulgaris* survival in 2003 when grazing was applied. Although 20% of the cover of plant community of the steppe is composed by *B. retusum*, more than 23% of it is composed of highly palatable species, such as *Dactylis glomerata* (5.6%), and annual species, such as *Brachypodium distachyon* (7.7%), *Bellis sylvestris* (5.1%), and *Aegilops ovata* (4.9%), which have been the sheep's main source of food (Meuret et al. 2013). As sheep are able to feed very selectively, the chamaephyte *T. vulgaris* was less preferred by the sheep and hence indirectly favored by neighboring plants.

When not grazed, neighbors in the melon field had a negative effect on both shoot and root biomass of both target species. The plant community of the melon field was composed of a dense sward of annual grasses (e.g. Bromus sp.) boosted by high nutrient concentrations. In other Mediterranean ecosystems, annual or biennial species have also been found to compete for resources with perennial species depending on their density and the season (Coiffait-Gombault 2011). Competition can be due to better soil water uptake (Clary et al. 2004; Moyes et al. 2005), light interception (Fehmi et al. 2004; Moyes et al. 2005), or litter interference (Lenz et al. 2003). In the ungrazed cereal field, T. vulgaris survival was lower when the rock cover was restored. After abandonment, this site was colonized by various legumes, such as Trifolium subterraneum, T. stellatum, T. scabrum, Trigonella monspellica, and Medicago minima. Rocks took the space that could have been colonized by these species and may have indirectly reduced the positive effect of these low-growing nitrogen-fixing neighbors on T. vulgaris.

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#### LITERATURE CITED

- Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B Methodological 57:289–300
- Bertness MD, Callaway R (1994) Positive interaction in communities. Trends in Ecology and Evolution 9:191–193
- Blumler MA (1992) Some myths about California grasslands and grazers. Fremontia 20:22–27
- Bourrelly M (1984) Contribution à l'étude écologique de la Crau (Bouches-du Rhône): caractéristiques floristiques, dynamique annuelle, production primaire de la Crau steppique. PhD dissertation. Aix-Marseille University, Marseille, France
- Briske DD (1996) Strategies of plant survival in grazed systems: a functional interpretation. Pages 37–67. In: Hodgson J, Illius AW (eds) The ecology

and management of grazing systems. CAB International, Wallingford, United Kingdom

- Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres LA, Kunstler G, et al. (2008) Facilitation in plant communities: the past, the present, and the future. Journal of Ecology 96:18–34
- Buisson E (2005) Ecological restoration of Mediterranean grassland plant communities: southern France and coastal California (U.S.A.). PhD dissertation. Université Paul Cézanne, Aix-Marseille III, Marseille, France
- Buisson E, Anderson S, Holl KD, Corcket E, Hayes GF, Peteers A, Dutoit T (2008) Re-introducing native perennial bunchgrass *Nassella pulchra* to degraded California coastal prairies. Applied Vegetation Science 11:195–204
- Buisson E, Dutoit T (2004) Colonisation by native species of abandoned farmland adjacent to a remnant patch of Mediterranean steppe. Plant Ecology 174:371–384
- Buisson E, Dutoit T (2006) Creation of the Natural Reserve of La Crau: implications for the creation and management of protected areas. Journal of Environmental Management 80:318–326
- Buisson E, Dutoit T, Torre T, Römermann C, Poschlod P (2006a) The implications of seed rain and seed bank patterns for plant succession at the edges of abandoned fields in Mediterranean landscapes. Agriculture, Ecosystems and Environment 115:6–14
- Buisson E, Holl KD, Anderson S, Corcket E, Hayes GF, Peteers A, Dutoit T (2006b) Using grazing, topsoil removal, and plant neighbor removal to restore degraded California coastal prairies. Restoration Ecology 14:569–577
- Bulot A, Provost E, Dutoit T (2014) A comparison of different soil transfer strategies for restoring a Mediterranean steppe after a pipeline leak (La Crau plain, South-eastern France). Ecological Engineering 71:690–702
- Cingolani AM, Posse G, Collantes MB (2005) Plant functional traits, herbivore selectivity and response to sheep grazing in Patagonian steppe grasslands. Journal of Applied Ecology 42:50–59
- Clary J, Savé R, Biel C, De Herralde F (2004) Water relations in competitive interactions of Mediterranean grasses and shrubs. Annals of Applied Biology 144:149–155
- Coiffait-Gombault C (2011) Règles d'assemblages et restauration écologique des communautés végétales herbacées méditerranéennes. PhD dissertation. University of Avignon, Avignon, France
- Coiffait-Gombault C, Buisson E, Dutoit T (2011) Hay transfer promotes establishment of Mediterranean steppe vegetation on soil disturbed by pipeline construction. Restoration Ecology 19:214–222
- Coiffait-Gombault C, Buisson E, Dutoit T (2012a) Secondary plant successional trajectories in a multi-secular grazed Mediterranean steppe: impacts of pipeline construction. Acta Oecologica 43:86–94
- Coiffait-Gombault C, Buisson E, Dutoit T (2012b) Using a two-phase sowing approach in restoration: sowing foundation species to restore, and subordinate species to evaluate, restoration success. Applied Vegetation Science 15:277–289
- Corcket E, Liancourt P, Callaway RM, Michalet R (2003) The relative importance of competition for two dominant grass species, as affected by environmental manipulations in the field. Ecoscience 10:186–194
- D'Antonio C, Meyerson LA (2002) Exotic plant species as problems and solutions in ecological restoration: a synthesis. Restoration Ecology 10:703-713
- Dutoit T, Buisson E, Fadda S, Henry F, Coiffait-Gombault C, Jaunatre R, Alignan JF, Masson S, Bulot A (2013*a*) The pseudo-steppe of La Crau (south-eastern France): origin, management and restoration of a Mediterranean rangeland. Pages 287–301. In: Traba J, Morales M (eds) Steppe ecosystems: biological diversity, management and restoration. Nova Science Publishers, Hauppauge, New York
- Dutoit T, Jaunatre R, Buisson E (2013b) Mediterranean steppe restoration in France. Pages 60–64. In: Clewell A, Aronson J (eds) Ecological restoration: principles, values, and structure of an emerging profession. 2<sup>nd</sup> ed. Island Press, Washington, D.C.

- Elmarsdottir A, Aradottir AL, Trlica MJ (2003) Microsite availability and establishment of native species on degraded and reclaimed sites. Journal of Applied Ecology 40:815–823
- Fehmi JS, Rice KJ, Laca EA (2004) Radial dispersion of neighbors and the small-scale competitive impact of two annual grasses on a native perennial grass. Restoration Ecology 12:63–69
- Fenn ME, Allen EB, Weiss SB, Jovan S, Geiser LH, Tonnesen GS, et al. (2010) Nitrogen critical loads and management alternatives for N-impacted ecosystems in California. Journal of Environmental Management 91:2404–2423
- Fowler NL (1988) What is a safe site? Neighbor, litter, germination date and patch effects. Ecology 69:947–961
- Gobron N, Pinty B, Mélin F, Taberner M, Verstraete MM, Belward A, Lavergne T, Widlowski JL (2005) The state of vegetation in Europe following the 2003 drought. International Journal of Remote Sensing 26:9
- Gómez-Aparicio L (2009) The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. Journal of Ecology 97:1202–1214
- Haussmann NS, McGeoch MA, Boelhouwers JC (2010) Contrasting nurse plants and nurse rocks: the spatial distribution of seedlings of two sub-Antarctic species. Acta Oecologica 36:299–305
- Hothorn T, Hornik K, Van de Wiel MA, Zeileis A (2008) Implementing a class of permutation tests: the coin package. Journal of Statistical Software 28:1–23
- Ingelmo F, Cuadrado S, Ibafiez A, Hernandez J (1994) Hydric properties of some Spanish soils in relation to their rock fragment content: implications for runoff and vegetation. Catena 23:73–85
- Jaunatre R, Buisson E, Dutoit T (2014a) Can ecological engineering restore Mediterranean rangeland after intensive cultivation? A large-scale experiment in southern France. Ecological Engineering 64: 202–212
- Jaunatre R, Buisson E, Dutoit T (2014b) Topsoil removal improves various restoration treatments of a Mediterranean steppe (La Crau, south-eastern France). Applied Vegetation Science 17:236–245
- Julander O (1945) Drought resistance in range and pasture grasses. Plant Physiology 20:573–599
- Lahav I, Steinberger Y (2001) The contribution of stone cover to biological activity in the Negev Desert, Israel. Land Degradation and Development 12:35–43
- Lenz TI, Moyle-Croft JL, Facelli JM (2003) Direct and indirect effects of exotic annual grasses on species composition of a South Australian grassland. Austral Ecology 28:23–32
- Liancourt P, Corcket E, Michalet R (2005) Stress tolerance abilities and competitive responses in a watering and fertilization field experiment. Journal of Vegetation Science 16:713–722
- Lopez BR, Tinoco-Ojanguren C, Bacilio M, Mendoza A, Bashan Y (2012) Endophytic bacteria of the rock-dwelling cactus *Mammillaria fraileana* affect plant growth and mobilization of elements from rocks. Environmental and Experimental Botany 81:26–36
- Mestre LAM, Cochrane MA, Barlow J (2013) Long-term changes in bird communities after wildfires in the Central Brazilian Amazon. Biotropica 45:480–488
- Meuret M, Gonzalez-Pech P, Agreil C, Wolff A, Minard R (2013) L'intelligence alimentaire des brebis conduites par les bergers au printemps sur la steppe. Pages 176–191. In: Tatin L, Wolff A, Boutin J, Colliot E, Dutoit T (eds) La Crau, Ecologie et conservation d'une steppe méditerranéenne. Quae Editions, Paris, France

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- Moyes AB, Witter MS, Gamon JA (2005) Restoration of native perennials in a California annual grassland after prescribed spring burning and solarization. Restoration Ecology 13:659–666
- Noy-Meir I (2001) Ecology of wild emmer wheat in Mediterranean grasslands in Galilee. Israel Journal of Plant Sciences 49:S43–S52
- Perrow MR, Davy AJ (2002) Handbook of restoration ecology: 2. Restoration in practice. Cambridge University Press, Cambridge, United Kingdom
- Peters EM, Martorell C, Ezcurra E (2008) Nurse rocks are more important than nurse plants in determining the distribution and establishment of globose cacti (*Mammillaria*) in the Tehuacán Valley, Mexico. Journal of Arid Environment 72:593–601
- R Core Team (2015) R: A Language and Environment for Statistical http://www.R-project.org 09 July 2015
- Römermann C, Dutoit T, Poschlod P, Buisson E (2005) Influence of former cultivation on the unique Mediterranean steppe of France and consequences for conservation management. Biological Conservation 121:21–33
- Sauguet F (2013) L'élevage ovin en Crau: situation économique, enjeux et perspectives. Pages 192–205. In: Tatin L, Wolff A, Boutin J, Colliot E, Dutoit T (eds) La Crau, Ecologie et conservation d'une steppe méditerranéenne. Quae Editions, Paris, France
- Stahlheber KA, D'Antonio CM (2013) Using livestock to manage plant composition: a meta-analysis of grazing in California Mediterranean grasslands. Biological Conservation 157:300–308
- Ugolini FC, Corti G, Dufey JE, Agnelli A, Certini G (2001) Exchangeable Ca, Mg and K of rock fragments and fine earth from sandstone and siltstone derived soils and their availability to grass. Journal of Plant Nutrition and Soil Science 164:309–315
- White RP, Murray S, Rohweder M (2000) Pilot analysis of global ecosystems: grassland ecosystems. World Resources Institute, Washington D.C.
- Wolff A, Tatin L, Dutoit T (2013) La Crau, une steppe méditerranéenne unique en France? Pages 13–28. In: Tatin L, Wolff A, Boutin J, Colliot E, Dutoit T (eds) La Crau, Ecologie et conservation d'une steppe méditerranéenne. Quae Editions, Paris, France

#### **Supporting Information**

The following information may be found in the online version of this article:

**Table S1.** List of species composing the plant communities of the three sites: the steppe, the abandoned cereal field, and the abandoned melon field. The composition of the plant community was assessed by visually estimating all species % cover in one 40 cm×40 cm quadrat in each subplot (n = 48/site). The numbers in the table are the mean % cover of each species at each site in the forty-eight 40 cm×40 cm quadrats. Numbers in bold are the dominant species mentioned in the text.

**Table S2.** Soil pH, N, C, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O, CaO (n=24); soil moisture (SM) (n=12); and plant community characteristics (n=48) at the three sites: steppe, abandoned cereal field, and abandoned melon field. In June 2004, 24 soil samples were randomly collected at each site, 12 inside and 12 outside the exclosures at each site and analyzed them for pH, Kjeldahl nitrogen, C, O.M., K<sub>2</sub>O, P<sub>2</sub>O<sub>5</sub>, MgO, and CaO. Soil moisture was measured after a dry period (n=12) and after a rainy period (n=12). Soil moisture was calculated using SM = [(weight<sub>humid</sub> – weight<sub>dry</sub>) × 100]/weight<sub>humid</sub>. At plant community level, aboveground biomass was assessed at each site by cutting all plants to the ground in four 50 × 50 cm quadrats in the vicinity of each experimental unit, in April 2004 ( $12 \text{ m}^2$  in 48 quadrats for each site) dried ( $70^{\circ}$ C) to constant mass and weighted. Before cutting, mean plant height was assessed in each quadrat. Species richness was calculated from the plant community data taken in one 40 cm × 40 cm quadrat in each subplot (n=48/site).

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## Effect of Seed Source, Topsoil Removal, and Plant Neighbor Removal on Restoring California Coastal Prairies

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#### Abstract

Grasslands are hot spots of biodiversity but are now widely threatened by changes in patterns of disturbances, such as grazing and fire regimes, exotic species invasions, and cultivation. The goal of this experiment was to find the most appropriate combination of treatments to reintroduce *Danthonia californica*, a formerly dominant perennial bunchgrass, into degraded California coastal prairies. *Danthonia californica* was sown from seed and transplanted at two sites and at two grazing intensities (grazed/ungrazed) in a multifactorial experiment testing the effects of (1) local versus nonlocal seed sources; (2) topsoil removal; and (3) reduction of plant neighbors. Seed emergence was very low, suggesting that transplanting may be a better option to reintroduce *D. californica*. Although transplants grown from nonlocal seeds survived

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Introduction

Grasslands were once widespread, species-rich ecosystems, representing more than 25% of the vegetation cover of the world (Henwood 1998) and approximately 25% of California's natural vegetation (Barbour & Major 1977). Both globally (Jacobs et al. 1999) and in California (Heady et al. 1988), primary grassland cover has drastically decreased due to development, agricultural intensification, and altered disturbance regimes (Hoekstra et al. 2005); the remaining primary grasslands are fragmented and degraded. This loss and degradation of grasslands has significant impacts on the conservation of biodiversity,

<sup>5</sup> Université Bordeaux 1, UMR INRA BIOdiversité, Gènes, ECOsystèmes, Avenue des Facultés, 33405 Talence Cedex, France better initially at both sites, transplants from local seeds had higher survival after 1.5 year at one site. This suggests that short-term plant establishment studies may be misleading. Topsoil removal greatly enhanced transplant survival, and neighbor removal primarily increased transplant growth. Our results suggest that removing topsoil prior to transplanting seedlings grown from local seeds is the most promising method to reintroduce *D. californica*. However, the benefits of removing topsoil to provide safe sites for plant establishment should be weighed carefully against potential negative effects on the native seed bank and microbial communities on a sitespecific basis.

Key words: competition, *Danthonia californica*, exotic annual species, grazing, nitrogen reduction, weeding.

particularly in California where primary grasslands are important habitats for wildlife and hot spots of plant diversity (Stromberg et al. 2001).

California grasslands evolved with a number of disturbances, including intentional high-frequency burning by Native Americans, seasonal grazing by native ungulates, soil disturbance and grazing by burrowing mammals, and periodic drought stress (Heady et al. 1988). In the late eighteenth century, after European settlement, year-round, intensive cattle grazing was introduced along with numerous exotic forage grasses, and fire intervals increased. These systems are now dominated by annual exotic grasses and forbs of Mediterranean origin (Heady et al. 1988; Stromberg et al. 2001; Hayes & Holl 2003a, 2003b). Some of these ecosystems have been affected not only by changes in disturbance regimes but also by cultivation and/or native N-fixing plant invasion (Maron & Jefferies 1999), both of which lead to soil nitrogen enrichment. Such areas support even fewer native species than those that have never been cultivated or invaded (Stromberg & Griffin 1996; Hamilton et al. 2002).

California coastal prairies, which have received much less study than inland California grasslands, have been sufficiently affected by humans that their recovery is extremely slow (Hamilton et al. 2002) or even unlikely (Stromberg & Griffin 1996), and will require human

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intervention. The restoration of coastal prairies may require a combination of treatments on varying time scales because these areas evolved with several endogenous disturbances and because single-management strategies, including reintroducing fire or grazing (Bartolome & Gemmill 1981; Dyer & Rice 1997; Hatch et al. 1999), sowing native plant seeds (Wilson & Gerry 1995), or reducing soil N (Corbin & D'Antonio 2004), have not been demonstrated to be successful methods by themselves (for review, see Corbin et al. 2004).

When ecosystems have been severely degraded, Whisenant et al. (1995) proposed that restoration be initiated by enhancing soil and microenvironmental conditions and by reintroducing some species to improve habitats. We propose the reintroduction of California oatgrass (Danthonia californica) to degraded coastal prairies as a first step in restoring the habitat. Although little is known about the composition and cover of these herbaceous ecosystems before degradation, it is likely that original coastal prairies had a greater cover of native perennial grasses, such as D. californica or California hairgrass (Deschampsia cespitosa) on the coast and Purple needlegrass (Nassella pulchra) further inland. Most studies have focused on restoring N. pulchra, although a number of authors have noted the need for information on other perennial grass species (Hatch et al. 1999; Hayes & Holl 2003a).

We tested topsoil removal as a restoration technique to reduce both competition from the exotic seed bank and soil nitrogen (Peeters & Janssens 1998; Marrs 2002; Wilson 2002). Indeed, the restoration of ecosystems invaded by annual exotic grasses and forbs without reducing exotic cover often has limited success (Corbin et al. 2004). Removing the soil surface seed bank may be expected to reduce competition because most of the exotic species in California grasslands are annuals. Reduced soil N in the establishment phase, while diminishing all plant growth, should favor slower growing native species that are adapted to low nutrient conditions as compared with faster growing exotics (Huenneke et al. 1990; Corbin et al. 2004). Other methods aiming at reducing soil nutrients, such as mowing and removing the cut biomass (Maron & Jefferies 2001) or carbon amendment (sawdust, sucrose, starch, cellulose: Wilson & Gerry 1995; Reever Morghan & Seastedt 1999; Alpert & Maron 2000; Török et al. 2000; Corbin & D'Antonio 2004), have shown limited positive effects on native species richness or biomass (Wilson 2002; Corbin et al. 2004). Moreover, topsoil removal has been shown to provide habitat for the endangered Ohlone Tiger Beetle (Cicindela ohlone), which is only found in coastal prairie (Knisley & Arnold 2004), but may also alter hydrology, soil texture, and microbial communities.

We propose an additional treatment, neighbor removal (mainly exotic annuals), to reduce exotic plants because reducing the seed bank may not be sufficient to overcome the competitive advantage of exotic annuals. Previous studies have shown that weeding (Dyer & Rice 1997) and late winter application of broad-leaved herbicide (Stromberg & Kephart 1996) early in the restoration can reduce exotic cover. A variety of other approaches have been proposed to reduce exotic cover and provide native seeds with suitable safe sites to germinate, including burning (Menke 1992; Dyer et al. 1996), or combining summer burning and intensive short-duration grazing or mowing in early spring (Menke 1992; Stromberg & Kephart 1996). Although these approaches frequently reduce cover of exotic species (Corbin et al. 2004), some studies show that they have limited value for prairie restoration (Dyer et al. 1996; Hatch et al. 1999), and prescribed burn permits can be hard to obtain (Edwards 1992) due to air quality and fire risk concerns (ARB 2002).

A major question facing restorationists is how locally seeds should be collected (McKay et al. 2005). This is a particularly important question for grass seeds because they have high genetic variation (Knapp & Rice 1996; Wilson 2002). It is commonly proposed to use local propagules because other ecotypes may be genetically unsuited to the local context and because commercial ecotypes may reduce local genetic biodiversity (Knapp & Rice 1994). However, local propagules are often hard to obtain. Few field studies on grassland species have tested the homefield advantage hypothesis (Wilson 2002), although it has been demonstrated in some species (e.g., Deerweed [*Lotus scoparius*] in California coastal sage scrub, Montalvo & Ellstrand 2000).

The goal of this experiment was to test the efficacy of various combinations of two levels of these treatments to enhance emergence of sown seeds (seedlings) and survival and growth of planted seedlings (transplants) of *D. californica*. Treatments included two seed sources (local or nonlocal), topsoil removal (topsoil removal or intact topsoil), and two neighbor removal intensities (neighbor removal or neighbors intact). We tested all combinations of the levels of these treatments at two sites and for two grazing intensities (moderately grazed or ungrazed) because grazing has showed mixed effects on native and exotic coastal prairie vegetation cover (Hatch et al. 1999; Hayes & Holl 2003*a*; Corbin et al. 2004).

#### Methods

#### Site Description

We conducted experiments at two coastal prairies in central California near Santa Cruz: Elkhorn (near Elkhorn Slough, South of Watsonville, lat 36°52′4.3″N, long 121°44′23.8″W, 7 km from the coast) and UCSC (on the University of California Santa Cruz campus, lat 36°59′5.5″N long 122°3′0.9″W, 3 km from the coast). Coastal prairies are found at less than 1,000-m elevation and within the area influenced by coastal fog (Heady et al. 1988). At both sites, the vegetation is dominated by annual European grasses (69% cover at Elkhorn and 63% cover at UCSC), such as Brome (*Bromus* spp.), Barley (*Hordeum* spp.), Italian ryegrass (*Lolium multiflorum*) and *Vulpia* spp., and by annual European forbs (15% cover at Elkhorn and 26% cover at UCSC), such as Plantain (Plantago lanceolata) and storksbill (Erodium spp.) (Hayes & Holl 2003b). There are also patches of native perennial grasses: 9% cover at Elkhorn and 2% cover at UCSC. Both sites have slopes of less than 10% facing South and sandy loam soil greater than 1 m deep (pH 4.9; sand 60%; silt 28%; clay 17%; see Hayes & Holl 2003b for details). Pre-European vegetation is not known, and cattle have grazed the sites at least since the beginning of the nineteenth century. Elkhorn was partly cultivated before 1931, when the first aerial photograph was taken, and has been grazed since the 1950s. Hay was cultivated at UCSC from at least the early 1940s and possibly prior to this time. Cultivation ceased between 1957 and 1962. This site has been grazed with variable intensity since then, except for a few year abandonment in the early 1990s (Hayes & Holl 2003b).

We compiled weather data (precipitation, air and soil temperature, relative humidity, evapotranspiration, and duration of summer drought and of drought after the first rain in autumn) from the closest (<5 km) weather station to each site (CDWR 2005). Mean annual air temperature was approximately 13.5°C at both sites. Elkhorn received less rainfall over the study period from January 2002 to June 2004 (901 mm) than UCSC (1,314 mm), with the majority of precipitation falling between November and March. During the experiment, both sites received relatively less rainfall and had higher evapotranspiration than during the 20 previous years in average.

In 2004, we collected 24 soil samples at both sites, 12 inside and 12 outside the exclosure, half with topsoil removal and half with intact topsoil. Each sample consisted of three 10-cmdiameter, 2-cm-deep soil cores on four  $1.5 \times 1.5$ -m plots; soil was analyzed for total Kjeldahl N at the Laboratory of Ecology of Louvain, Belgium (Baize 2000), and a oneway analysis of variance (ANOVA) was run on the data.

#### Main Experimental Design

At each site, a  $52 \times 52$ -m cattle exclosure was installed in fall 1998 (Hayes & Holl 2003b). The areas outside the exclosure were grazed and inside the exclosure were mowed twice a year, once in spring and once in fall, until the start of the current study in 2002. This did not induce differences in community composition between the plots inside and outside the exclosure (Hayes & Holl 2003b).

We randomly allocated 12 plots inside (ungrazed) and 12 plots outside (grazed) the exclosure (see Fig. 1 for details). In each plot, we experimentally manipulated seed sources, topsoil, and plant interactions in a split–split plot design. Grazed plots were located in an area similar in size adjacent to the exclosure. During the experiment, cattle grazed Elkhorn at a stocking rate of six animals/ha for approximately four days at 45- to 60-day intervals from December to June and UCSC at a stocking rate of three animals/ha continuously from March to May.

Two seed sources were tested: local seeds collected in June 2002 in the hills around Santa Cruz, 40 km from Elkhorn and 15 km from UCSC, and seeds purchased from S&S Seeds, Inc. (Carpinteria, CA, U.S.A.), grown from source populations located at Fern Ridge Reservoir near Eugene, Oregon, and harvested in summer 2002, as



Figure 1. Experimental design layout. Each site had 12 paired  $3 \times 1.5$  m–plots inside and outside the exclosure, with one plot from each pair allocated to one seed source (local, nonlocal). Each plot was split in two  $1.5 \times 1.5$ –m topsoil plots: topsoil intact (white) and topsoil removal (shaded). Each subplot was split in two  $0.75 \times 1.5$ –m neighbor subplots (separated by dashed line). Four transplanted plants are indicated with four large dots. Solid squares correspond to plant neighbor removal and dashed squares to intact neighbors. Only nonlocal seeds were sown at UCSC in the remaining space.

well. Seeds were stored dry at room temperature in paper bags until used.

We removed the topsoil layer (0–10 cm: litter layer and part of the Ah horizon) in one  $1.5 \times 1.5$ -m split plot in each plot in August 2002 by tilling and then scraping (Fig. 1). Topsoil removal also eliminated neighbors and the top layer of the seed bank. In January 2003, at transplanting, 25–35% of the ground cover was recolonized by plant species, and in March 2003 ground cover was greater than 70% on split plots with topsoil removal versus 90– 100% on plots with intact topsoil.

To test the effect of plant neighbor interactions on seedlings and transplants, we allocated one half of each split plot to neighbor removal and we left neighbors intact in the other half (Fig. 1). Neighbor removal was performed by hand-pulling all small seedlings (native and exotic) and clipping all larger plants to the ground within a 25-cmdiameter area surrounding the four *Danthonia californica* transplants; we chose a 25-cm diameter because Davies et al. (1999) showed that some grassland plants responded to neighbor removal in areas greater than 15-cm diameter. We removed neighbors before sowing and transplanting, twice in spring 2003 and twice in winter 2003–2004.

#### Transplanting

In January 2003, we transplanted four *D. californica* plants into each of the 12 replicates (Fig. 1) of each seed source  $\times$ topsoil  $\times$  neighbor treatment (total of 1,536 transplants). These plants had been grown outdoors in individual containers for four months, watered as needed, and not fertilized. The four transplants were planted 50 cm apart to minimize interactions and were watered once when outplanted. Transplants that died within the first month were replaced.

#### Seeding Experimental Design

Using the same experimental design as in the main experiment described above, we seeded *D. californica* at UCSC only because there were patches of *D. californica* at Elkhorn that would have made it difficult to distinguish natural recruits from seedlings emerging from seeds we sowed. We only sowed purchased seeds because they are sorted through a machine to select seeds with embryos, whereas local seeds contained a sufficiently large number of seeds without embryos that we did not have enough apparently viable seeds for seeding experiments. In the nonlocal seed split plots, we sowed 25 purchased *D. californica* seeds, on 12 and 13 October 2002, before the first autumn rain, which occurred on 31 October 2002 (total of 2,400 seeds). Seeds were sown 6.25 cm apart in  $5 \times 5$  seed grid, and pushed into the soil to a depth about equal to their size (Laude 1949).

#### Data Collection

We recorded seedling emergence by replacing grids on 12 and 13 December 2002, 6 and 7 January 2003, and 26 and

27 February 2003, 6, 10, and 17 weeks after the first rain. All seedlings (out of 25 seeds) recorded as emerged on any of the three sampling dates and alive at the last sample date were summed in each split plot to obtain the combined emergence and survival of the seedlings over 17 weeks. We monitored transplanted D. californica survival and growth over 1.5 years, which included one complete growth season and two grazing cycles. We measured survival in March 2003, June 2003, December 2003, and June 2004, after which we collected the aboveground and belowground biomass of all the transplants by sampling the same volume of soil around each plant. We washed the roots very carefully by holding them at the base of the stem, below the soil line. We dried the aboveground and belowground biomass at 70°C to a constant mass, weighed, and calculated an average biomass for each set of four transplants.

#### Statistical Analyses

For survival and biomass data, we conducted ANOVA for split-split plot design on four separate models: one for each site  $\times$  grazing treatment combination (R Statistical Computing version 2.0.1 2005). Seed source, topsoil removal, and neighbor removal were treated as categorical explanatory variables, with seed source as the wholeplot treatment, topsoil removal the first split plot, and neighbor removal the second split plot (Fig.1). Only results of survival three months after transplanting (March 2003) and of survival and biomass 1.5 years after transplanting (June 2004) are presented here because analyses from other sampling dates were similar. Arcsine square root transformations were applied to survival data and log transformations to biomass measurements (Sokal & Rohlf 1998). Because grazing was not replicated within site, we could not evaluate grazing effects statistically. The number of seedlings that emerged in the field at UCSC was extremely low (<1%) although seed viability reached 96% (tetrazolium test). We thus could not carry out statistical analyses on emergence data.

#### Results

#### Soil Nitrogen

Overall, topsoil removal decreased soil N at both sites (topsoil removal:  $0.21 \pm 0.01$  mg N/g of soil vs. intact topsoil:  $0.25 \pm 0.01$  mg N/g of soil) although this effect was significant only on the Elkhorn grazed plots (topsoil removal:  $0.23 \pm 0.02$  mg N/g of soil vs. intact topsoil:  $0.31 \pm 0.02$  mg N/g of soil).

#### Survival Three Months after Transplanting

Topsoil removal increased three-month survival substantially in all grazed plots at UCSC (topsoil removal: 88.5% vs. intact topsoil: 72.9%; Table 1) and in intact neighbor plots

	Elkhorn								UC	CSC		
	Grazed				Ungrazed			Graze	d	Ungrazed		
	df	F	р	df	F	р	df	F	р	df	F	р
Replication	11			11			11			11		
Seed source	1	0.2	0.701	1	9.3	0.011	1	< 0.1	1.000	1	15.3	0.002
Error (whole plot)	11			11			11			11		
Topsoil removal	1	0.2	0.651	1	1.3	0.267	1	8.5	0.008	1	1.0	0.319
Seed source $\times$ topsoil	1	1.9	0.182	1	0.2	0.630	1	0.4	0.532	1	0.6	0.463
Error (subplot)	22			22			22			22		
Neighbor removal	1	3.7	0.061	1	0.3	0.616	1	< 0.1	0.889	1	0.1	0.817
Seed source $\times$ neighbor	1	0.1	0.803	1	< 0.1	0.867	1	0.2	0.676	1	0.7	0.399
Topsoil $\times$ neighbor	1	4.4	0.042	1	< 0.1	0.867	1	2.8	0.099	1	< 0.1	0.939
Seed source $\times$ topsoil $\times$ neighbor	1	< 0.1	0.933	1	0.7	0.404	1	0.7	0.404	1	1.0	0.319
Error (split subplot)	44			44			44			44		

that were grazed at Elkhorn (94.8% on topsoil removal  $\times$  intact neighbors vs. 84.4% on intact topsoil  $\times$  intact neighbors; Table 1).

Topsoil removal did not affect three-month survival in ungrazed areas (Table 1). In ungrazed areas, however, transplants grown from nonlocal seed survived slightly better than transplants grown from local seeds (Elkhorn: nonlocal seeds 99.5% vs. local seeds 95.31%; UCSC: nonlocal seeds 91.7% vs. local seeds 76.6%; Table 1). No other factors studied affected three-month survival (Table 1).

#### Survival 1.5 Years after Transplanting

Topsoil removal strongly increased *Danthonia californica* survival after 1.5 years whatever the site or grazing treatment (topsoil removed: 38.5% vs. topsoil intact: 12.1%; Table 2; Fig. 2). Neighbor removal significantly increased survival only at Elkhorn in both grazed (Table 2) and ungrazed areas (Table 2; Fig. 2) (neighbor removal: 42.7% vs. neighbor intact: 23.2%; Fig. 2). In the grazed area, once topsoil was removed, neighbor removal did not increase survival anymore (Table 2; Fig. 2).

After 1.5 year, seed source influenced survival only at Elkhorn, but contrary to earlier data, transplants grown from local seeds survived substantially better (Table 2; Fig. 2). On the grazed plots, survival of transplants grown from local seeds was 41.2% as compared with 25.5% for nonlocal seed; on the ungrazed plots, survival of transplants grown from local seeds  $\times$  neighbor removal reached 55.2% versus 31.3% on nonlocal seeds  $\times$  neighbor removal.

#### Biomass

Overall, neighbor removal greatly increased the biomass of transplants whatever the site or grazing treatment (e.g., at Elkhorn in the grazed area; neighbor removal: 1.18 g vs. neighbor intact: 0.11 g; Table 3; Fig. 3). Topsoil removal slightly increased the biomass of transplants (Table 3; Fig. 3). Seed source had a minimal effect on biomass of surviving transplants (e.g., at Elkhorn in the grazed area; local: 0.78 g vs. nonlocal: 0.52 g; Table 3; Fig. 3).

#### Discussion

Our results indicated that topsoil removal served to maximize Danthonia californica transplant survival and to slightly increase their biomass. Topsoil removal simultaneously manipulates available nutrients and biotic relations through the reduction of both the large exotic annual seed bank and the sparse perennial aboveground vegetation. The decrease in soil N may not have played a large role in our study. The fact that topsoil removal had no negative effect on D. californica biomass suggests that low N availability did not affect D. californica either because it is adapted to low nutrient availability or because our topsoil removal treatment did not remove enough N. The direct effects of available N, N dynamics, and other nutrient availability, as well as the effects of changes in hydrology, on both establishment and perennial/annual species interactions should be further investigated to understand the effects of topsoil manipulation.

In addition, topsoil removal had a slight positive effect on *D. californica* biomass, suggesting that topsoil removal mainly contributed to *D. californica* establishment by decreasing competition. This was consistent with the effect of neighbor removal, which also reduced competition, although topsoil removal was more efficient. The efficacy of topsoil removal versus an herbicide treatment (Stromberg & Kephart 1996), to lower the exotic seed bank prior to planting, should be compared, including the potential negative/positive impacts of both these restoration techniques on soil microbial communities, soil texture, and other native grasses and forbs.

Danthonia californica transplants on subplots with intact topsoil and neighbors survived three months after planting, but they grew little aboveground or belowground

	Elkhorn							UG	CSC				
	Grazed				Ungrazed			Grazed			Ungrazed		
	df	F	р	df	F	р	df	F	р	df	F	р	
Replication	11			11			11			11			
Seed source	1	8.8	0.013	1	8.7	0.013	1	0.9	0.368	1	< 0.1	0.905	
Error (whole plot)	11			11			11			11			
Topsoil removal	1	22.1	< 0.001	1	66.7	< 0.001	1	16.3	< 0.001	1	30.7	< 0.001	
Seed source $\times$ topsoil	1	0.1	0.797	1	0.3	0.567	1	1.1	0.307	1	0.2	0.674	
Error (subplot)	22			22			22			22			
Neighbor removal	1	11.9	0.001	1	23.5	< 0.001	1	0.4	0.542	1	< 0.1	1.000	
Seed source $\times$ neighbor	1	0.1	0.792	1	6.5	0.014	1	2.4	0.131	1	0.6	0.462	
Topsoil $\times$ neighbor	1	7.0	0.011	1	0.6	0.432	1	3.4	0.072	1	4.2	0.047	
Seed source $\times$ topsoil $\times$ neighbor	1	4.5	0.040	1	0.2	0.662	1	1.5	0.225	1	1.7	0.201	
Error (split subplot)	44			44			44			44			

Table 2. ANOVA of treatment effects on survival of	D. californica plants 1.	5 years after transplanting	in grazed and ungrazed areas	at two sites.
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biomass before summer and may not have been able to withstand summer drought. Past research concurred that perennial grasses cannot survive dry conditions when grown with exotic annual grasses because annuals outcompete perennials for light and perennials are thus unable to develop sufficient root biomass before summer to use deep soil moisture (Bartolome 1981; Dyer & Rice 1997, 1999). Hamilton et al. (1999) suggested that exotics and native perennial grasses compete for water at all stages. Therefore, in our study, when the annual seed bank, the main potential source of competition, was not reduced, neighbor removal has a positive effect on *D. californica*  survival. The small scale at which this project was carried out, and particularly the modest size of areas from which neighbors and topsoil were removed, did not prevent exotic annuals from rapidly recolonizing inwards from edges. Neighbor removal may thus be useful when topsoil has been removed but when there is no grazing to reduce annual biomass. If the project was carried out on a larger scale, surrounding exotic annual seeds would likely recolonize the area more slowly and neighbor removal may thus not be necessary.

Topsoil removal is a resource-intensive restoration project that has been carried out elsewhere: in Europe,



Figure 2. Survival of *D. californica* transplants after 1.5 years (see Table 2 for ANOVA results). Ti = topsoil intact, Tr = topsoil removal, Ni = neighbors intact, Nr = neighbor removal. Values are medians  $\pm$  quartiles.

	Elkhorn					UCSC							
	Grazed				Ungrazed			Grazed			Ungrazed		
	df	F	р	df	F	р	df	F	р	df	F	р	
Replication	11			11			11			11			
Seed source	1	6.1	0.031	1	4.2	0.065	1	< 0.1	0.869	1	0.4	0.520	
Error (whole plot)	11			11			11			11			
Topsoil removal	1	5.1	0.034	1	35.5	< 0.001	1	4.8	0.040	1	16.8	< 0.001	
Seed source $\times$ topsoil	1	1.0	0.336	1	1.6	0.227	1	0.1	0.728	1	0.5	0.474	
Error (subplot)	22			22			22			22			
Neighbor removal	1	65.8	< 0.001	1	67.6	< 0.001	1	32.7	< 0.001	1	9.1	0.004	
Seed source $\times$ neighbor	1	3.3	0.075	1	3.0	0.092	1	< 0.1	0.934	1	0.3	0.601	
Topsoil $\times$ neighbor	1	< 0.1	0.872	1	7.1	0.011	1	0.1	0.732	1	0.3	0.595	
Seed source $\times$ topsoil $\times$ neighbor	1	3.3	0.077	1	3.5	0.069	1	0.6	0.461	1	4.9	0.033	
Error (split subplot)	44			44			44			44			

Table 3. ANOVA of treatment effects on biomass of D. californica plants 1.5 years after transplanting in grazed and ungrazed areas at two sites.

topsoil is removed from areas covering between 2 and 30 ha for published work only (Verhagen et al. 2001; Grootjans et al. 2002). Once removed, topsoil can be sold and this income used toward restoration (Marrs 1985, 2002). As topsoil removal is an intensive restoration technique, it should be considered carefully, in highly degraded prairies and only after testing the seed bank for native seeds (Grootjans et al. 2002; Marrs 2002). Another alternative for large-scale projects would be to remove topsoil in patches instead of throughout a site or in combination with other methods for reducing rapid reinvasion of exotic seeds, such as herbiciding or carefully timed mowing. Interestingly, early survival of transplants grown from nonlocal seeds was slightly higher than that of transplants grown from local seeds for both sites. Ultimately, however, transplants grown from local seed survived better at one site, whereas there was no difference at the other site. Although the use of locally adapted seeds for this species could not be confirmed in this study, it may not be the case over its entire range. *Danthonia californica* is distributed throughout western Canada and the western United States; within California, it can be found in bioregions as varied as the North Coast, the Sierra Nevada Foothills, the High Sierra Nevada, the Central Coast, or San Francisco Bay (Hickman 1993). Although California-based



Figure 3. Biomass of *D. californica* transplants after 1.5 years (see Table 3 for ANOVA results). Ti = topsoil intact, Tr = topsoil removal, Ni = neighbors intact, Nr = neighbor removal. Values are medians  $\pm$  quartiles.

seed companies often grow plants from California, seeds may be locally adapted at much smaller scales. *Nassella pulchra* has been shown to be locally adapted across a coastal to interior climatic gradient (Knapp & Dyer 1998). Our purchased seeds came from southern Oregon, a location with a generally less severe summer drought than the central coast of California. This result suggests that studies on plant establishment that are carried out on short periods may be misleading.

Seedling emergence in the field was very low, and seeding D. californica may not be the most appropriate restoration technique. Our results should be treated tentatively because we only have emergence data from one site and the year of the experiment was drier than average. Studies of other native perennial California grasses showed that they could be restored by seeding (Seabloom et al. 2003); even in cases with low establishment (<1%), some grass species, such as N. pulchra (Dyer et al. 1996), could be seeded in large quantity because they are affordable (\$79/ kg; Pacific Coast Seed 2005) to produce densities of surviving grasses similar to those achieved by more costly transplanting seedlings. However, ours was one of the few studies of reintroducing D. californica, which seemed to have much lower emergence than N. pulchra and for which seeds are much more expensive (\$331/kg; Pacific Coast Seed 2005). Therefore, transplanting D. californica may be a better option, but additional work on the emergence requirements of this species is needed.

#### **Implications for Practice**

This study suggests that restoring *D. californica* to highly degraded California coastal prairie will be challenging. The ideal combination of treatments to plant *D. californica* is

- to remove topsoil from degraded prairies to provide a suitable establishment environment for *D. californica* seedlings
- to transplant D. californica
- to collect seeds locally because it may help to increase survival, although more field research is needed
- to remove neighbors to increase survival and ensure maximum growth, if necessary

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#### LITERATURE CITED

- Alpert, P., and J. L. Maron. 2000. Carbon addition as a countermeasure against biological invasion by plants. Biological Invasions 2:33–40.
- ARB (Air Resources Board). 2002. Smoke management guidelines for agricultural and prescribed burning. Air Resources Board. URL http://www.arb.ca.gov/smp/regs/regs.htm [accessed 24 September 2006].

Baize, D. 2000. Guide des analyses en pédologie. INRA, Paris, France.

- Barbour, M. G., and J. Major. 1977. Terrestrial vegetation of California. Wiley, New York.
- Bartolome, J. W. 1981. Stipa pulchra, a survivor from the pristine past. Fremontia 9:3–6.
- Bartolome, J., and B. Gemmill. 1981. The ecological status of *Stipa pulchra* (Poaceae) in California. Madroño 28:172–184.
- CDWR (California Department of Water Resources). 2005. Weather Data and Products. California Department of Water Resources. URL http://www.ipm.ucdavis.edu/WEATHER/wxretrieve.html [accessed 24 September 2006].
- Corbin, J. D., and C. M. D'Antonio. 2004. Can carbon addition increase competitiveness of native grasses? A case study from California. Restoration Ecology 12:36–43.
- Corbin, J. D., C. M. D'Antonio, and S. Bainbridge. 2004. Tipping the balance in the restoration of native plants: experimental approaches to changing the exotic:native ratio in California grassland. Pages 154–179 in M. Gordon and S. Bartol, editors. Experimental approaches to conservation biology. University of California Press, Berkeley.
- Davies, A., N. P. Dunnett, and T. Kendel. 1999. The importance of transplant size and gap with in the botanical enrichment of species-poor grasslands in Britain. Restoration Ecology 7:271–280.
- Dyer, A. R., H. C. Fossum, and J. W. Menke. 1996. Emergence and survival of *Nassella pulchra* in a California grassland. Madroño 43:316–333.
- Dyer, A. R., and J. R. Rice. 1997. Intraspecific and diffuse competition: the response of *Nassella pulchra* in a California grassland. Ecological Applications 7:484–492.
- Dyer, A. R., and J. R. Rice. 1999. Effects of competition on resource availability and growth of a California bunchgrass. Ecology 80: 2697–2716.
- Edwards, S. W. 1992. Observation on the prehistory and ecology of grazing in California. Fremontia **20:**3–9.
- Grootjans, A. P., J. P. Bakker, A. J. M. Jansen, and R. H. Kemmers. 2002. Restoration of brook valley meadows in the Netherlands. Hydrobiologia 478:149–170.
- Hamilton, J. G., J. R. Griffin, and M. R. Stromberg. 2002. Long-term population dynamics of native *Nassella* (Poaceae) bunchgrasses in Central California. Madroño 49:274–284.
- Hamilton, J. G., C. Holzapfel, and B. E. Mahall. 1999. Coexistence and interference between a native perennial grass and non-native annual grasses in California. Oecologia 121:518–526.
- Hatch, D. A., J. W. Bartolome, J. S. Fehmi, and D. S. Hillyard. 1999. Effects of burning and grazing on a coastal California grassland. Restoration Ecology 7:376–381.
- Hayes, G. F., and K. D. Holl. 2003a. Cattle grazing impacts on annual forbs and vegetation composition of mesic grasslands in California. Conservation Biology 17:1694–1702.
- Hayes, G. F., and K. D. Holl. 2003b. Site-specific responses of native and exotic species to clipping frequency, litter accumulation and soil disturbance in a mesic grassland community. Journal of Applied Vegetation Science 6:235–244.
- Heady, J. H., T. C. Foin, M. M. Hektner, M. G. Barbour, D. W. Taylor, and W. J. Barry. 1988. Coastal prairies and northern coastal scrub. Pages 733–760 in M. G. Barbour and J. Major, editors. Terrestrial vegetation of California. UC Davis and CNPS, Sacramento.
- Henwood, W. D. 1998. An overview of protected areas in the temperate grasslands biome. Parks 8:3–8.

- Hickman, J. C. 1993. The Jepson manual: higher plants of California. University of California Press, Berkeley.
- Hoekstra, J. M., T. M. Boucher, T. H. Ricketts, and C. Roberts. 2005. Confronting a biome crisis: global disparities of habitat loss and protection. Ecology Letters 8:23–29.
- Huenneke, L. F., S. P. Hamburg, and R. Koide. 1990. Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology **71**:478–491.
- Jacobs, B. F., J. Kingston, and L. L. Jacobs. 1999. Origin of grass-dominated ecosystems. Annals of the Missouri Botanical Garden 86:590–643.
- Knapp, E. E., and A. R. Dyer. 1998. When do genetic considerations require special approaches to ecological restoration? Pages 345–363 in P. L. Fiedler and P. M. Karieva, editors. Conservation biology for the coming decade. Chapman & Hall, New York.
- Knapp, E. E., and K. J. Rice. 1994. Starting from seeds genetic issues in using native grasses for restoration. Restoration and Management Notes 12:40–45.
- Knapp, E. E., and K. J. Rice. 1996. Genetic structure and gene flow in *Elymus glaucus* (blue wildrye): implications for native grassland restoration. Restoration Ecology 4:1–10.
- Knisley, C. B., and R. A. Arnold. 2004. Biology and conservation of *Cicindela ohlone* the Ohlone tiger beetle. Report of the U.S. Fish and Wildlife Service Ventura Field Office, Ventura.
- Laude, H. M. 1949. Delayed germination of California oatgrass, *Danthonia californica*. Agronomy Journal **41**:404–408.
- Maron, J. L., and R. L. Jefferies. 1999. Bush lupine mortality, altered resource availability, and alternative vegetation states. Ecology 80:443–454.
- Maron, J. L., and R. L. Jefferies. 2001. Restoring enriched grasslands: effects of mowing on species richness, productivity, and nitrogen retention. Ecological Applications 11:1088–1100.
- Marrs, R. H. 1985. Techniques for reducing soil fertility for nature conservation purposes: a review in relation to research at Roper's Heath, Suffolk, England. Biological Conservation 34:301–332.
- Marrs, R. H. 2002. Manipulating the chemical environment of the soil. Pages 155–183 in M. R. Perrow and A. J. Davy, editors. Handbook of restoration ecology: 2. Restoration in practice. Cambridge University Press, United Kingdom.
- McKay, J. K., C. E. Christian, S. Harrison, and K. J. Rice. 2005. How local is local? A review of practical and conceptual issues in the genetics of restoration. Restoration Ecology 13:432–440.
- Menke, J. W. 1992. Grazing and fire management for native perennial grass restoration in California grasslands. Fremontia 20:22–25.
- Montalvo, A. M., and N. C. Ellstrand. 2000. Transplantation of the subshrub Lotus scoparius: testing the home-site advantage hypothesis. Conservation Biology 14:1034–1045.

- Pacific Coast Seed. 2005. Pacific Coast Seed Inc. URL http://www.pcseed. com/ [accessed 24 September 2006].
- Peeters, A., and F. Janssens. 1998. Talus et prés fleuris: Mode d'emploi. Un guide pour l'aménagement écologique des espaces verts. Brochure technique n°7. Direction générale des ressources naturelles et de l'environnement, Liège, Belgium.
- R Statistical Computing version 2.0.1. 2005. The R project for statistical computing. URL http://www.r-project.org/ [accessed 24 September 2006].
- Reever Morghan, K. J., and T. R. Seastedt. 1999. Effects of soil nitrogen reduction on nonnative plants in restored grasslands. Restoration Ecology 7:51–55.
- Seabloom, E. W., W. S. Harpole, O. J. Reichman, and D. Tilman. 2003. Invasion, competitive dominance, and resource use by exotic and native California grassland species. Proceedings of the National Academy of Sciences of the United States of America 100:13384– 13389.
- Sokal, R. R., and F. J. Rohlf. 1998. Biometry: the principles and practice of statistics in biological research. 3rd edition. W. H. Freeman Company, New York.
- Stromberg, M. R., and J. R. Griffin. 1996. Long-term patterns in coastal California grasslands in relation to cultivation, gophers, and grazing. Ecological Applications 6:1189–1211.
- Stromberg, M. R., and P. Kephart. 1996. Restoring native grasses in California old fields. Restoration and Management Notes 14: 102–111.
- Stromberg, M. R., P. Kephart, and V. Yadon. 2001. Composition, invasibility, and diversity in coastal California grasslands. Madroño 48:236–252.
- Török, K., T. Szili-Kovàcs, M. Halassy, T. Toth, Zs. Hayek, and M. K. Paschke. 2000. Immobilization of soil nitrogen as a possible method for the restoration of sandy grassland. Applied Vegetation Science 3:7–14.
- Verhagen, R., J. Klooker, J. P. Bakker, and R. van Diggelen. 2001. Restoration success of low-production plant communities on former agricultural soils after topsoil removal. Applied Vegetation Science 4:75–82.
- Whisenant, S. G., T. L. Thurow, and S. J. Maranz. 1995. Initiating autogenic restoration on shallow semiarid sites. Restoration Ecology 3:61–67.
- Wilson, S. D. 2002. Prairies. Pages 443–465 in M. R. Perrow and A. J. Davy, editors. Handbook of restoration ecology: 2. Restoration in practice. Cambridge University Press, United Kingdom.
- Wilson, S. D., and A. K. Gerry. 1995. Strategies for mixed-grass prairie restoration: herbicide, tilling, and nitrogen manipulation. Restoration Ecology 3:290–298.

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# Soil phosphorus constrains biodiversity across European grasslands

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#### Abstract

Nutrient pollution presents a serious threat to biodiversity conservation. In terrestrial ecosystems, the deleterious effects of nitrogen pollution are increasingly understood and several mitigating environmental policies have been developed. Compared to nitrogen, the effects of increased phosphorus have received far less attention, although some studies have indicated that phosphorus pollution may be detrimental for biodiversity as well. On the basis of a data-set covering 501 grassland plots throughout Europe, we demonstrate that, independent of the level of atmospheric nitrogen deposition and soil acidity, plant species richness was consistently negatively related to soil phosphorus. We also identified thresholds in soil phosphorus above which biodiversity appears to remain at a constant low level. Our results indicate that nutrient management policies biased toward reducing nitrogen pollution will fail to preserve biodiversity. As soil phosphorus is known to be extremely persistent and we found no evidence for a critical threshold below which no environmental harm is expected, we suggest that agro-environmental schemes should include grasslands that are permanently free from phosphorus fertilization.

Keywords: atmospheric nitrogen deposition, environmental policy, grassland, nutrient enrichment, phosphorus

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#### Introduction

Since the beginning of the industrial revolution, anthropogenic activities have caused an unprecedented input of nitrogen (N) and phosphorus (P) in the biosphere (from 15.3 to 259 Tg N yr<sup>-1</sup> and <0.3 to 16 Tg P yr<sup>-1</sup>; Peñuelas *et al.*, 2012). Increased nitrogen flows originate primarily from the combustion of fossil fuels and the use of industrial fertilizers manufactured from airborne N<sub>2</sub> via the Haber-Bosh procedure. Increased phosphorus flows on the other hand, originate mainly

Correspondence: Tobias Ceulemans, tel. +32 16 32 15 20, fax +32 16 32 19 68, e-mail: tobias.ceulemans@bio.kuleuven.be from the application of mineral fertilizers from mined rock-reserves and the use of livestock slurry and manure (Vance *et al.*, 2002; Peñuelas *et al.*, 2012, 2013). Excess agricultural fertilizer can subsequently be redistributed into adjacent ecosystems via runoff or transport via freshwater bodies. Atmospheric deposition of volatilized nitrogenous compounds and, to a lesser extent, mineral aerosols of dust from phosphorus fertilizers, further contribute to nutrient pollution of natural ecosystems (Newman, 1995; Peñuelas *et al.*, 2013). Finally, anthropogenic changes to the soil biogeochemical conditions that affect soil phosphorus sequestration, such as sulfurous pollution, can disrupt natural phosphorus balances leading to increased soil phosphorus availability (Addiscot and Thomas 2000, Hinsinger 2001, Turner & Haygarth, 2001).

The consequences of increased nutrient flows in the biosphere have become one of the major components of global environmental change (Sala et al. 2000, Foley et al., 2005; Galloway et al., 2008). Indeed, a large number of studies identify increased nitrogen inputs, particularly via atmospheric nitrogen deposition, as a major culprit of biodiversity loss in both terrestrial and aquatic ecosystems worldwide (e.g., Stevens et al., 2004; Phoenix et al., 2006; Clark & Tilman, 2008; Conley et al., 2009; Cleland & Harpole, 2010). Particular attention has been paid to the industrialized parts of the world, as the atmospheric nitrogen deposition here may exceed more than a 10-fold of the expected natural background deposition (~5 kg N ha<sup>-1</sup> yr<sup>-1</sup>; Galloway *et al.*, 2008). To mitigate the deleterious impact of nitrogen pollution, a suite of nitrogen management policies have been developed and attempts have been made to identify critical loads of nitrogen deposition, below which there should be no adverse effects on biodiversity (Bobbink et al., 2010; Sutton et al., 2011; Payne et al., 2012).

Similar to nitrogen, there is a risk of phosphorus pollution in the industrialized countries, owing to the high agricultural application of phosphorus fertilizer, frequently associated with intensive livestock production (Reijneveld et al., 2010; Obersteiner et al., 2013). For instance, 47% of the tested agricultural soils in New York state and over 50% of agricultural soils in the Netherlands, Sweden and Belgium showed soil phosphorus levels well above the recommended ranges (Djodjic et al., 2004; BDB, 2005; Ketterings et al., 2005; Reijneveld et al., 2010). Compared to nitrogen pollution, the environmental consequences of increased phosphorus have received far less attention (Elser & Bennet, 2011). Furthermore, environmental policies aimed at mitigating phosphorus pollution have mainly targeted aquatic ecosystems (e.g. Schindler et al., 2008; Conley et al., 2009). Nevertheless, some studies have indicated that phosphorus pollution may be at least equally detrimental for biodiversity in terrestrial ecosystems, independent of the level of nitrogen pollution (Wassen et al., 2005; Ceulemans et al., 2013; Fujita et al., 2013). To develop appropriate environmental policies with respect to nutrient enrichment in terrestrial ecosystems, there is a need to disentangle the contribution of nitrogen and phosphorus pollution to biodiversity loss.

In this study, we aimed at identifying the relationships between nitrogen and phosphorus on the one hand and plant species richness in European grasslands on the other. It has been established that European grasslands are susceptible to increased nitrogen deposition (e.g., Stevens *et al.*, 2010), but owing to agricultural intensification, particularly live stock production (Reijneveld *et al.*, 2010), they may also be at risk of phosphorus enrichment. This is one of the first large scale studies that directly and simultaneously takes indicators of both nitrogen and phosphorus pollution into account when investigating species diversity. Furthermore, we tested whether the observed relationships differ between different grassland types or could be generalized. Finally, to provide tools for developing environmental policies, we aimed at identifying possible critical thresholds in soil phosphorus levels with respect to biodiversity.

#### Materials and methods

#### Data collection

We surveyed three common grassland types in Europe (314 *Nardus* grasslands in 10 European countries and 105 lowland hay meadows and 82 calcareous grasslands spread across five European countries; Fig. 1). These grasslands are often components of agricultural landscapes, providing grazing areas for cattle and sheep (Veen *et al.* 2009). Species rich examples of these three grassland communities are protected by the European Habitat Directive, the European legislative



**Fig. 1** Map of the European regions where grassland quadrats were surveyed (N = 501). Nardus grasslands: green, N = 314; lowland hay meadows: blue, N = 105 and calcareous grasslands: red, N = 82.

instrument for the conservation of wild animal and plant species and natural habitats of community importance (Nardus grasslands: Habitat type 6230, lowland hay meadows: Habitat type 6510 and calcareous grasslands, Habitat types 6210 and 2130). For the purposes of this research, we compiled a dataset consisting of two methodologically similar datasets collected to investigate the effects of nutrient pollution on species composition and richness of Nardus grasslands (Stevens et al. 2011, N = 153 and Ceulemans *et al.*, 2011, N = 132). Using the same methodology, we additionally surveyed 29 Nardus grasslands, 105 lowland hay meadows and 82 calcareous grasslands. As Nardus grasslands were subject of two previous studies with a larger time span, sampling of lowland hay meadows and calcareous grasslands did not cover the same spatial range as Nardus grasslands. Based on their high similarity in species composition, calcareous Atlantic dune grasslands were lumped with inland calcareous grasslands. Access permission for all the sites was gained through landowners and responsible agencies for protected areas. Grasslands were surveyed from late May to early August, between 2002 and 2013.

To ensure consistent habitat selection, surveyed grasslands needed to meet three main criteria. Firstly, we drew up lists of indicative plant species for all three grassland types, based on the Interpretation Manual of European Union Habitats (European Commission, 2007). We only sampled grasslands meeting the general description of the habitat type and containing at least four indicative species (Table S1). Secondly, to avoid extinction debt, we only surveyed grasslands that did not receive direct agricultural fertilizer application in the recent past. Nevertheless, nutrient enrichment of these grasslands may still occur through inflow from adjacent arable land (Nardus and calcareous grasslands on slopes), via polluted freshwater bodies (lowland hay meadows in valleys) or via redistribution by livestock. Finally, to minimize possible effects of agricultural intensification on biodiversity other than nutrient enrichment, surveyed grassland were not ploughed or reseeded and received continuous extensive management by cutting or cutting and grazing. To control for the latter two criteria, we inquired for site management history dating back at least 17 years prior to sampling. We then selected areas in Europe in a stratified manner to cover as much of the typical range of atmospheric nitrogen deposition as possible (Fig. 1). Based on information from national datasets, vegetation maps and local conservation agencies, we randomly chose grassland sites in these areas meeting the above mentioned selection criteria.

Each grassland site was surveyed for plant species presence before establishing a quadrat of 2 m by 2 m containing as much of the observed dominant and characteristic plant species of the site as possible. For a subset of the data (*Nardus* grasslands N = 153 and lowland hay meadows N = 105), between three and five randomly placed replicate quadrats within a 1-ha sampling area of the desired grassland type were placed. To give one record per site, the median species richness and mean of the environmental variables were calculated. In each quadrat, we recorded plant species richness and then took between two and ten top-soil samples (0–10 cm, on shallower soils as deep as possible) with an auger (2–5 cm diameter). These samples were then air-dried, stones and roots were removed, and the samples were thoroughly homogenized prior to soil analyses.

Soil phosphorus was determined using the Olsen-P extraction and subsequent colorimetrical analysis using the molybdenum blue method (Robertson et al., 1999). Although this method has been identified as the most suitable extraction method to assess plant available phosphorus (Gilbert et al., 2009), we also performed soil phosphorus extraction on a subset of the quadrats with anion exchange membranes and oxalic acid extraction to compare the results (N = 84 and N = 36respectively; Robertson et al., 1999). We found very high Pearson correlations across the entire pH range and similar relationships with plant species richness across all three phosphorus extraction methods (Figure S1). Although we used soil extractable phosphorus as indicator for phosphorus enrichment in this study, some of the observed variation in soil phosphorus levels may be attributed to inherent and natural differences in soil mineralogy. Nevertheless, reports of soil phosphorus levels in unfertilized or unimproved similar grassland communities vary from 4 mg P kg<sup>-1</sup> to 25 mg P kg<sup>-1</sup> (Olsen-P method; Critchley et al., 2002; Silvertown et al., 2006; Gilbert et al., 2009), and levels above 20-30 mg P kg<sup>-1</sup> are considered to be an indication of anthropogenic phosphorus enrichment (Critchley et al., 2002). It is therefore likely that most of these elevated levels of soil phosphorus in our study represent a gradient in phosphorus enrichment.

As indicator for nitrogen pollution, we obtained data on total atmospheric nitrogen deposition, consisting of wet and dry deposition of oxidized and reduced nitrogen, from the best available local estimation model. National models were used for Germany (Gauger, 2002), the Netherlands (Asman & van Jaarsveld, 2002), Great Britain (NEGTAP, 2001) and the northern part of Belgium (Flanders, VMM, 2010). For all other countries the EMEP-based IDEM models were used (Pieterse et al., 2007). The different models use similar approaches to model nitrogen deposition. For all of the models, deposition was calculated as a three-year average to provide a more robust estimate of long-term nitrogen inputs (years 2000-2002). We did not determine soil nitrogen for all quadrats, as it does not always provide a good approximation of plant available nitrogen (1 M KCl method, Robertson et al., 1999). It proved unfeasible to determine nitrogen mineralization rates, which provides a better measure, on all 501 grasslands. Nevertheless, we report the results including soil nitrogen for a subset of the data in supplementary information indicating no significant relationships with soil phosphorus or plant species richness (Table S2; N = 283).

Finally, we also determined soil acidity using a pH glass electrode in a soil-water mix that was shaken for 30 min (soil/distilled water: 1/2.5).

#### Statistical analyses

First we calculated Pearson correlations between soil phosphorus, soil pH and total nitrogen deposition to check for possible relationships between the explanatory variables. Next, as species richness is a count variable, Poisson regression models were used to investigate the relationship between soil phosphorus, soil pH and total nitrogen deposition on the one side and species richness on the other. To account for differences in geography and climate, we also included longitude and latitude of each quadrat as explanatory variables. Grassland type was used as a categorical variable to account for differences in species pools and species composition between the grassland types. We included all first order interactions between the explanatory variables in a first full factorial model. Next, the interaction factors were one by one removed to build a suite of reduced models (the fully reduced model contained the six main variables mentioned above). Out of the models whit all possible combinations of interaction factors, we selected the most parsimonious model using the Akaike Information Criterion (AIC). As grassland quadrats were sampled over a long time period, we also checked whether year of sampling may influence our analyses. We found no significant effect of year of sampling on plant species richness and including year of sampling as variable did not change any of the other relationships we found in this study (Table S3).

To identify possible critical thresholds in soil phosphorus, piecewise Poisson regression analyses were performed for each grassland type separately, with soil phosphorus as explanatory variable and species richness as response variable. In these analyses, possible thresholds were identified based on a significant nonlinear change in the response of plant species richness to soil phosphorus and were estimated by a 'breakpoint' or 'knot' connecting two regression lines (Toms & Lesperance, 2003). We built three different piecewise regression models to allow for different possible responses of species richness to soil phosphorus. In the first model, both regressions were allowed to follow a linear path according to the equations (a):

- (i) Ln(Plant species richness) =  $\beta_0 + \beta_1^*$  soilP for soilP  $\leq$  knot (intercept =  $\beta_0$ ; slope =  $\beta_1$ )
- (ii) Ln(Plant species richness) =  $(\beta_0 \beta_2^* \text{knot}) + (\beta_1 + \beta_2)^* \text{soilP}$ for soilP  $\geq$  knot (intercept =  $\beta_0 - \beta_2^* \text{knot}$ ; slope =  $\beta_1 + \beta_2$ )

in the second model, the first regression was modeled as a constant and the second regression was allowed a linear path according to the equations (b):

- (i) Ln(Plant species richness) =  $\beta_0$ for soilP  $\leq$  knot (intercept =  $\beta_0$ ; slope = 0)
- (ii) Ln(Plant species richness) =  $\beta_0 + \beta_1^*$ (soilP knot) for soilP > knot (intercept =  $\beta_0$ ; slope =  $\beta_1$ )

and in the third model the first regression was allowed a linear path and the second regression was modeled as a constant according to the equations (c):

- (i) Ln(Plant species richness) =  $\beta_0 + \beta_1^*$  soilP
- for soilP  $\geq$  knot (intercept =  $\beta_0$ ; slope =  $\beta_1$ )
- (ii) Ln(Plant species richness) =  $\beta_0 + \beta_1^*$ knot
  - for soilP  $\leq$  knot (intercept =  $\beta_0 + \beta_1^*$  knot; slope = 0)

Note that the latter two sets of equations are special cases of the first set where  $\beta_0$  equals 0 and  $-\beta_1$  respectively. All regression models calculated the results based on maximizing the

likelihood of all parameters (two possible slopes and a break point). Out of the three piecewise regression models and a single linear Poisson regression (without a breakpoint), the model that best described the data was selected based on the lowest AIC value. The SAS programs used to perform the piecewise regression analyses are included in supporting information.

#### Results

A total of 571 plant species were found in the 501 grassland quadrats. Frequent species in *Nardus* grasslands were *Agrostis capillaris*, *Danthonia decumbens*, *Nardus stricta*, *Molinia caerulea*, *Potentilla erecta*, *Succisa pratensis* and *Galium saxatile*. In lowland hay meadows frequent species were *Holcus lanatus*, *Alopecurus pratensis*, *Cynosurus cristatus*, *Cardamine pratensis* and *Sanguisorba officinalis*. Frequent species in calcareous grasslands included *Brachypodium pinnatum*, *Bromus erectus*, *Koeleria pyramidata* s.l., *Carex flacca*, *Sanguisorba minor*, *Teucrium chamaedrys*, *Ononis repens* and *Helianthemum nummularium*. Details of the environmental variables used in the Poisson regression models can be found in Table 1.

Invariably, the highest species richness occurred at lower levels of soil phosphorus. Maximum observed species richness did not exceed 20 species per 4 m<sup>2</sup> beyond 80 mg P kg<sup>-1</sup>, compared to more than 40 species below 40 mg P kg<sup>-1</sup> (Fig. 2). The Poisson regression analysis with the lowest AIC showed a highly significant negative relationship between soil phosphorus and species richness across all grassland types (Wald  $\chi 2 = 208.36$ , P < 0.0001, Table 2). This negative relationship was independent of nitrogen deposition and soil pH as we found no significant Pearson correlations between soil phosphorus and nitrogen deposition or soil pH (r = -0.02, P = 0.63; r = 0.01, P = 0.77respectively, Figure S2) and no significant interactions with soil phosphorus (Table 2). Nitrogen deposition and soil pH were also significantly negatively related to species richness, reflected by the only remaining interaction term in the model showing a gradually stronger negative relationship between nitrogen deposition and species richness in more acidic sites (Wald  $\chi^2 = 57.48$ , P < 0.0001, Table 2). In addition, we found that lowland hay meadows had significantly lower species richness than calcareous grasslands and Nardus grasslands (Wald  $\chi^2 = 83.7$ , *P* < 0.0001, Table 2). Geographical location was not significantly related to species richness (Table 2).

Piecewise regression analyses with the lowest AIC's invariably showed that the relationship between soil phosphorus and plant species richness was best described by an initial log-linear decrease in species richness until a 'threshold' in soil phosphorus has been
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**Table 1** Details of variables used in the Poisson regression models. Unit for species number is: count 4 m<sup>-2</sup>; soil phosphorus (Olsen method): mg kg<sup>-1</sup> and total nitrogen deposition: kg ha<sup>-1</sup> yr<sup>-1</sup>

	Mean	Range	Mean	Range	
Variable	All grasslands	(N = 501)	Hay meadows ( $N = 105$ )		
Species number	21.57	4–49	17.28	5–31	
Soil P	31.82	0-305.45	28.17	0-266.48	
Soil pH	5.44	3.69-8.27	5.58	4.26-7.93	
Total N deposition	18.50	2.3-43.5	21.50	10.9-27.8	
-	Calc. grassland	s (N = 82)	<i>Nardus</i> grasslands ( $N = 314$ )		
	Mean	Range	Mean	Range	
Species number	24.44	12–40	22.25	4–49	
Soil P	38.01	0.22-200.33	31.45	0-305.45	
Soil pH	6.90	4.83-8.27	5.01	3.69-7.37	
Total N deposition	18.02	10.19–25	17.6	2.3-43.5	



**Fig. 2** Relationship between soil phosphorus and plant species richness across three types of European grasslands (N = 501). Nardus grasslands: green, N = 314; lowland hay meadows: blue, N = 105 and calcareous grasslands: red, N = 82. The inset depicts the piecewise Poisson regression models with breakpoints (vertical reference lines depict the breakpoints, see Table 3).

reached, after which species richness remained at a constant level (Fig. 2). In *Nardus* grasslands, no further decreases in species richness were observed beyond 127.6 mg P kg<sup>-1</sup>, remaining at an average of 12.5 species per quadrat. In calcareous grasslands, species richness stabilized at 17.2 species beyond 104.9 mg P kg<sup>-1</sup> and in lowland hay meadows at 9.8 species beyond 124.3 mg P kg<sup>-1</sup> (Fig. 2; Table 3).

# Discussion

Our results suggest that, independent of the environmental impacts of nitrogen pollution, soil phosphorus constrains plant species richness in European grasslands. Indeed, we found no relationship between soil phosphorus on the one hand and nitrogen deposition on the other. Furthermore, at any level of nitrogen deposition or soil pH, quadrats with high soil phosphorus consistently showed lower species richness. This was not restricted to a specific grassland community, as average species richness dropped at similar rates across all three grassland types. Although we cannot infer a direct causal relationship, our observations are consistent with grassland fertilization experiments indicating long-lasting negative effects of phosphorus amendment on plant species richness and characteristic plant Table 2 Detailed results of the Poisson regression model with lowest AIC examining the relationships between soil phosphorus, soil pH, total nitrogen deposition, longitude, latitude and grassland type and plant species richness (N = 501). For interpretation of the interaction between nitrogen deposition and soil pH, estimated slopes at shifted intercepts of nitrogen deposition and soil pH are included. Shifted intercepts are: <sup>1</sup>pH<sub>observed</sub>-3, <sup>2</sup>pH<sub>observed</sub>-5, <sup>3</sup>pH<sub>observed</sub>-6.5 and <sup>a</sup>Ndep<sub>observed</sub>-10, <sup>b</sup>Ndep<sub>observed</sub>-20, <sup>c</sup>Ndep<sub>observed</sub>-30. We shifted the intercepts by subtracting 3, 5 and 6.5 respectively from all observed pH values and then recalculated the estimated slopes of total N deposition (vice versa for Ndep). This was necessary as the default intercept corresponds to pH = 0or Ndep = 0, which is ecologically senseless. Unit for species number is: ln(count) 4 m<sup>-2</sup>; soil phosphorus: mg kg<sup>-1</sup> and total nitrogen deposition: kg ha<sup>-1</sup> yr<sup>-1</sup>.  $\delta$ AIC compared with the fully reduced model without interaction factor is 56.6

		Standard		
	Estimate	Error	Wald $\chi^2$	<i>P</i> -value
Intercept	4.570	0.255	322.29	< 0.0001
Soil phosphorus	-0.004	0.000	208.36	< 0.0001
Total N deposition *Soil pH	0.012	0.002	57.48	< 0.0001
Total N deposition	-0.075	0.008	85.51	< 0.0001
Soil pH	-0.148	0.031	22.53	< 0.0001
Latitude	-0.006	0.003	3.80	0.05
Longitude	0.001	0.002	0.24	0.67
type = Lowland	-0.288	0.032	83.70	< 0.0001
hay meadows				
type = Calcareous grasslands	-0.004	0.038	0.01	0.92
type = Nardus grasslands	0	0		
Total N deposition <sup>1</sup>	-0.040	0.004	116.96	< 0.0001
Total N deposition <sup>2</sup>	-0.016	0.002	22.53	< 0.0001
Total N deposition <sup>3</sup>	0.002	0.003	0.53	0.47
Soil pH <sup>a</sup>	-0.029	0.019	2.35	0.13
Soil pH <sup>b</sup>	0.090	0.015	34.90	< 0.0001
Soil pH <sup>c</sup>	0.209	0.025	72.58	< 0.0001

species (Crawley *et al.*, 2005; Hejcman *et al.*, 2005, 2010); as well as with local observations of lower species richness in grasslands with higher phosphorus availability (Janssens *et al.*, 1998; Härdtle *et al.*, 2006; Kleijn & Müller-Schärer, 2006). Nevertheless, the results of this study do not contradict findings of negative effects of nitrogen pollution on biodiversity, particularly through high rates of atmospheric nitrogen deposition (e.g., Stevens *et al.*, 2004; Cleland & Harpole, 2010). Indeed, we found a clear negative relationship between atmospheric nitrogen deposition and plant species richness, mediated by soil pH, suggesting a gradually higher loss of plant species richness in more acidic grasslands. This supports a suite of studies demonstrating that the negative effects of nitrogen deposition on biodiversity are primarily connected with soil acidification (e.g. Horswill *et al.*, 2008; Stevens *et al.*, 2010).

According to our results, we can expect that current nutrient management policies in terrestrial ecosystems, primarily biased toward reducing nitrogen pollution, will fail to preserve biodiversity. A key policy tool for mitigating nitrogen pollution has been the critical load of nitrogen input, calculated based on fertilization experiments and observations along gradients of nitrogen pollution (Sutton et al., 2011; Payne et al., 2012). A critical load indicates the level of pollution that can be tolerated by an ecosystem without harmful effects. In this study, we aimed at identifying similar critical loads for levels of soil phosphorus. We found indications that loss of plant species richness following phosphorus pollution may only occur below certain soil phosphorus levels. Indeed, average species richness in all three grassland types remained at a constant low level in quadrats with soil phosphorus exceeding 104-130 mg  $P \text{ kg}^{-1}$  (Fig. 2, Table 3). However, these thresholds do not show a critical level below which no harm on biodiversity is to be expected, but indicate that the least harm to biodiversity can be expected when no phosphorus pollution takes place. A drawback of our dataset is the relatively poor representation of grasslands with high soil phosphorus levels. This is due to site selection criteria demanding no sites under direct fertilization and which is based on the presence of characteristic plant species that may already have been lost at these levels of soil phosphorus (e.g. Ceulemans et al., 2011). Nevertheless, combined with the general log-linear relationship between soil phosphorus and plant species richness, these thresholds suggest that the largest loss of biodiversity may already occur at small rates of phosphorus enrichment. Therefore, and consistent with recent suggestions regarding nitrogen pollution (Payne et al., 2012), it seems necessary that environmental policy decisions are based on a sliding scale of phosphorus pollution, rather than on critical loads that assume no harm below certain levels of pollution.

The strong negative relationship between soil phosphorus and plant species richness, similar across the three different grassland types, suggests particular ecological mechanisms determining this plant species loss. Loss of plant diversity following nutrient enrichment may be caused by increased productivity and subsequent competitive exclusion of species (Hautier *et al.*, 2009). However, the impact of increased productivity is likely to be weak as previous work on a subset of our grasslands showed only a small effect on species richness, and enhanced productivity following increased soil phosphorus was primarily restricted to phosphorus limited grasslands (Ceulemans *et al.*, 2013). Alternatively, it has been hypothesized that the large

**Table 3** Detailed results of the piecewise Poisson regression models with lowest AIC examining a possible threshold (knot) of soil phosphorus with respect to plant species richness per grassland type. Unit for species number is: Ln(count) 4 m<sup>-2</sup> and soil phosphorus: mg kg<sup>-1</sup>.  $\delta$ AIC compared with a single regression model without breakpoint was 4.7 for lowland hay meadows, 4.0 for calcareous grasslands and 10.2 for *Nardus* grasslands

		Standard			
	Estimate	error	t Value	<i>P</i> -value	DF
Lowland hay	y meadows				
Intercept	2.952	0.029	103.10	< 0.0001	105
Slope	-0.005	0.001	-3.70	< 0.0001	105
Knot	124.28	36.80	3.38	< 0.001	105
Calcareous g	rasslands				
Intercept	3.350	0.033	102.51	< 0.0001	82
Slope	-0.005	0.001	-6.04	< 0.0001	82
Knot	104.86	0.049	2119.52	< 0.0001	82
Nardus grass	lands				
Intercept	3.250	0.016	200.58	< 0.0001	314
Slope	-0.006	0.000	-12.37	< 0.0001	314
Knot	127.64	0.04	2898.11	< 0.0001	314

variety of chemical phosphorus compounds in the soil (complexes with Ca, Fe, Mg, Al or organic compounds) may facilitate high resource partitioning for phosphorus uptake and subsequently promote species coexistence in phosphorus poor soils (Turner, 2008; Olde Venterink, 2011). In this context, it is also noteworthy that mycotrophic species, which are able to tap into phosphorus pools otherwise unavailable for plant uptake through their mycorrhizal partner, appear susceptible to increased soil phosphorus (Ceulemans *et al.*, 2011). However, although resource partitioning has been demonstrated for uptake of nitrogen compounds (McKane *et al.*, 2002), ecologists have yet to demonstrate this with respect to phosphorus uptake.

Based on this study, it appears that both nutrients may contribute to biodiversity loss in terrestrial ecosystems. However, anthropogenic nutrient enrichment tends to fertilize the biosphere with phosphorus more locally and at a smaller rate than nitrogen (Peñuelas et al., 2012). Indeed, nitrogen pollution originates mainly from seemingly unlimited sources (fossil fuels, industrial fertilizers manufactured from airborne N2 via the Haber-Bosh procedure), whereas phosphorus pollution originates mainly from the application of fertilizer manufactured from dwindling rock-reserves (Vance et al., 2002; Peñuelas et al., 2012, 2013). Furthermore, both airborne nitrogenous compounds and soil nitrogen are more mobile than soil phosphorus compounds or mineral phosphorus aerosols (Gough & Marrs, 1990; Newman, 1995; Peñuelas et al., 2012). Therefore, terrestrial ecosystems in or near agricultural areas are the most likely to suffer from phosphorus pollution, indirectly through runoff from nearby arable land or mineral aerosol deposition or directly through phosphorus fertilization (Newman, 1995; Peñuelas et al., 2012). Important in this context is that phosphorus fertilizer recommendations for grasslands used to produce live stock frequently exceed the apparent phosphorus thresholds we identified (~100-130 mg P kg<sup>-1</sup>; e.g., Reijneveld *et al.*, 2010). In addition, phosphorus fertilization causes chronically enhanced soil phosphorus owing to tight sequestration in the soil (Sattari et al., 2012). Consequently, even low levels of phosphorus inputs can be damaging in the long term through cumulative effects. Therefore, high biodiversity cannot be expected in fertilized sites in the foreseeable future. even after implementing agroenvironmental schemes reducing phosphorus fertilization in favor of nature conservation.

To conclude, and despite our support for efforts to reduce nitrogen pollution (e.g. Phoenix et al., 2006), we highlight the need to develop a global perspective regarding the effects of phosphorus pollution on terrestrial biodiversity. Noteworthy in this context is that the negative impact of soil phosphorus on biodiversity, may not be exclusively restricted to Europe. Indeed, two studies indicated that plant species richness and the occurrence of characteristic plant species is restricted by high soil phosphorus in both Australian savanna and in Northeast American pastures (Tracy & Sanderson 2000, Dorrough & Scroggie, 2008). Finally, based on the long term enhanced soil phosphorus following fertilization and the lack of a critical threshold below which no environmental harm is expected, we strongly advocate that agro-environmental schemes should include grasslands permanently free from phosphorus fertilization.

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#### References

- Addiscott TM & Thomas D (2000) Tillage, mineralization and leaching: phosphate. Soil and Tillage Research, 53, 255–273.
- Asman WAH, van Jaarsveld JA (2002) A variable-resolution transport model applied for NH<sub>x</sub> in Europe. Atmospheric Environment, 26A, 445–464.
- BDB (2005) Chemical Soil Fertility of Arable Land and Grassland in Belgium 2000–2003. Bodemkundige Dienst België, Leuven, Belgium.

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- Bobbink R, Hicks K, Galloway J et al. (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications, 20, 30–59.
- Ceulemans T, Merckx R, Hens M, Honnay O (2011) A trait based analysis of the role of phosphorus vs. nitrogen enrichment in plant species loss across Northwest European grasslands. *Journal of Applied Ecology*, 48, 1145–1163.
- Ceulemans T, Merckx R, Hens M, Honnay O (2013) Plant species loss from European semi-natural grasslands following nutrient enrichment – Is it nitrogen or is it phosphorus? *Global Ecology and Biogeography*, **22**, 73–82.
- Clark CM, Tilman D (2008) Loss of plant species after chronic low level nitrogen deposition to prairie grasslands. *Nature*, 451, 712–715.
- Cleland EE, Harpole WS (2010) Nitrogen enrichment and plant communities. *Annals* of the New York Academy of Sciences, **1195**, 46–61.
- Conley DJ, Paerl HW, Howarth RW et al. (2009) Controlling eutrophication: nitrogen and phosphorus. Science, 323, 1014–1015.
- Crawley MJ, Johnston AE, Silvertown J et al. (2005) Determinants of species richness in the park grass experiment. The American Naturalist, 165, 179–192.
- Critchley CNR, Chambers BJ, Fowbert JA, Bhogal A, Rose SC, Sanderson RA (2002) Plant species richness, functional type and soil properties of grasslands and allied vegetation in english environmentally sensitive areas. *Grass and Forage Science*, 57, 82–92.
- Djodjic F, Börling K, Bergström L (2004) Phosphorus leaching in relation to soil type and soil phosphorus content. Journal of Environmental Quality, 33–2, 678–684.
- Dorrough J, Scroggie MP (2008) Plant responses to agricultural intensification. Journal of Applied Ecology, 45, 1274–1283.
- Elser J, Bennet E (2011) Phosphorus cycle: a broken biogeochemical cycle. *Nature*, **478**, 29–31.
- European Commission, DG Environment (2007) Interpretation Manual of European Union Habitats EUR27. European Commission, Brussels, Belgium.
- Foley JA, Defries R, Asner GP et al. (2005) Global consequences of land use. Science, 309, 570–574.
- Fujita Y, Olde Venterink H, van Bodegom PM et al. (2014) Low investment in sexual reproduction threatens plants adapted to phosphorus limitation. Nature, 505, 82–86.
- Galloway JN, Towsend AR, Erisman JW *et al.* (2008) Transformation of the nitrogen cycle: recent trends, questions and potential solutions. *Science*, **320**, 889–892.
- Gauger T (2002) Mapping of Ecosystems Specific Long-Term Trends in Deposition Loads and Concentrations of Air Pollutants in Germany and their Comparison with Critical Loads and Critical Levels. Institut Fur Navigation, University of Stuttgart, Germany.
- Gilbert J, Gowing D, Wallace H (2009) Available soil phosphorus in seminatural grasslands: assessment methods and community tolerances. *Biological Conservation*, **142**, 1074–1083.
- Gough MW, Marrs RH (1990) Trends in soil chemistry and floristics associated with the establishment of a low input meadow system on an arable clay soil in Essex. *Biological Conservation*, **52**, 135–146.
- Härdtle W, Redecker B, Assmann T, Meyer H (2006) Vegetation responses to environmental conditions in floodplain grasslands: prerequisites for preserving plant species diversity. *Basic and Applied Ecology*, 7, 280–288.
- Hautier Y, Niklaus PA, Hector A (2009) Competition for light causes plant biodiversity loss after eutrophication. *Science*, 324, 636–638.
- Hejcman M, Klaudisova M, Stursa J, Pavlu V, Schellberg J, Hejcmanova P, Hakl J, Rauch O, Vacek S (2005) Revisiting a 37 years abandoned fertilizer experiment on *Nardus* grassland in the Czech Republic. *Agriculture, Ecosystems and Environment*, 118, 231–236.
- Hejcman M, Schellberg J, Pavlu V (2010) Dactylorhiza maculata, Platanthera bifolia and Listera ovata survive N application under P limitation. Acta Oecologia, 36, 684–688.
- Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root induced chemical changes: a review. *Plant and Soil*, 237, 173–195.
- Horswill P, O'Sullivan O, Phoenix GK, Lee JA, Leake JR (2008) Base cation depletion, eutrophication and acidification of species-rich grasslands in response to longterm simulated nitrogen deposition. *Environmental Pollution*, **155**, 336–349.
- Janssens H, Peeters A, Tallowin JRB, Bakker JP, Bekker RM, Fillat F, Oomes JMM (1998) Relationship between soil chemical factors and grassland diversity. *Plant* and Soil, 202, 69–78.
- Ketterings QM, Kahabka JE, Reid WS (2005) Trends in phosphorus fertility of New York agricultural land. Journal of Soil and Water Conservation, 60, 10–20.
- Kleijn D, Müller-Schärer H (2006) The relation between unpalatable species, nutrients and plant species richness in Swiss montane pastures. *Biodiversity and Conservation*, 15, 3971–3982.
- McKane RB, Johnson LC, Shaver GR *et al.* (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature*, **415**, 68–71.

- NEGTAP (2001) Transboundary Air Pollution: Acidification, Eutrophication and Groundlevel Ozone in the UK. CEH, Edinburgh, UK.
- Newman EI (1995) Phosphorus inputs to terrestrial ecosystems. Journal of Ecology, 83, 713–726.
- Obersteiner M, Peñuelas J, Ciais P, van der Velde M, Janssens IA (2013) The phosphorus trillema. Nature Geoscience, 6, 897–898.
- Olde Venterink H (2011) Does phosphorus limitation promote species-rich plant communities? Plant and Soil, 345, 1–9.
- Payne RJ, Dise ND, Stevens CJ, Gowing D, the BEGIN partners. (2012) Impact of nitrogen deposition at the species level. *Proceedings of the National Academy of Sciences*, **110**, 984–987.
- Peñuelas J, Sardans J, Rivas-Ubach IA, Janssens A (2012) The human-induced imbalance between C, N and P in Earth's life system. *Global Change Biology*, 18, 3–6.
- Peñuelas J, Poulter B, Sardans J et al. (2013) Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. Nature Communications, 4, 2934.
- Phoenix GK, Hicks WK, Cinderby S et al. (2006) Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. Global Change Biology, 12, 470–476.
- Pieterse G, Bleeker A, Vermeulen AT et al. (2007) High resolution modeling of atmosphere–canopy exchange of acidifying and eutrophying components and carbon dioxide for European forests. *Tellus*, 59B, 412–424.
- Reijneveld JA, Ehlert PAI, Termorshuizen AJ, Oenema O (2010) Changes in the soil phosphorus status of agricultural land in the Netherlands during the 20th century. Soil use and management, 26, 399–411.
- Robertson GP, Coleman DC, Bledsoe CS, Sollins P. (eds). 1999. Soil phosphorus. In: Standard Methods in Long Term Ecological Research, pp. 1–462. Oxford University Press, New York.
- Sala OE, Chapin FS III, Armesto JJ (2000) Biodiversity global biodiversity scenarios for the year 2100. Science, 287, 1770–1774.
- Sattari SZ, Bouwman AF, Giller KE, van Ittersum M (2012) Residual soil phosphorus as the missing piece in the global phosphorus crisis puzzle. *Proceedings of the National Academy of Science*, **109**, 6348–6353.
- Schindler DW, Hecky RE, Findlay DL et al. (2008) Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proceedings of the National Academy of Sciences USA, 105, 11254– 11258.
- Silvertown J, Poulton P, Johnston E, Edwards G, Heard M, Biss PM (2006) The park grass experiment 1856–2006: its contribution to ecology. *Journal of Ecology*, 94, 801–814.
- Stevens CJ, Dise NB, Mountford JO, Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. *Science*, 303, 1876–1879.
- Stevens CJ, Thompson K, Grime PJ, Long CJ, Gowing DJG (2010) Contribution of acidification and eutrophication in species richness of calcifuge grasslands along a gradient of atmospheric nitrogen deposition. *Functional Ecology*, 24, 478–484.
- Stevens CJ, Dupré C, Dorland E et al. (2011) Grassland species composition and biogeochemistry in 153 sites along environmental gradients in Europe. Ecology, 92, 1544–1544.
- Sutton MA, Howard CM, Erisman JW et al. (eds) (2011) The European Nitrogen Assessment. Sources, Effects and Policy Perspectives, Cambridge University Press, Cambridge.
- Toms JD, Lesperance MJ (2003) Piecewise regression: a tool for identifying ecological thresholds. *Ecology*, 84, 2034–2014.
- Tracy BF, Sanderson MA (2000) Patterns of plant species richness in pasture lands of the northeast United States. *Plant Ecology*, **149**, 169–180.
- Turner BL (2008) Resource partitioning for phosphorus: a hypothesis. *Journal of Ecology*, **82**, 946–954.
- Turner BL, Haygarth PM (2001) Phosphorus solubilisation in rewetted soils. Nature, 411, 258.
- Vance CP, Uhde Stone C, Allan DL (2002) Phosphorus acquisition and use: critical adaptations by plants for securing a non renewable resource. *New Phytologist*, 157, 423–447.
- Veen P, Jefferson R, de Smidt J, van der Straaten J (eds) (2009) Grasslands in Europe of High Nature Value. KNNV Publishing, Zeist, the Netherlands.
- VMM (2010) 'Zure regen' in Vlaanderen, Depositiemeetnet verzuring 2009. Erembodegem, Belgium.
- Wassen MJ, Olde Venterink H, Lapshina ED, Tanneberger F (2005) Endangered plants persist under phosphorus limitation. *Nature*, 437, 547–550.

# **Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Relationships between the different phosphorus extraction methods and plant species richness (N = 84 for Olson-P and AEM-P, N = 36 for Oxalic acid-P). Red triangles represent grassland quadrats with pH < 5.5, blue circles 5.5 < pH < 7, green squares pH > 7. Respective Pearson correlation coefficients are from top left to top right: r = -0.56, P < 0.001; r = -0.59, P < 0.001; r = -0.60, P < 0.001 and from bottom left to bottom right: r = 0.81, P < 0.001; r = 0.78, P < 0.001; r = 0.80, P < 0.001.

**Figure S2**. Relationship between plant species richness, soil phosphorus and total nitrogen deposition across three types of European grasslands. Plane equation:  $\ln(\text{Plant species richness}) = 3.53 - 0.004(\text{soil P}) - 0.022(\text{N deposition})$ . *Nardus* grasslands: green, N = 314; lowland hay meadows: blue, N = 105 and calcareous grasslands: red, N = 82. We found no significant Pearson correlation between soil phosphorus and nitrogen deposition (r = -0.02, P = 0.63).

Table S1. List of the indicative species that were used to identify the surveyed grassland types.

**Table S2.** Detailed results of the Poisson regression model examining the relationships between soil phosphorus, soil pH, total nitrogen deposition, longitude, latitude, grassland type and soil nitrogen (1  $\bowtie$  KCl extraction, NH<sub>4</sub><sup>+</sup> + NO<sub>3</sub><sup>-</sup>) and plant species richness (N = 293). Including soil nitrogen did not change the results of our general analyses (Table 2) and had no significant effect on plant species richness. Soil nitrogen showed significant Pearson correlation coefficients with total nitrogen deposition (r = 0.18, P = 0.02) and soil pH (r = -0.35, P < 0.001) but not with soil phosphorus (r = -0.1, P = 0.09).

**Table S3.** Detailed results of the Poisson regression model examining the relationships between soil phosphorus, soil pH, total nitrogen deposition, longitude, latitude, grassland type and year of sampling and plant species richness (N = 490). Year of sampling did not change the results of our general analyses (Table 2) and had no significant effect on plant species richness. For 11 quadrats we had no data on the year of sampling.



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# Insect herbivory and grass competition in a calcareous grassland: results from a plant removal experiment

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#### Abstract

We compared the effects of herbivory by grasshoppers and neighbourhood competition on two dominant grasses, *Bromus erectus* and *Brachypodium pinnatum*, in a calcareous grassland in the French Alps. In a fully factorial design, herbivory was reduced by insecticide spraying and competition was reduced by removal of neighbouring plants. The effects of herbivory and competition were species-dependent. *Bromus*, a stress-tolerant species, was strongly affected by competition, but not by herbivory. In contrast, the more competitive species, *Brachypodium*, was negatively affected by herbivory, but only when neighbouring vegetation was removed. The greatest herbivory pressure on isolated targets of *Brachypodium* is likely to be due to the indirect effects of experimental gaps, i.e. more favourable microclimatic and foraging conditions for grasshoppers. This suggests that herbivory by insects may be a confounding factor in many plant removal experiments. Field experiments designed to study the combined effects of competition and herbivory should take into account the indirect effects induced by experimental gaps.

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Keywords: Brachypodium pinnatum; Bromus erectus; Competition; Grasshopper; Removal experiment

# 1. Introduction

The effects of insect herbivory on ecosystem processes and plant community structure have been widely documented and include increased nutrient turnover (Blumer and Diemer, 1996; Mulder et al., 1999; Belovsky and Slade, 2000), shifts in community composition and altered plant performances (Brown, 1985; Olff and Ritchie, 1998; Escarré et al., 1999). The course of plant succession may also be altered by insect herbivory (McBrien et al., 1983; Brown, 1984; Brown and Gange, 1992) due to the preference of particular insect types for habitats of different successional stages (Brown, 1984) or to the successional status of target plants (Leps et al., 2001).

Competition is also a major force in plant communities, determining vegetation composition (Grime, 1973; Reader et al., 1994), plant performance (Goldberg, 1987), and patterns of succession in many habitats (Tilman, 1990). The most

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common manner to measure the intensity of competition in the field is to perform neighbour removal experiments (Goldberg and Scheiner, 1993; Markham and Chanway, 1996), i.e. to compare responses of target individuals with or without neighbouring vegetation. Some experimental studies have combined the effects of herbivory and plant competition (Rodriguez and Brown, 1998; Van et al., 1998), and typically use a factorial design of competitor removal and herbivory exclusion with cages or insecticide (Rees and Brown, 1992; Thébaud et al., 1996; Dormann et al., 2000; Fowler, 2002; Olofsson et al., 2002). The comparative importance of herbivory and competition has been shown to vary among study systems, particular target plant species, and the species of herbivore investigated (Dormann et al., 2000; Corcket et al., 2002). Very few studies have focused specifically on insect herbivory and competition among grass species (Clay et al., 1993).

In an abandoned pasture in Canada, Reader (1992) demonstrated that herbivory by slugs may confound measurements of competition intensity determined by removal experiments. In that study, plant survival was reduced by

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herbivory when the plant was surrounded by its neighbours, suggesting that removing a plant's neighbours may reduce food and shelter for slugs. In this case, herbivory mimicked the effects of competition since it decreased the performance of targets with neighbours, which may be described as "apparent competition" (Holt, 1977). This may create serious bias in studies assessing plant interactions using removal experiments. Even so, few competition studies have explicitly considered this confounding effect (Van der Wal et al., 2000).

The purpose of our study was to compare the effects of foliar herbivory by insects and the effects of plant competition on two target grasses which are dominant in calcareous grasslands of the French Alps. We tested the responses of the dominant grasses to herbivory and competition with plants neighbouring, and whether herbivory might be a confounding factor in our experiment. Finally, we attempted to assess the long-term effects of insect herbivory and plant competition on plant dominance in calcareous grasslands.

# 2. Materials and methods

The field site, "Liche Petet" (45°10'N, 5°50'E, 700 m a.s.l.), is a calcareous grassland located near Grenoble, in the foothills of the French Alps. The vegetation structure is typical of prairies classed as Mesobromion communities in phytosociological classifications (Braun-Blanquet and Moor, 1938); this vegetation type is widespread in Western Europe (Willems, 1982; Royer, 1985). At Liche Petet, vegetation is dominated by Bromus erectus and Brachypodium pinnatum (respectively, 54% and 14% of vegetation density; see data in Corcket et al., 2002). Although these two perennial grasses may co-exist in the same habitat, B. erectus is an early-successional species in comparison to B. pinnatum (Royer, 1972; Barbaro and Cozic, 1998; Fekete et al., 2000). The impact of grazing mammals in the grasslands at Liche Petet is minimal, with only several days of sheep grazing permitted per year. Grasshoppers are often abundant in Mesobromion grasslands (Voisin, 1986), and when they are they cause significant damage to plants (Voisin, 1995; Corcket et al., 2002). This is the case in Liche Petet, where the dominant grasshopper species are Euchorthippus declivus Brisout and Chortippus biguttulus Linné (Corcket et al., 2002). Grasshoppers are "edge-eaters" and leave rounded, scallopedmarks on the edge of the grass blades they eat. Herbivory marks from grasshoppers are unlikely to be confused with marks from other herbivores such as small mammals or gastropods, which were not detected at the experimental site. Herbivory by large mammals, particularly sheep, was prevented by erecting fences around the experimental area. No evidence of herbivory by other invertebrates (snails or slugs) or vertebrates (rodents) was detected in the experimental site.

The loss of leaf tissue to herbivores may lead to a loss of photosynthetic production. If the incision is deep enough (more than half of the width of the blade), the leaf is greatly weakened and the top end of the blade may be cut. Herbivory damage may also increase water loss at the edge of the herbivory marks. Overall, we assumed that an increase in the number of marks of herbivory on an individual plant may lead to reduced fitness and decreased growth, especially in stressful environments. For each dominant grass, *B. erectus* and *B. pinnatum*, 60 target individuals were randomly sampled. When a randomly chosen target was close to another conspecific clump, we checked to be sure that the individual was not connected to the clump, especially for *B. pinnatum*, which is a clonal species. Each target individual was randomly assigned to an experimental treatment, which included two levels of competition and two levels of herbivory. Each treatment combination was replicated 15 times. Target individuals were at least 30 cm from other targets.

Competition intensity was assessed through neighbour removals. For half of the targets, we clipped all aboveground vegetation at the ground level within a radius of 15 cm. We clipped all regrowth as often as necessary. In such field experiments, we were able to measure diffuse competition experienced by target species, i.e. summed intra-and interspecific effects of neighbourhood. When the growth of target species without neighbours is significantly greater than the growth of target species with neighbours, competitive effects are assumed.

To decrease herbivory, we sprayed insecticide on individual target grasses (15 with neighbours and 15 without neighbours for each species). We did not spray both targets and neighbours in order to avoid confounding indirect effects of herbivory which could have masked the direct effect of herbivory on our targets (Crawley, 1989; Escarré et al., 1999). Shoots were included inside a plastic bottle with the bottom removed, 9-cm in width, and insecticide was sprayed through the bottleneck. Every 10–15 days from June to October 1999, treated target individuals received five sprays of a 0.5 ml/1 water solution of insecticide (Decis<sup>TM</sup>; active ingredient: Deltamethrin dosed 25 g/l). This treatment provided a negligible amount of water to target plants (0.2 ml per spray), in comparison to natural rainfall (ca. 605 mm during the course of the experiment; Météo-France, 1999).

Measurements of the intensity of leaf damage experienced by the grasses were conducted using a relative scale graduated from 0 to 5 (described in Corcket et al., 2002). This subjective non-linear scale was especially developed to assess herbivory non-destructively in the field. The level "0" indicated no damage, "1" indicated very few marks on the blades, and "5" indicated that more than half of the estimated foliar area was eaten. Levels "2–4" were assigned as intermediate levels of herbivory, according to the number and the width of herbivory marks on the blades. We recorded the damage experienced by *Bromus* and *Brachypodium* in June, August, and October 1999. These data did not meet the assumptions of normality and were square root transformed prior to analysis.

We quantified the growth of target individuals by counting the number of leaves with at least half of their blade area remaining green. This non-destructive measurement allowed us to collect data on the growth of our target individuals at the different dates throughout the season. Previous studies have shown that, for *B. erectus* and *B. pinnatum*, leaf number is significantly related to total biomass (Corcket et al., 2003). These data were not normally distributed (Shapiro-Wilk tests), and therefore, were also square root-transformed.

We tested the effects of insecticide and neighbourhood removal on damage and leaf numbers using two-way ANO-VAs (neighbour  $\times$  insecticide). Six models of analysis were designed, combining each species (*B. erectus*, *B. pinnatum*) and each date (June, i.e. before the experimental treatments; August; October). Tukey tests were also performed within each model.

Microclimate at the ground level was measured within clumps of *Bromus* and *Brachypodium* and on bare soil, where vegetation was removed within a 30 cm diameter to simulate removal conditions. Hobo® H8 Pro Series climatic sensors (Onset Computer Corporation, Bourne, MA, USA) were placed 2 cm above the ground, with two replications for each microsite. Air temperature and vapour pressure deficit (VPD) were recorded each hour for 22 days from late June to late July 1999. We used data at 16:00 h, which corresponded to mean maximal temperature and VPD during the period of record. Data of the two replicates per microsite (*Bromus*, *Brachypodium*, bare soil) were plotted. Results were expressed as mean  $\pm 1$  standard error (S.E.) for these 22 days.

Phytotoxic or stimulatory effects of insecticide (Crawley, 1989) on *Bromus* and *Brachypodium* were tested in a common garden experiment. Thirty individuals of each species were grown from local seeds and transplanted into pots. For each species, half of the target individuals were sprayed with insecticide, as described above. Shoot length and leaf number were measured in July (before spraying) and September 1999, and differences in plant performances between September and July were calculated. Shoot length and leaf number variations were normal without any transformation (Shapiro–Wilk test), allowing us to perform a two-way MANOVA (species  $\times$  insecticide, with shoot length and leaf number as dependent variables).

### 3. Results

In the common garden experiment, neither shoot length nor leaf number of the target grasses were affected by insecticide treatments (P > 0.4), indicating that the insecticide did not have phytotoxic or stimulatory effects on *Bromus* and *Brachypodium*. In the field, insecticide decreased herbivory levels on treated plants, but was not sufficient to completely exclude herbivory on targets. Damage measured on targets sprayed with insecticide decreased during the course of the experiment (from  $1.15 \pm 0.14$  in June to  $0.47 \pm 0.11$  in October, Z = -3.80, P < 0.001, Wilcoxon signed ranks test), whereas no significant changes were observed for targets not sprayed (from  $0.85 \pm 0.11$  in June to  $0.83 \pm 0.15$  in October, Z = -0.60, P = 0.952, Wilcoxon signed ranks test).

Leaf numbers of target species were very similar in June 1999, before the experimental treatments were applied (Table 1 and Fig. 1A). For *Bromus*, leaf number was not affected by experimental treatments in August, but increased significantly in October in the neighbour removal treatment. There was no effect of insecticide on *Bromus* leaf number, neither as main effect nor first order interaction (Table 1 and Fig. 1B,C). In contrast, leaf number of *Brachypodium* increased significantly in August for targets sprayed by insecticide (Table 1 and Fig. 1B) and in October for *Brachypodium* individuals without neighbours and sprayed with insecticide (Table 1 and Fig. 1C).

Prior to initiation of the experiment, Bromus targets showed differences in herbivory damage among the treatment groups. Specifically, the targets, which were randomly assigned to the neighbourhood removal treatment, had significantly more herbivory marks than the others (Table 2 and Fig. 2A). Nevertheless, this difference disappeared when experimental treatments were performed, suggesting that leaf damage was not affected by experimental treatments over the course of the experiment (Table 2 and Fig. 2B,C). Prior to initiation of the experiment, targets of Brachypodium were very similar among treatments (Table 2 and Fig. 2A). The insecticide treatment significantly decreased damage to Brachypodium at the end of the experiment. In October, damage experienced by Brachypodium increased with the neighbour removal treatment but the interaction neighbour × insecticide was not significant (Table 2 and Fig. 2C). Damage increased to a maximum intensity in August for both species (Fig. 2).

Mean maximal aboveground temperatures were  $35.0 \pm 0.9$  °C on bare soil,  $33.4 \pm 0.7$  °C in *Bromus* swards, and  $33.3 \pm 0.8$  °C in *Brachypodium* swards. Mean maximal VPDs were  $2.67 \pm 0.20$  kPa on bare soil,  $1.78 \pm 0.14$  kPa in

Table 1

Results of two-way ANOVAs (neighbour × insecticide) on the growth (square root of the number of leaves) of *Bromus* and *Brachypodium*, in June, August and October 1999. \*, \* \*, \* \* \* denote significance at *P* level <0.05, <0.01 and 0.001, respectively

Species	Source	d.f.	<i>F</i> -value			
			June	August	October	
Bromus	Neighbour	1	1.6	3.1	16.7 * * *	
	Insecticide	1	0.2	0.5	0.2	
	Neighbour × insecticide	1	0.0	0.0	0.0	
Brachypodium	Neighbour	1	0.7	2.2	2.7	
	Insecticide	1	2.2	4.2 *	8.7 * *	
	$Neighbour \times insecticide$	1	0.7	0.7	4.6 *	





Control

Insecticide

#### Table 2

Results of two-way ANOVAs (neighbour  $\times$  insecticide) on the intensity of herbivory (square root of damage) of *Bromus* and *Brachypodium*, in June, August and October 1999. \*, \* \*, \* \* \* denote significance at *P* level <0.05, <0.01 and 0.001, respectively

Species	Source	d.f.	F-value		
			June	August	October
Bromus	Neighbour	1	8.7 * *	3.1	0.1
	Insecticide	1	1.5	2.9	0.1
	Neighbour × insecticide	1	2.2	2.1	0.2
Brachypodium	Neighbour	1	0.0	1.1	4.4 *
	Insecticide	1	0.7	1.7	4.7 *
	Neighbour $\times$ insecticide	1	0.3	1.9	1.8

*Bromus* swards, and 2.24  $\pm$  0.19 kPa in *Brachypodium* swards. Summarised, temperature was 1.5 °C warmer and VPD was at least 0.43 kPa higher at the bare site than within the grasses. VPD was 0.46 kPa higher within *Brachypodium* than within *Bromus* swards.

Control

# 4. Discussion

# 4.1. Species-dependent responses

Each species responded differently to the experimental treatments. *B. erectus* was strongly affected by plant neigh-

bourhood removal but not by insecticide treatment, whereas *B. pinnatum* was above all affected by insecticide spraying in combination with neighbour removal. These results contrast with those of other field experiments, which found that neighbour removal affected all species while the effects of reducing herbivory treatments were specific to only a few species (Dormann et al., 2000; Greiling and Kichanan, 2002).

Insecticide

The greater sensitivity of *Brachypodium* to herbivory suggests that these grasshoppers may be somewhat specific in their host choice, as documented in several studies (Joern and Lawlor, 1980; Cottam, 1985; Le Gall, 1989; Corcket et al.,





Fig. 2. Means (±1 S.E.) of damage for B. erectus and B. pinnatum in June (A), August (B), and October (C) 1999 for control and sprayed individuals, with (black bars  $\square$ ) and without (white bars  $\square$ ) neighbours.

2002). The presence of defence chemicals in target plants (Szentesi, 2002), and particularly trade-offs between secondary metabolites and nutrient contents of leaves (Behmer et al., 2002) may determine the foraging decisions of herbivores. Therefore, stress-tolerant species are often grazed less than fast growing species because they generally allocate more to defence (Grime, 1977; Taylor et al., 1990; Fraser and Grime, 1999; Le Gall, 1989; Jones and Coleman, 1991). This perspective is consistent with our results: B. erectus has been shown to be more stress-tolerant than B. pinnatum (Barbaro and Cozic, 1998; Corcket et al., 2003). In arctic tundra, McGraw and Chapin (1989) found that a more competitive Eriophorum species was more frequently eaten than a less competitive congener. Convergence between stress tolerance and resistance to herbivory was also emphasised by Oksanen and Ranta (1992), who found a consistent relationship between traits for stress tolerance and those involved in grazing tolerance. The overall positive effect of neighbour removal on leaf number was only significant for B. erectus. This is consistent with previous results, which emphasised the important effects of plant competition on the growth of B. erectus in Mesobromion grasslands (Corcket et al., 2003). In contrast, the response of B. pinnatum to neighbourhood competition was not strong and depended on whether or not herbivory was manipulated.

# 4.2. Herbivory and plant removal: two related factors

The increase in leaf number for *B. pinnatum* when the effects of neighbours and grasshoppers were reduced appeared to be caused by the indirect effects of vegetation removal.

Typically, plant interactions are evaluated in the field by removing neighbouring species or all surrounding vegetation. We found that grasshopper herbivory was higher on isolated plants than on plants surrounded by neighbours. The reduced growth of *Brachypodium* without neighbours in the high herbivory treatment is consistent with general densitydependent hypotheses of herbivory intensity in which plants with neighbours' experience less herbivory than isolated individuals. Several studies have shown that the effects of invertebrate herbivores are stronger in low-density vegetation (Solomon, 1981; Parmesan, 2000). For grasshoppers, this may be explained in part by their use of visual and olfactory signals to locate their primary resource (Le Gall, 1989; Szentesi, 2002). Grasshoppers may preferentially eat isolated plants (our results) or more common plant species (Cottam, 1985) simply because preferred species are easier to locate.

Total removal of vegetation may also induce important changes in microclimate (Reader, 1992; our results), which may directly affect herbivore populations. Several studies have shown that grasshoppers are favoured by an increase in VPD and air temperature (Chopard, 1951; Ritchie, 2000; Corcket et al., 2002).

Our results are consistent with Reader's (Reader, 1992) statement that herbivory may be a confounding factor in plant removal experiments, but Reader found that predation was higher on plants surrounded by neighbours than in the openings and concluded that herbivory established conditions of "apparent competition". In his system, the herbivores were slugs, which are animals requiring mesic habitats and shelter from predators. In our study, we found that Brachypodium individuals without neighbours experienced significantly higher herbivory rates. Such a confounding effect in competition studies will overestimate the intensity of competition, and should be avoided or alleviated by minimising the strong indirect effects due to large areas without vegetation. Ideally, targets should be protected against herbivory by caging and/or insecticide spraying, depending on the herbivore involved, or experimental designs should apply intermediate levels of plant removal, such as performed by Goldberg (1987) and Parmesan (2000).

# 4.3. Herbivory damage and plant growth

The only differences in herbivory damage among our experimental treatments were recorded for *Brachypodium* in October. The significant decrease in damage in the insecticide treatment led to an increase in leaf number for *Brachypodium* targets, but only those without neighbours. This suggests that the negative effects of plant neighbourhood on *Brachypodium* growth were stronger than the positive effects of neighbourhood in reducing herbivory. In contrast, the significant increase in damage without neighbours is consistent with a significant decrease of plant leaf number in these conditions, as compared with insecticide conditions. This indicates that our insecticide treatment was not sufficient to reduce the increase in damage experienced by isolated *Brachypodium* targets, which as mentioned were preferentially

eaten by grasshoppers. However, these data also suggest that measurable, but low levels of herbivory will not significantly reduce *Brachypodium* growth. When we examined the level of damage in relation to differences in plant growth responses, we found that an increase in plant damage from "level 1" (for example, *Brachypodium* sprayed with insecticide and without neighbours in October) to 1.5 (*Brachypodium* without neighbours in October, but not sprayed with insecticide) led to a significant decrease in leaf number. This suggests that the "crucial level" of herbivory, i.e. the level of herbivory, which is necessary to induce significant damage on plants, is between a level of 1 and a level of 1.5 in our relative scale of damage.

### 4.4. Relationships with other grassland processes

In our study the species, which was negatively affected by herbivory, *B. pinnatum*, is a late successional species in *Mesobromion* communities (Bobbink and Willems, 1993; Barbaro and Cozic, 1998). Therefore, our results are consistent with field approaches showing that herbivory by foliar feeding insects may slow the rate of plant succession (McBrien et al., 1983; Brown, 1985). In contrast, other studies involving belowground invertebrate herbivores (Brown and Gange, 1992; Verschoor et al., 2002) or artificial groups of phytometers (Fraser and Grime, 1999), have shown a converse effect of herbivory on plant secondary succession. In a literature survey, Davidson (1993) found a general effect of herbivores slowing secondary succession in old-fields dominated by grass species.

In our experiment, we excluded sheep from the experimental area and focused on the effects of grasshoppers. However, sheep grazing may strongly interact with grasshoppers. In particular, sheep grazing may dramatically reduce grasshopper populations by increasing larval mortality (Guéguen-Genest and Guéguen, 1987). Sheep and grasshopper herbivory may also have opposite effects, depending on the identity of perennial grasses (Gibson et al., 1987). This may be the case in our study, because grasshoppers eat preferentially *B. pinnatum*, which is usually less preferred by cattle in comparison to *B. erectus* (Delescaille, 1999; Barbaro et al., 2000). Although rarely compared directly, when both types of herbivores co-exist the overall effect will likely be determined by large vertebrate grazers (Gibson et al., 1987).

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# References

- Barbaro, L., Corcket, E., Dutoit, T., Peltier, J.-P., 2000. Réponses fonctionnelles des communautés de pelouses calcicoles aux facteurs agroécologiques dans les Préalpes françaises. Can. J. Bot. 78, 1010–1020.
- Barbaro, L., Cozic, P., 1998. Organisation agro-écologique des pelouses et landes calcicoles du Parc Naturel Régional du Vercors. Écologie 29, 443–457.
- Behmer, S.T., Simpson, S.J., Raubenheimer, D., 2002. Herbivore foraging in chemically heterogeneous environments: nutrients and secondary metabolites. Ecology 83, 2489–2501.
- Belovsky, G.E., Slade, J.B., 2000. Insect herbivory accelerates nutrient cycling and increases plant production. Proc. Natl. Acad. Sci. USA 97, 14412–14417.
- Blumer, P., Diemer, M., 1996. The occurrence and consequences of grasshopper herbivory in an alpine grassland, Swiss Central Alps. Arctic Alpine Res. 28, 435–440.
- Bobbink, R., Willems, J.H., 1993. Restoration management of abandoned chalk grassland in the Netherlands. Biodivers. Conserv. 2, 616–626.
- Braun-Blanquet, J., Moor, M., 1938. Prodromus der Pflanzengesellschaften. Verband des *Bromion erecti*. SIGMA 5, 64 p.
- Brown, V.K., 1984. Secondary succession: insect-plant relationships. Bioscience 34, 710–716.
- Brown, V.K., 1985. Insect herbivores and plant succession. Oikos 44, 17-22.
- Brown, V.K., Gange, A.C., 1992. Secondary plant succession: how is it modified by insect herbivory? Vegetatio 101, 3–13.
- Chopard, L., 1951. Orthoptéroïdes. Paul Lechevalier, Paris.
- Clay, K., Marks, S., Cheplick, G.P., 1993. Effects of insect herbivory and fungal endophyte infection on competitive interactions among grasses. Ecology 74, 1767–1777.
- Corcket, E., Chintauen-Marquier, I., Callaway, R.M., Michalet, R., 2002. Sélectivité et variations environnementales de l'herbivorie par les Orthoptères. C. R. Biol. 325, 155–164.
- Corcket, E., Liancourt, P., Callaway, R.M., Michalet, R., 2003. The relative importance of competition for two dominant grass species, as affected by environmental manipulations in the field. Ecoscience in press.
- Cottam, D.A., 1985. Frequency-dependent grazing by slugs and grasshoppers. J. Ecol. 73, 92–933.
- Crawley, M.J., 1989. Insect herbivores and plant population dynamics. Ann. Rev. Entomol. 34, 531–564.
- Davidson, D.W., 1993. The effects of herbivory and granivory on terrestrial plant succession. Oikos 68, 23–35.
- Delescaille, L.M., 1999. La gestion conservatoire des pelouses sèches par le pâturage ovin. Aspects théoriques et pratiques. Parcs et Réserves 54, 2–9.
- Dormann, C.F., Van der Wal, R., Bakker, J.P., 2000. Competition and herbivory during salt marsh succession: the importance of forb growth strategy. J. Ecol. 88, 571–583.
- Escarré, J., Lepart, J., Sans, X., Sentuc, J.J., Gorse, V., 1999. Effects of herbivory on the growth and reproduction of *Picris hieracioides* in the Mediterranean region. J. Veg. Sci. 10, 101–110.
- Fekete, G., Viragh, K., Aszalos, R., Précsényi, I., 2000. Static and dynamic approaches to landscape heterogeneity in the Hungarian forest-steppe zone. J. Veg. Sci. 11, 375–382.
- Fowler, N.L., 2002. The joint effect of grazing, competition, and topographic position on six savanna grasses. Ecology 83, 2477–2488.
- Fraser, L.H., Grime, J.P., 1999. Interacting effects of herbivory and fertility on a synthesized plant community. J. Ecol. 87, 514–525.
- Gibson, C.W.D., Brown, V.K., Jepsen, M., 1987. Relationships between the effects of insect herbivory and sheep grazing on seasonal changes in an early successional plant community. Oecologia 71, 245–253.
- Goldberg, D.E., 1987. Neighborhood competition in an old-field plant community. Ecology 68, 1211–1223.
- Goldberg, D.E., Scheiner, S.M., 1993. ANOVA and ANCOVA: field competition experiments. In: Gurevitch, M.S.J (Ed.), Design and Analysis of Ecological Experiments. Chapman & Hall, New York, pp. 69–93.

- Greiling, D.A., Kichanan, N., 2002. Old-field seedling responses to insecticide, seed addition, and competition. Plant Ecol. 159, 175–183.
- Grime, J.P., 1973. Competitive exclusion in herbaceous vegetation. Nature 242, 344–347.
- Grime, J.P., 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. 111, 1169–1194.
- Guéguen-Genest, M.-C., Guéguen, A., 1987. Effet du pâturage ovin sur la dynamique de population du criquet de Sibérie *Gomphocerus sibiricus* Finot Orthoptère, *Acrididae* dans une formation paturée d'altitude. C. R. Acad. Sci. 304 (Série III), 443–447.
- Holt, R.D., 1977. Predation, apparent competition, and the structure of prey communities. Theor. Population Biol. 12, 197–229.
- Joern, A., Lawlor, L.R., 1980. Food and microhabitat utilization by grasshoppers from arid grasslands: comparisons with neutral models. Ecology 61, 591–599.
- Jones, C.G., Coleman, J.S., 1991. Plants stress and insect herbivory: toward an integrated perspective. Response of Plants to Multiple Stresses. Academic Press, pp. 249–280.
- Le Gall, P., 1989. Le choix des plantes nourricières et la spécialisation trophique chez les Acrididae (Orthoptères). Bull. Ecol. 20, 245–261.
- Leps, J., Novotny, V., Basset, Y., 2001. Habitat and successional status of plants in relation to the communities of their leaf-chewing herbivores in Papua New Guinea. J. Ecol. 89, 186–199.
- Markham, J.H., Chanway, C.P., 1996. Measuring plant neighbour effects. Funct. Ecol. 10, 548–549.
- McBrien, H., Harmsen, R., Crowder, A., 1983. A case of insect grazing affecting plant succession. Ecology 64, 1035–1039.
- McGraw, J.B., Chapin III, F.S., 1989. Competitive ability and adaptation to fertile and infertile soils in two *Eriophorum* species. Ecology 70, 736–749.
- Météo-France, 1999. Bulletin climatologique annuel de l'Isère. Grenoble/Saint-Martin d'Hères.
- Mulder, C.P.H., Koricheva, J., Huss-Danell, K., Högberg, P., Joshi, J., 1999. Insects affect relationships between plant species richness and ecosystem processes. Ecol. Lett. 2, 237–246.
- Oksanen, L., Ranta, E., 1992. Plant strategies along mountain vegetation gradients: a test of two theories. J. Veg. Sci. 3, 175–186.
- Olff, H., Ritchie, M.E., 1998. Effects of herbivores on grassland plant diversity. Trends Ecol. Evol. 13, 261–265.
- Olofsson, J., Moen, J., Oksanen, L., 2002. Effects of herbivory on competition intensity in two Arctic-Alpine tundra communities with different productivity. Oikos 96, 265–272.
- Parmesan, C., 2000. Unexpected density-dependent effects of herbivory in a wild population of the annual *Collinsia torreyi*. J. Ecol. 88, 392–400.
- Reader, R.J., Wilson, S.D., Tilman, D., Morris, E.C., Grace, J.B., McGraw, J.B., Olff, H., Turkington, R., Klein, E., Leun, Y., Shipley, B., Van Hulst, R., Johansson, M.E., Nilsson, C., Gurevitch, J., Grigulis, K., Beisner, B.E., 1994. Plant competition in relation to neighbor biomass: an intercontinental study with *Poa pratensis*. Ecology 75, 1753–1760.
- Reader, R.J., 1992. Herbivory as confounding factor in an experiment measuring competition among plants. Ecology 73, 373–376.
- Rees, M., Brown, V.K., 1992. Interactions between invertebrate herbivores and plant competition. J. Ecol. 80, 353–360.
- Ritchie, M.E., 2000. Nitrogen limitation and trophic vs. abiotic influences on insect herbivores in a temperate grassland. Ecology 81, 1601–1612.
- Rodriguez, M.A., Brown, V.K., 1998. Plant competition and slug herbivory: effects on the yield and biomass allocation pattern of *Poa annua* L. Acta Oecol. 19, 37–46.
- Royer, J.-M., 1985. Liens entre chorologie et différenciation de quelques associations du *Mesobromion erecti* d'Europe occidentale et centrale. Vegetatio 59, 85–96.
- Royer, J.-M., 1972. Essai de synthèse sur les groupements végétaux de pelouses, d'éboulis et rochers de Bourgogne et Champagne méridionale. Annales Scientifiques de l'université de Besançon 3<sup>e</sup> série, vol. B, 127–316.

- Solomon, B.P., 1981. Response of a host-specific herbivore to resource density, relative abundance, and phenology. Ecology 62, 1205–1214.
- Szentesi, A., 2002. Insect-plant relationship—chance and necessity. Acta Zool. Acad. Sci. Hungaricae 48, 55–71.
- Taylor, D.R., Aarssen, L.W., Loehle, C., 1990. On the relationship between r/K selection and environmental carrying capacity: a new habitat templet for plant life-history strategies. Oikos 58, 239–250.
- Thébaud, C., Finzi, A.D., Affre, L., Debussche, M., Escarre, J., 1996. Assessing why two introduced *Coniza* differ in their ability to invade Mediterranean old fields. Ecology 77, 791–804.
- Tilman, D., 1990. Constraints and tradeoffs: toward a predictive theory of competition and succession. Oikos 58, 3–15.
- Van der Wal, R., Egas, M., Van der Veen, A., Bakker, J., 2000. Effects of resource competition and herbivory on plant performance along a natural productivity gradient. J. Ecol. 88, 317–330.

- Van, T.K., Wheeler, G.S., Center, T.D., 1998. Competitive interactions between Hydrilla (*Hydrilla verticillata*) and Vallisneria (*Vallisneria americana*) as influenced by insect herbivory. Biol. Control 11, 185–192.
- Verschoor, B.C., Pronk, T.E., De Goede, R.G.M., Brussaard, L., 2002. Could plant-feeding nematodes affect the competition between grass species during succession in grasslands under restoration management? J. Ecol. 90, 753–761.
- Voisin, J.-F., 1986. Évolution des peuplements d'Orthoptères dans le canton d'Aime (Savoie). Travaux Scientifiques du Parc National de la Vanoise XV, 229–254.
- Voisin, J.-F., 1995. Observations sur une pullulation d'Aeropus sibiricus (Insecta, Orthoptera) en Grande Sassière (Savoie): évolution pendant les années 1987 et 1988 Travaux Scientifiques du Parc National de la Vanoise XIX.
- Willems, J.H., 1982. Phytosociological and geographical survey of *Meso-bromion* communities in Western Europe. Vegetatio 48, 227–240.

Écologie / Ecology

# Sélectivité et variations environnementales de l'herbivorie par les Orthoptères

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**Abstract – Species-dependence and environmental changes in grasshoppers herbivory.** The aim of this study is 1°) to quantify environmental changes in herbivory due to *Orthoptera* on two perennial grasses and 2°) to assess the processes involved in the control of herbivory. Herbivory varies strongly according to shade, drought and mowing, and is positively related to vapour pressure deficit and temperature. Besides the hypothesis of a trophic control of herbivory, our results are consistent with a microclimatic control of herbivory by *Orthoptera*. The coexistence of different hypothesis of herbivory control may depend on the studied system and specifically on the type of herbivore involved. *To cite this article: E. Corcket et al., C. R. Biologies 325 (2002) 155–164.* © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

# bottom-up control / *Bromus erectus / Brachypodium pinnatum /* environmental gradients / Grasshopper / herbivory / microclimate

**Résumé** – L'objet de cette étude est de 1°) quantifier les variations environnementales de l'herbivorie causée par les Orthoptères sur deux graminées pérennes et 2°) d'appréhender les mécanismes de régulation de l'herbivorie. L'herbivorie varie fortement en fonction de l'ombrage, la sécheresse et la fauche, et est positivement corrélée avec le déficit en vapeur saturante et la température. Nos résultats sont partiellement cohérents avec les hypothèses de régulation trophique de l'herbivorie et suggèrent en outre une régulation microclimatique de l'herbivorie due aux Orthoptères. Une claire distinction entre les différents types d'herbivores permet d'expliquer la coexistence de différentes hypothèses de régulation de l'herbivorie. *Pour citer cet article : E. Corcket et al., C. R. Biologies 325* (2002) 155–164. © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

*Bromus erectus | Brachypodium pinnatum |* gradients environnementaux / herbivorie / microclimat / Orthoptère / régulation ascendante

# . Abridged version

Herbivory in ecosystems is not a static phenomenon, but varies over space and time. Variation in herbivory intensity has generally been studied along natural gradients such as those created by variation in soil fertility, biomass, or productivity. These gradients are complex, i.e. they include changes in several components of ecosystems (such as soil and vegetation) and numerous abiotic factors (such as temperature and soil

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moisture) at the same time. Studies performed along complex gradients have improved our knowledge of how the intensity of herbivory varies, but the large number of correlated factors incorporated in natural gradients does not allow a clear mechanistic manipulations of processes driving herbivory. Studies along complex gradients have led to conflicting results, making generalisations about the patterns of herbivory variation difficult. However, some general models suggest that the intensity of herbivory along natural gradients (especially biomass) may be somewhat predictable. According to food web theory, herbivory should be limited in productive habitats by 'top-down' control, i.e. by predation on herbivores, and in low productive habitats by 'bottom-up' control, i.e. by a shortage in the primary resource supporting herbivores. In addition to studies involving complex gradients, other investigations have focused on changes in the intensity of herbivory with simple experimental factors, such as CO<sub>2</sub> enrichment or nutrient addition. These studies have yielded insight into the influence of particular factors on herbivory, but our understanding of the relative importance of various factors in combination remains. We established three treatments in the field (shade, drought and mowing), by which particular abiotic factors were varied in order to create a complex experimental gradient of biomass. The aim of our work is to study variation in the intensity herbivory caused by grasshoppers (Orthoptera) on two perennial grasses, Bromus erectus and Brachypodium pinnatum. Specifically, we have 1) quantified herbivory experienced by Bromus and Brachypodium, 2) compared the intensity of herbivory in different treatments of shade, drought, mowing, 3) compared the intensity of herbivory with variation in plant biomass, and 4) considered our results in the context of the dominant hypotheses for how the importance of herbivory varies in natural communities. Shade, drought and mowing were manipulated in a calcareous grassland to simulate canopy effects of encroaching trees in grasslands, drought, and management for hay. These treatments were performed on eight 32-m<sup>2</sup> plots (Control; Shade; Drought; Mowing; Shade and Drought; Shade and Mowing; Drought and Mowing; Shade, Drought and Mowing), following a fully factorial design. In each plot, we recorded a range of abiotic environmental changes that occurred with experimental treatments: vapour pressure deficit (VPD), air and soil temperature, soil moisture, soil nitrogen content. We also recorded variation in biotic factors among the treatment plots including the total density of all vegetation, and the density of Bromus erectus, Brachypodium pinnatum, and the main functional types of plants. Herbivory experienced by Bromus erectus

and *Brachypodium pinnatum* was measured using a relative scale of damage graduated from 0 (no damage) to 5 (more than 50% of the leaf area were eaten). To assess mechanisms driving grasshopper herbivory in calcareous grasslands, damage by herbivory on target species were correlated with abiotic and biotic conditions that occurred in the different experimental plots.

Herbivory was selective and Brachypodium was 2-4 times more eaten than Bromus. Shade sharply decreased herbivory experience by both species (F-values > 7). Drought showed contrasting effects, increasing herbivory on Bromus in July but decreasing herbivory on Brachypodium in August. Herbivory experienced by Brachypodium decreased substantially with mowing (P < 0.001), whereas herbivory experienced by *Bromus* was not affected by mowing. When plots were not mowed, herbivory was positively related to VPD and temperature, and negatively related to soil nitrogen availability, density of Brachypodium, and density of other grasses (e.g. Dactylis glomerata, Holcus lanatus). After mowing, herbivory was only weakly related to total density of vegetation density and not correlated with any other environmental measurements.

The preference of grasshoppers for *Brachypodium* was not consistent with the frequency-dependence of herbivory by grasshoppers which states that the dominant plant species in communities is the more eaten, but suggest chemical or physical defences of the studied grasses. Unlike grasshoppers, cattle preferentially eat *Bromus*, especially when plants are mature, which may be due to silica inclusions in the leaves of *Brachypo-dium*.

The positive correlation between herbivory and microclimate is consistent with the ecological requirements of grasshoppers, which prefer warm and dry habitats. The decrease in herbivory in shaded plots may be explained by the decrease in VPD and temperature under the shade cloth, leading to an unfavourable microclimate for grasshoppers. However, the increase in herbivory with drought in July occurred with an increase in VPD. The decrease in herbivory in mowed and dry plots in August corresponds with the 'bottomup' hypothesis for herbivory. Mowing, and the long drought in August, may have led to a decrease in the quality and quantity of vegetation available for herbivores.

Besides the trophic control hypothesis of herbivory, alternative hypothesis such as control of herbivory by microclimate or herbivores foraging have to be consider. These conflicting hypotheses for the importance of herbivory in plant communities may be explained by differences among herbivore species involved in studies. Large herbivores are not highly restricted by microclimate and damage large amounts of vegetation in a relatively indiscriminate manner. In contrast, small ectothermic herbivores such as grasshoppers are highly constrained by microclimate, and sometimes in opposite ways depending on the species: grasshoppers prefer warm, dry habitats, whereas slugs prefer wet habitats. Microclimate effects and species-specific effects may create conditions where herbivory is highly selective, therefore have great consequences on community composition. This perspective is apparent in the literature on herbivory intensity and hypotheses for how herbi-

# 1. Introduction

L'herbivorie n'est pas une composante stable des l'écosystèmes, comme le suggèrerait une lecture statique des réseaux trophiques, mais elle varie de façon spatiale et temporelle [1]. Ces variations ont souvent été étudiées le long de gradients complexes de l'environnement, c'est-à-dire de gradients le long desquels de nombreux facteurs directs (qualité et quantité de ressource végétale pour l'herbivore, teneur du sol en azote, température de l'air, etc.) varient de façon concomitante. Ce sont en général des gradients naturels qui intègrent différentes communautés végétales se succédant sur le terrain. L'approche par gradients complexes apporte une meilleure connaissance des variations d'herbivorie qui se produisent dans le milieu naturel, mais le grand nombre de facteurs impliqués dans ces gradients ne permet pas d'avoir une approche déterministe fine de l'herbivorie. Les gradients complexes les plus étudiés sont les gradients de succession végétale [2–4], de fertilité des sols [5,6] ou la conjonction des deux [7–9]. Ils sont assimilables aux gradients de biomasse végétale étudiés par Oksanen et al. [10], Belsky [11] et Bonser et Reader [12]. D'autres gradients complexes tels que des gradients de stress végétal [13,14], de microstructures de végétation [14] ou de topographie [15] ont également servi de base à des études de variations d'herbivorie.

Des conclusions parfois contradictoires émergent de ces études. Rand [14] trouve une intensité d'herbivorie maximale à faible niveau de biomasse végétale alors que Bonser et Reader [12] ainsi que Fraser et Grime [9] montrent l'inverse. Par ailleurs, Oksanen et al. [10] ont proposé un modèle selon lequel l'intensité d'herbivorie est maximale à un niveau intermédiaire de biomasse végétale. Ce modèle est basé sur les réseaux trophiques, et plus particulièrement sur le contrôle ascendant ou descendant des populations d'herbivores [16,17]. Le contrôle ascendant des populations d'herbivores a lieu lorsque la ressource primaire, c'est-à-dire la biomasse vory affects the composition of plant communities. In general, trophic hypotheses for herbivory are based on studies of large vertebrates herbivores, microclimate hypotheses are based on studies involving arthropods, and support for the importance of foraging strategies are based on studies of small vertebrate herbivores. Specific comparisons of herbivore identity and the functional traits of herbivores may help to explain contradictions in literature for how herbivory may control community structure and ecosystem function.

végétale, est trop limitée pour entretenir une population suffisante d'herbivores. Le contrôle descendant des populations d'herbivores intervient lorsque la quantité d'herbivores est suffisante pour entretenir une population de prédateurs susceptible de réguler, en rétroaction, le nombre d'herbivores. Par extension, le modèle d'Oksanen et al. [10] prédit une baisse d'herbivorie dans les faibles niveaux de biomasse due à un manque de ressource végétale, et une baisse d'herbivorie dans les forts niveaux de biomasse due à une forte pression de prédation sur les herbivores.

D'autres études se sont focalisées sur l'influence de facteurs directs, tels que la concentration en  $CO_2$  atmosphérique [18,19] ou la quantité de nutriments du sol [20,21], sur l'herbivorie. Ces approches expérimentales permettent une meilleure compréhension des mécanismes déterminant l'herbivorie, mais la prédiction des variations d'herbivorie dans les milieux naturels nécessite de relativiser l'importance de ces facteurs directs dans le fonctionnement de l'écosystème. Une troisième approche, qui à notre connaissance a été peu ou pas explorée, consisterait à scinder des gradients complexes en une gamme étendue de facteurs directs abiotiques et biotiques et de les corréler aux variations d'herbivorie observées dans le milieu naturel.

Lors d'une expérimentation en pelouse calcicole [22], trois traitements expérimentaux simulant le fonctionnement de l'écosystème ont été appliqués (l'ombrage, la sécheresse et la fauche) et ont induit un gradient complexe de biomasse. Ces milieux de pelouses sèches collinéennes sont inféodés à de fortes pressions d'herbivorie de la part du bétail, de rongeurs, de mollusques ou d'insectes [18,19,23–25]. Les populations d'Orthoptères, notamment, sont bien représentées [26] et sont susceptibles d'infliger des pertes importantes à la végétation [27]. A partir de ce système modèle, l'objectif est alors de 1) évaluer la pression d'herbivorie exercée par la guilde des Orthoptères phytophages sur les espèces dominantes de la pelouse calcicole, 2) de mesurer les variations d'herbivorie de ce groupe d'espèces en fonction de l'ombrage, de la sécheresse, de la fauche, 3) de caractériser ces variations d'herbivorie le long d'un gradient de biomasse végétale, et 4) de relier nos résultats avec les principales hypothèses de régulation de l'herbivorie.

# 2. Matériel et méthodes

# 2.1. Site d'étude et espèces dominantes

La pelouse étudiée, Liche Petet (45°10' N, 5°50' E, 700 m d'altitude), se situe à 15 km à l'est de Grenoble, sur les balcons de Belledonne (chaîne montagneuse des Alpes externes du nord françaises). Les précipitation annuelles à Grenoble (210 m d'altitude) sont importantes (1000 mm environ) et réparties régulièrement tout au long de l'année. La température annuelle moyenne y est de 10,9 °C [28]. La pelouse se rattache au Mesobromion [29] : elle est caractérisée par la dominance de deux Poacées pérennes, Bromus erectus et Brachypodium pinnatum (respectivement 55% et 15% de la densité végétale totale), et par la présence d'espèces telles que Plantago media, Salvia pratensis (Tableau 1). Le Brome érigé présente des feuilles plus appétentes pour le bétail que celles du Brachypode penné [25,30]. La dynamique du Mesobromion est guidée par trois processus principaux [31]: la colonisation par les ligneux (ici : Rosa sp., Juniperus communis, Pinus silvestris, Fraxinus excelsior), la sécheresse (due ici à une exposition sud-sud-ouest, une pente assez importante et un sol drainant contenant 38% de sable) et le pâturage extensif par les ovins (depuis 15 à 50 ans).

La caractérisation du peuplement d'Orthoptères phytophages de la pelouse (Acrididae, Tettigoniidae) a été réalisée lors de 9 journées de prélèvement en août 1999 au filet fauchoir sur six placettes de 1 m<sup>2</sup> situées aléatoirement dans les lots de notre dispositif expérimental. En l'absence de traitements expérimentaux, le peuplement est fortement dominé par Euchorthippus declivus (50% des imago) et Chorthippus biguttulus (33% des imago). Chorthippus brunneus est plus rare (10% des imago) tandis que Metrioptera bicolor, Omocestus haemorrhoidalis, O. ventralis sont occasionnels (2,5% des imago pour chaque espèce). À Liche Petet, les dégâts d'herbivorie sur le Brome et le Brachypode sont attribués essentiellement aux Orthoptères : ceux-ci constituent la population d'insectes phytophages dominante et les seules observations directes d'herbivorie sur les espèces cibles qui ont pu être faites ont concerné des Orthoptères (observations personnelles). De plus, les dégâts d'herbivorie sur le Brome et le Brachypode sont significativement corrélés à la densité d'Orthoptères dans les différentes lots expérimentaux (r = 0.92,

Tableau 1. Composition floristique et pourcentage de recouvrement des principales espèces du site d'étude en l'absence de traitements expérimentaux (relevé linéaire 100 points)

Espèces	Bromus erectus Huds	54,3%
dominantes :	Brachypodium pinnatum (L.) P. Beauv.	14,5%
Espèces	Carex flacca Schreb.	7,3%
fréquentes :	Origanum vulgare L.	4,2%
	Medicago sativa L.	3,6%
	Carex caryophyllea Latour.	3,4%
Espèces peu	Potentilla tabernaemontani Asch.	2,3%
fréquentes :	Dactylis glomerata L.	1,8%
	Teucrium chamaedrys L.	1,3%
	Thymus serpillum L.	1,3%
Espèces rares :	Clinopodium vulgare L.	0,8%
	Daucus carota L.	0,8%
	Linum catharticum L.	0,8%
	Lotus corniculatus L.	0,8%
	Plantago media L.	0,8%
	Festuca gr. ovina L.	0,5%
	Arrhenatherum elatius (L.) P. Beauv.	0,3%
	Hippocrepis comosa L.	0,3%
	Poa pratensis L.	0,3%
	Salvia pratensis L.	0,3%
	Sanguisorba minor Scop.	0,3%
	Verbena officinalis L.	0,3%

P = 0,001 pour le Brome et r = 0,68, P = 0,031 pour le Brachypode ; test de corrélation de Pearson, n = 8). Contrairement à des études menées en Suisse ou en Angleterre sur des milieux similaires [18,19], aucun Gastéropode n'a été détecté.

# 2.2. Dispositif expérimental

Au milieu de la pelouse, un enclos de  $30 \times 30$  m a été mis en place, à l'intérieur duquel huit lots de 32 m<sup>2</sup> ont été délimités. Trois types de traitements expérimentaux, étroitement liés au fonctionnement de la pelouse, ont été combinés et appliqués pendant 2 saisons de végétation (1998 et 1999) sur les lots : l'ombrage simulant l'embroussaillement, la sécheresse simulant les aléas climatiques, et la fauche simulant la gestion. La disposition des traitements au sein des lots a été étudiée afin d'éviter toute interférence entre les traitements (Fig. 1). L'ombrage a été réalisé du côté de la chaîne de Belledonne (à l'est) qui capte la lumière rasante du matin et évite ainsi de projeter l'ombrage expérimental sur des lots voisins ne devant pas être ombrés. La sécheresse a été réalisée sur les lots les plus bas afin de ne pas affecter l'hydrologie des lots non asséchés.

Un filet d'ombrage d'environ  $350 \text{ m}^2$  a été installé à 1,70 m du sol, de fin avril à fin octobre 1998 et 1999, pour simuler une canopée décidue. Les mailles du filet captaient 50% de rayonnement lumineux et permettaient le passage de l'eau de pluie. Le filet était



Fig. 1. Schéma du dispositif expérimental.

interrompu à 50 cm au dessus du sol pour permettre la circulation d'air et d'animaux de petite taille (dont les Orthoptères) entre l'extérieur et l'intérieur de l'ombrage. La sécheresse a été créée en déployant au dessus de la végétation une bâche plastique transparente de  $15 \times 13$  m avant chaque averse. La bâche était repliée après l'averse pour éviter les effets d'échauffement avec le soleil. La sécheresse expérimentale a été appliquée de fin avril à fin juin 1998, et de début mai à fin septembre 1999. Des tranchées de 1 m de profondeur ont été creusées en amont des lots asséchés afin d'intercepter les eaux de ruissellement (Fig. 1). L'étroitesse des tranchées (50 cm) permettait le passage des Orthoptères. Enfin, la fauche a été réalisée fin avril 1998 et mi-juillet 1999, réduisant la longueur de l'appareil végétatif aérien des plantes cibles à environ 5 cm.

# 2.3. Paramètres environnementaux

Les effets des traitements expérimentaux sur les composantes abiotiques (microclimat, sol) et biotiques (végétation) de l'environnement ont été mesurés pour chaque lot expérimental.

Au milieu de chaque lot, la température de l'air, du sol, et l'humidité atmosphérique ont été mesurées heure par heure grâce à une station de mesure (Hydroemac, Seyssinet-Pariset, Isère, France) de mai à août 1999. Le capteur aérien a été placé à 30 cm au dessus du sol alors que le capteur thermique souterrain a été enterré à 10 cm de profondeur. Les données climatiques ont été moyennées sur toute la période de mesure au pas de temps horaire, et seuls les maxima ont été retenus pour les analyses (16 h pour l'humidité et la température de l'air, 19 h pour la température du sol). L'humidité de l'air a été transformée en déficit de vapeur saturante

[32]. Les teneurs en eau et en azote disponible  $(NO_3^- \text{ et } NH_4^+)$  ont été quantifiées en juin 1999 à partir de 3 échantillons de 250 g de terre prélevés à 10–15 cm de profondeur [22]. Les moyennes d'humidité de sol et d'azote total disponible ont été calculées à partir des trois échantillons prélevés.

Les densités de végétation ont été déterminées à l'aide de relevés linéaires positionnés diagonalement au sein de chaque lot. Tous les 10 cm, le long de 88 à 93 points d'échantillonnage, le nombre de contacts avec les parties vivantes des différentes espèces végétales a été relevé. La densité de végétation a été calculée comme le nombre de contacts ramené à 100 points d'échantillonnage.

# 2.4. Biométries

Les mesures d'intensité d'herbivorie ont été effectuées sur des plantes en place au sein de chaque lot expérimental. 12 individus de chaque espèce par lot ont été suivis.

Les Orthoptères consomment le bord des limbes [33] et y laissent des impacts plus ou moins ovales ou allongés. Les traces les plus profondes peuvent dépasser la nervure centrale du limbe d'une graminée, et les plus longues peuvent excéder 1 cm de longueur sur le bord du limbe. Les impacts d'herbivorie sur les feuilles ont été mesurés l'été 1999, avant et après la fauche expérimentale (respectivement début juillet et mi-août). Pour quantifier ces impacts variés, nous avons mis au point une échelle relative d'herbivorie, graduée de 0 à 5 en fonction des conséquences prévisibles sur les plantes (Tableau 2). Quand les impacts d'herbivorie sur une feuille sont suffisamment nombreux et quand le limbe est entaillé suffisamment profondément ou sur une longueur suffisante, les feuilles deviennent fragiles et peuvent se casser.

### 2.5. Analyses statistiques

Des analyses de variance à une voie sur les dégâts en juillet et en août permettent de tester les différences d'herbivorie subie par le Brome et le Brachypode (effet espèce). Ensuite, des analyses de variance à 3 voies (ombrage × sécheresse × fauche) ont été réalisées sur les dégâts en juillet et en août pour chacune des deux espèces. La relation entre l'intensité d'herbivorie et les facteurs environnementaux a été analysée par corrélations (coefficient de Pearson) entre la moyenne des dégâts d'herbivorie et les paramètres environnementaux des huit lots expérimentaux (déficit en vapeur saturante, températures de l'air et du sol, humidité du sol, teneur en azote du sol, densité de végétation, de Brome, de Brachypode, d'autres graminoïdes, de légumineuses et d'autres Dicotylédones).

Tableau 2. Echelle relative utilisée pour quantifier les dégâts causés par les Orthoptères sur les graminées

Graduation	Dégâts associés
0	Aucun.
1	Quelques impacts sur l'ensemble de la plante.
2	1 impact par feuille en moyenne,
	ou 1–2 impacts de 1,5 cm de long,
	<i>ou</i> 1–2 impacts entaillant la feuille sur <sup>1</sup> / <sub>4</sub> à la moitié de sa largeur.
3	2-4 impacts par feuille en moyenne,
	ou plus de 3 impacts de 1,5 cm de long,
	<i>ou</i> plus de 3 impacts entaillant la feuille sur <sup>1</sup> / <sub>4</sub> à la moitié de sa largeur,
	<i>ou</i> 1–2 impacts entaillant la feuille sur plus de la moitié de sa largeur.
4	plus de 5 impacts par feuille en moyenne,
	<i>ou</i> plus de 3 impacts entaillant la feuille sur plus de la moitié de sa largeur.
5	Plus de la moitié de la surface foliaire estimée a été broutée.

# 3. Résultats

L'herbivorie par les Orthoptères affecte d'avantage le Brachypode que le Brome, avec des dégâts deux à trois fois supérieurs en juillet (F = 17,6, P < 0,001) et trois à quatre fois supérieurs en août (F = 31,0, P < 0,001) dans le cas du Brachypode.

L'herbivorie est fortement influencée par l'ombrage et de manière négative, que ce soit pour le Brome (F > 7) ou pour le Brachypode (F > 13; Tableaux 3 et 4). Quasiment aucune trace d'herbivorie n'est visible sur le Brome lorsqu'il est ombré, et l'intensité d'herbivorie mesurée sur le Brachypode par notre échelle relative diminue en général d'au moins de moitié avec l'ombrage (Figs. 2 et 3). La sécheresse influence aussi l'herbivorie subit par les deux espèces (Tableaux 3 et 4), mais de façon plus différenciée que dans le cas de l'ombre ; l'herbivorie subie par le Brome augmente en juillet alors qu'elle diminue pour le Brachypode en août (Figs. 2 et 3). Le fauche n'influence pas le niveau d'herbivorie subie par le Brome (Tableau 3) alors qu'elle entraîne une diminution d'herbivorie très significative pour le Brachypode, surtout au mois d'août  $(F_{août} = 18,8$ ; Tableau 4, Fig. 3). Lorsqu'elle est appliquée sous l'ombrage, la fauche entraîne une diminution d'herbivorie significativement plus importante que lorsqu'elle est appliquée en dehors de l'ombrage (interactions ombrage  $\times$  fauche et ombrage  $\times$  sécheresse  $\times$ fauche significatives, Tableau 4), ce qui se traduit par une absence complète d'herbivorie en août sur le Brachypode dans les lots Ombré - Fauché et Ombré -Asséché - Fauché (Fig. 3). La sécheresse en juillet augmente significativement l'herbivorie subie par le Brachypode sous l'ombrage (ce qui corrobore l'aug-

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Tableau 3. Résultats des analyses de variance à 3 voies (ombrage, sécheresse, fauche) effectuées sur les dégâts mesurées en juillet et août 1999 sur le Brome

		Juillet	Août
Effets	dl	Valeurs de F	Valeurs de F
Ombrage	1	12,18 **	7,17 **
Sécheresse	1	5,50 *	1,12
Fauche	1	1,25	1,63
Ombrage × sécheresse	1	1,80	1,12
Ombrage $\times$ fauche	1	0,01	1,63
Sécheresse $\times$ fauche	1	0,18	0,54
Ombrage × sécheresse × fauch	e 1	2,03	0,54

\*\*\*, \*\*, \* significativités aux seuils respectifs de P < 0.001; 0.01 et 0.05.

Tableau 4. Résultats des analyses de variances à 3 voies (ombrage, sécheresse, fauche) effectuées sur les dégâts mesurées en juillet et août 1999 sur le Brachypode

		Juillet	Août
Effets	dl	Valeurs de F	Valeurs de F
Ombrage	1	13,93 ***	14,29 ***
Sécheresse	1	0,56	4,60 *
Fauche	1	4,88 *	18,82 ***
Ombrage × sécheresse	1	2,21	0,63
Ombrage × fauche	1	1,46	4,36 *
Sécheresse × fauche	1	2,90	2,06
Ombrage × sécheresse × fauche	1	5,15 *	0,01

\*\*\*, \*\*, \* significativités aux seuils respectifs de P < 0,001; 0,01 et 0,05.

mentation d'herbivorie constatée pour le Brome asséché en juillet) mais pas à l'extérieur de l'ombrage (interaction ombrage × sécheresse × fauche significative; Tableau 4, Fig. 3). Les espèces d'Orthoptères phytophages dominantes en situations témoin, séchée et fauchée sont avant tout *Euchorthippus declivus* et *Chorthippus biguttulus*. Seul l'ombrage engendre une composition différente avec la dominance secondaire de *Homorocoryphus nitidulus*.

Les corrélations entre herbivorie et facteurs environnementaux sont très similaires pour les deux espèces, que ce soit au mois de juillet ou au mois d'août (Tableau 5). En juillet, les dégâts sur le Brome et le Brachypode sont positivement corrélés au microclimat (D.V.S., températures de l'air et du sol) et négativement corrélés à la fois aux ressources du sol (eau et azote), à la densité de Brachypode et à la densité d'autres graminoïdes (telles que *Dactylis glomerata*, *Holcus lanatus*). En août, après la fauche, seule la densité de végétation est négativement corrélée aux dommages subis par le Brome. Aucune corrélation significative entre facteurs environnementaux et herbivorie n'appa



Fig. 2. Dégâts moyens d'herbivorie ( $\pm$  erreur standard) sur *Bromus erectus* en juillet et août 1999 sur les différents lots expérimentaux. T : lot témoin, O : lot ombré, S : lot asséché, F : lot fauché, OS : lot ombré et asséché, OF : lot ombré et fauché, SF : lot asséché et fauché, OSF : lot ombré, asséché et fauché.



Fig. 3. Dégâts moyens d'herbivorie ( $\pm$  erreur standard) sur *Brachypodium pinnatum* en juillet et août 1999 sur les différents lots expérimentaux. T : lot témoin, O : lot ombré, S : lot asséché, F : lot fauché, OS : lot ombré et asséché, OF : lot ombré et fauché, SF : lot asséché et fauché, OSF : lot ombré, asséché et fauché.

raît au mois d'août (Tableau 5), à l'exception de la densité de végétation.

# 4. Discussion

# 4.1. Sélectivité de l'herbivorie

Les Orthoptères consomment avant tout le Brachypode et montrent ainsi une préférence alimentaire, en cohérence avec Joern et Lawlor [34], Cottam [35] et Le Gall [36]. La préférence alimentaire chez les Orthoptères peut être liée à la dominance de l'espèce végétale dans le milieu : Cottam [35] a montré que le criquet Omocestus viridulus consommait de préférence la plante la plus abondante. Dans notre étude, l'herbivorie n'est pas fréquence-dépendante puisque la pression d'herbivorie est maximale sur la graminée la moins dominante. La sélectivité alimentaire des Orthoptères pourrait alors s'expliquer soit par le développement de défenses physiques chez le Brome, soit par la présence de composés chimiques (métabolites secondaires, sucres, nutriments) susceptibles d'altérer l'appétence du Brome voire d'augmenter celle du Brachypode [36–39]. Quoique plus consommé par les Orthoptères, le Brachypode présente une appétence pour le bétail inférieure à celle du Brome [25,30]. Toutes les espèces du genre Brachypodium [40] ainsi que d'autres graminées parmi lesquelles ne figure pas le Brome érigé [41], possèdent des grains de silice dans leurs tissus foliaires, ce qui est souvent interprété comme une adaptation des plantes à l'herbivorie, notamment par les ongulés [42]. Contrairement au bétail, les Orthoptères sont peu affectés par la teneur des feuilles en silice du fait de leurs mandibules coriaces et des adaptations de leur tractus digestif; ils consomment en outre préférentiellement les pousses de graminées matures c'est-à-dire les plus riches en silice [36].

#### 4.2. Déterminisme de l'herbivorie

Les dégâts d'herbivorie diminuent fortement avec l'ombrage pour les deux espèces, et avec la fauche pour le Brachypode. La sécheresse engendre une réponse en deux temps : l'herbivorie augmente en juillet sur le Brome mais diminue en août sur le Brachypode. Les dégâts d'herbivorie sont, pour les deux espèces, positivement corrélés au microclimat (D.V.S., températures de l'air et du sol) mais négativement à la fertilité du sol et à la densité de Brachypode et d'autres graminoïdes.

La relation entre herbivorie et microclimat a déjà été mentionnée, notamment par Coll et Bottrell [43] et Schoonhoven et al. [44], mais à notre connaissance, aucune étude ne l'avait quantifiée à la suite de manipulations environnementales en conditions naturelles. Nos résultats sont cohérents avec le fait que les Orthoptères soient habituellement associés à des environnements secs et chauds [45,46]. Sous l'ombrage, la diminution du déficit en vapeur saturante et de la température [22] engendre un microclimat défavorable aux Orthoptères, ce qui pourrait expliquer la diminution d'herbivorie. Cette diminution d'herbivorie avec

Tableau 5.	Relations	entre her	bivorie e	et facteurs	environne	ementaux	en j	juillet e	et août	1999.	Résultats	des	corrélations	(coefficient	de
Pearson) e	ntre les dég	âts subits	par les	plantes ét	udiées (Br	ome, Bra	chyp	oode) e	t les fa	acteurs	abiotiques	s (a)	ou biotiques	( <i>b</i> )	

			Juillet		Août
		Bromus	Brachypodium	Bromus	Brachypodium
	n	r	r	r	r
a. Facteurs abiotiques					
Déficit en vapeur saturante	8	0,82 *	0,82 *	0,61	0,43
Température de l'air	8	0,85 **	0,87 **	0,65	0,54
Température du sol	8	0,79 *	0,85 **	0,62	0,46
Humidité du sol	8	-0,87 **	-0,76 *	-0,06	0,05
Azote total disponible	8	-0,81 *	-0,93 **	-0,47	-0,26
b. Facteurs biotiques					
Densité de végétation	8	-0,62	-0,62	-0,71 *	-0,60
Densité de Brome	8	0,27	0,34	-0,43	-0,42
Densité de Brachypode	8	-0,79 *	-0,81 *	-0,53	-0,37
Densité d'autres graminoïdes	8	-0,81 *	-0,84 **	-0,63	-0,49
Densité de légumineuses	8	0,15	0,02	-0,01	-0,06
Densité d'autres dicotylédones	8	-0,31	-0,33	0,12	0,01

\*\*\*, \*\*, \* significativités aux seuils respectifs de P < 0,001; 0,01 et 0,05.

l'ombrage est confirmé par Murcia [47], mais Meiners et al. [48] mettent en évidence une variation non linéaire de l'herbivorie par les insectes sur Acer rubrum en fonction de l'ouverture forestière. Ainsi, l'herbivorie augmente fortement de la forêt vers la lisière forestière, confirmant les résultats de Murcia [47], mais diminue de la lisière à la prairie. La forte herbivorie en lisière semble être davantage liée à un 'effet bordure' qu'à l'effet propre de la différence d'ouverture des milieux [48]. Ces deux études mentionnent l'importance des modifications microclimatiques engendrées par l'ouverture mais ne les relient pas explicitement aux variations d'herbivorie observées. L'amélioration des conditions microclimatiques pour les Orthoptères, avec l'augmentation du déficit en vapeur saturante [22], permet également d'expliquer l'augmentation d'herbivorie sur le Brome au mois de juillet dans les lots asséchés.

Le modèle de variation d'herbivorie proposé par Oksanen et al. [10] est avant tout basé sur la ressource primaire de l'écosystème. Les herbivores ne peuvent se maintenir que si la biomasse végétale est suffisamment abondante (contrôle ascendant de l'herbivorie), et l'abondance de ressource primaire permet la présence de fortes populations d'herbivores qui favorisent les prédateurs, responsables du contrôle descendant de l'herbivorie. Dans notre étude, la diminution d'herbivorie du Brachypode dans les lots fauchés et asséchés peut s'expliquer par une régulation ascendante de l'herbivorie et s'intègrent donc dans le modèle d'Oksanen et al. [10]. La fauche réduit fortement la ressource pour les herbivores et la diminution d'herbivorie qui en découle est particulièrement sensible sur le Brachypode

Orthoptères. L'influence de la fauche sur l'intensité d'herbivorie est d'autre part suggérée par la disparition des corrélations entre herbivorie et microclimat au mois d'août 1999 (c'est-à-dire après la fauche). L'effet négatif de la sécheresse au mois d'août sur l'herbivorie pourrait s'expliquer par une diminution qualitative et quantitative de la biomasse végétale due au déficit hydrique prolongé (depuis début mai). Cependant, la corrélation négative de l'herbivorie avec la densité de Brachypode, qui est pourtant la ressource préférentielle des Orthoptères, nous indique que la régulation trophique ne serait pas l'unique déterminisme des variations d'herbivorie. Dans le milieu le plus productif (lots ombrés), la prédation est plausible de par la présence importante d'araignées, susceptibles de causer de fortes mortalités dans les populations d'Orthoptères [49,50]. Mais la prédation n'est pas exclusive des lots ombrés puisque des prédateurs potentiels pour les Orthoptères, comme par exemple les oiseaux [46,51], agissent en dehors du voile d'ombrage. Des alternatives à l'hypothèse trophique de régulation de l'herbivorie sont à considérer. Notre étude suggère une régulation microclimatique de l'herbivorie, s'appuyant sur la corrélation positive de l'herbivorie avec le déficit en vapeur saturante et la température. D'autres investigations [7] expliquent la diminution d'herbivorie dans les milieux productifs par une faible efficacité de prospection de petit vertébrés tels que les lièvres, les lapins et les oies sauvages et ne retiennent pas la prédation comme un élément régulateur de l'herbivorie dans les milieux à végétation haute.

car c'est l'espèce dominante la plus appétente pour les

# **4.3.** Vers une approche plus fonctionnelle de l'herbivorie

Dans la présentation de leur modèle, Oksanen et al. [10] reconnaissent une alternative à la régulation trophique de l'herbivorie en invoquant la sensibilité des Arthropodes à la température. Ceci est confirmé par la corrélation positive de l'herbivorie par les Orthoptères vis-à-vis de la température mais aussi du déficit en vapeur saturante qui ressort de notre étude et d'autres travaux faisant état des exigences écologiques des populations d'Orthoptères [45,46]. Par ailleurs, Coll et Bottrell [43] ont souligné l'hétérogénéité des résultats des études portant sur les interactions plante-herbivore qui semblent liés à la large gamme d'herbivores impliqués dans les études. L'assimilation du niveau trophique « herbivore » à une entité écologique à part entière est sans doute trop réductionniste (Oksanen et al. [10]). Une approche fonctionnelle de l'herbivorie, tenant compte des traits biologiques des espèces d'herbivores, serait de nature à résoudre un certain nombre de contradictions apparentes. Ainsi, deux grands types d'herbivores peuvent être distingués, en fonction de leur taille et de leur métabolisme énergétique. Les herbivores vertébrés, tels que le bétail [52-54], les grands mammifères sauvages [11,55] et les micromammifères [6,56–58] sont de plutôt de grande taille et sont homéothermes, alors que les herbivores invertébrés tels que les limaces [9,18,35,59] ou les insectes [18,60,61] sont de petite taille et sont ectothermes.

La grande taille des vertébrés implique un fort prélèvement de végétation, une sélectivité relativement faible (à l'échelle de la touffe d'herbe) et des pertes de biomasse importante pour les plantes. L'herbivorie par les vertébrés dépend fortement de la quantité de biomasse végétale présente dans le milieu pour subvenir aux besoins de l'herbivore. Ce type d'herbivorie est susceptible d'être dirigé ou au moins d'être fortement influencée par les activités humaines (conduite de troupeaux, parcage de bétail, chasse). A l'intérieur du type « vertébrés », les herbivores tels que les rongeurs ou les oies doivent être distingués du fait de leur petite taille, réduisant leur efficacité dans les milieux productifs, et de leur plus grande spécialisation par rapport aux ongulés et au bétail [62].

La petite taille des invertébrés permet une herbivorie plus sélective (à l'échelle du brin d'herbe), plus discrète et qui se manifeste par une quantité importante d'impacts sur la végétation. Les herbivores invertébrés sont relativement indépendants des activités humaines et constituent une des seules populations non domestiques agissant dans les milieux anthropisés, comme par exemple les pâtures. Les invertébrés sont essentiellement ectothermes et leur activité est particulièrement dépendante du microclimat, parfois de manière opposée : ainsi, les Acrididés sont liés aux habitats chauds et surtout secs et les mollusques terrestres (limaces, escargots) aux milieux humides.

Cette distinction entre types d'herbivores permet d'expliquer les différents modèles de régulation d'herbivorie et certains résultats contradictoires. Ainsi, l'hypothèse de régulation descendante de l'herbivorie développée par Oksanen et al. [10] s'appuie essentiellement sur les vertébrés, notre hypothèse de régulation microclimatique sur les Arthropodes, et l'hypothèse de régulation par efficacité de prospection sur de petits vertébrés. En outre, l'herbivorie due aux Arthropodes décroît lorsque la biomasse augmente ([14,63], nos résultats), alors que l'herbivorie causée par les grands vertébrés augmente avec la biomasse [20]. Malgré leur faible prélèvement, en comparaison des grands mammifères, les Arthropodes peuvent cependant avoir de fortes répercussions sur la structure et la dynamique des communautés végétales de par leur sélectivité et les variabilités démographiques qui les affectent [27].

# Références

[1] Pimm S.L., Lawton J.H., Cohen J.E., Food web patterns and their consequences, Nature 350 (1991) 669-674.

[2] McBrien H., Harmsen R., Crowder A., A case of insect grazing affecting plant succession, Ecology 64 (1983) 1035–1039.

[3] Brown V.K., Gange A.C., Secondary plant succession : how is it modified by insect herbivory? Vegetatio 101 (1992) 3–13.

[4] Dormann C.F., van der Wal R., Bakker J.P., Competition and herbivory during salt marsh succession: the importance of forb growth strategy, J. Ecol. 88 (2000) 571–583.

[5] Proulx M., Mazumder A., Reversal of grazing impact on plant species richness in nutrient-poor vs. nutrient rich ecosystems, Ecology 79 (1998) 2581–2592.

[6] van der Wal R., Egas M., van der Veen A., Bakker J., Effects of resource competition and herbivory on plant performance along a natural productivity gradient, J. Ecol. 88 (2000) 317–330.

[7] van de Koppel J., Huisman J., van der Wal R., Olff H., Patterns of herbivory along a productivity gradient: an empirical and theorical investigation, Ecology 77 (1996) 736–745.

[8] Fraser L.H., Grime J.P., Top-down control and its effects on the biomass and composition of three grasses at high and low soil fertility in out door microcosms, Oecologia 113 (1998) 239–246.

[9] Fraser L.H., Grime J.P., Interacting effects of herbivory and fertility on a synthesized plant community, J. Ecol. 87 (1999) 514–525.

[10] Oksanen L., Fretwell S.D., Arruda J., Niemelä P., Exploitation ecosystems in gradients of primary productivity, Am. Nat. 118 (1981) 240–261.

[11] Belsky A.J., Effects of grazing, competition, disturbance and fire on species composition and diversity in grassland communities, J. Veg. Sci. 3 (1992) 187–200.

[12] Bonser S.P., Reader R.J., Plant competition and herbivory in relation to vegetation biomass, Ecology 76 (1995) 2176–2183.

[13] Jones C.G., Coleman J.S., Plants stress and insect herbivory: toward an integrated perspective, in : Editors (Ed.), Response of Plants to Multiple Stresses, Academic Press, 1991, pp. 249–280.

[14] Rand T.A., Effects of environmental context on the susceptibility of *Atriplex patula* to attack by herbivorous beetles, Oecologia 121 (1999) 39–46.

[15] Reader R.J., Herbivory, competition, plant mortality and reproduction on a topographic gradient in an abandoned pasture, Oikos 65 (1992) 414–418.

[16] Louda S.M., Differential predation pressure : a general mechanism for structuring communities along complex environmental gradients? Trends Ecol. Evol. 4 (1989) 158–159.

[17] Crawley M.J., Plant-herbivore dynamics, in : Editors (Ed.), Plant Ecology, Blackwell Science, Oxford, 1997, pp. 401–474.

[18] Ledergerber S., Thommen G.H., Baur B., Grazing damage to plants and gastropod and grasshopper densities in a COenrichement experiment on calcareous grassland, Acta Oecol. 18 (1997) 255–261.

[19] Peters H.A., Baur B., Bazzaz F., Körner C., Consumption rates and food preferences of slugs in a calcareous grassland under current and future CO<sub>2</sub> conditions, Oecologia 125 (2000) 72–81.

[20] Gough L., Grace J.B., Herbivore effects on plant species density at varying productivity levels, Ecology 79 (1998) 1586–1594.

[21] Jarzomski C.M., Stamp N.E., Bowers M.D., Effects of plant phenology, nutrients and herbivory on growth and defensive chemistry of plantain, *Plantago lanceolata*, Oikos 88 (2000) 371–379.

[22] Corcket E., Approche expérimentale de la compétition entre graminées dominantes et de l'herbivorie par les Orthoptères dans une pelouse sèche collinéenne, thèse, université Joseph-Fourier, Grenoble, 2001.

[23] Tansley A.G., Adamson R.S., Studies of the vegetation of the English chalk. III. The chalk grasslands of the Hampshire-Sussex border, J. Ecol. 13 (1925) 177–223.

[24] Dutoit T., Alard D., Lambert J., Frileux P.N., Biodiversité et valeur agronomique des pelouses calcicoles : effets du pâturage ovin, Fourrages 142 (1995) 145–158.

[25] Delescaille L.M., La gestion conservatoire des pelouses sèches par le pâturage ovin. Aspects théoriques et pratiques, Parcs et Réserves 54 (1999) 2–9.

[26] Voisin J.F., Evolution des peuplements d'Orthoptères dans le canton d'Aime (Savoie), Trav. Sci. Parc Nation. Vanoise XV (1986) 229–254.

[27] Voisin J.F., Observations sur une pullulation d'Aeropus sibiricus (Insecta, Orthoptera) en Grande Sassière (Savoie) : évolution pendant les années 1987 et 1988, Trav. Sci. Parc Nation. Vanoise XIX (1995) 183–190.

[28] Météo-France, Bulletin climatologique mensuel de l'Isère, décembre.[29] Braun-Blanquet J., Moor M., Prodromus des Pflantzengesellschaften.Verbrand des *Bromion erecti*, SIGMA 5 (1938) 64 p.

[30] Barbaro L., Dynamique agro-écologique des communautés de pelouses sèches calcicoles du Vercors méridional : application à la gestion conservatoire de la biodiversité par le pastoralisme, université Joseph-Fourier, Grenoble, 1999.

[31] Dutoit T., Alard D., Les pelouses calcicoles du nord-ouest de l'Europe (*Brometalia erecti* Br. Bl. 1936) : analyse bibliographique, Ecologie 27 (1996) 5–34.

[32] Besnard G., Carlier G., Le régime hydrique de l'Aulne blanc (*Alnus incana* (L.) Moench) dans une forêt riveraine du Haut Rhône, Bull. Ecol. 19 (1988) 1–11.

[33] Verkaar H.J., Are defoliators beneficial for their host plants in terrestrial ecosytems. A review, Acta Bot. Neerl. 37 (1988) 137–152.

[34] Joern A., Lawlor L.R., Food and microhabitat utilization by grass-hoppers from arid grasslands: comparisons with neutral models, Ecology 61 (1980) 591–599.

[35] Cottam D.A., Frequency-dependent grazing by slugs and grasshoppers, J. Ecol. 73 (1985) 92–933.

[36] Le Gall P., Le choix des plantes nourricières et la spécialisation trophique chez les *Acrididea* (Orthoptères), Bull. Ecol. 20 (1989) 245–261.

[37] Hartley S.E., Jones C.G., Plant chemistry and herbivory, or why the world is green, in : Editors (Ed.), Plant Ecology, Blackwell Science, Oxford, 1997, pp. 284–324.

[38] Agrawal A.A., Rutter M.T., Dynamic anti-herbivore defense in ant-plants : the role of induced responses, Oikos 83 (1998) 227–236.

[39] Linhart Y.B., Chaouni-Benabdallah L., Parry J.M., Thompson J.D., Selective herbivory of the thyme chemotypes by a mollusk and a grasshopper, Ecologia Mediterranea 25 (1999) 147–151.

[40] Schippmann U., Revision der europäischen Arten der Gattung Brachypodium Palisot de Bauvois (Poaceae), Boissieria 45 (1991) 249.

[41] Cherouvier A., Gueguen A., Lefeuvre J.C., Essai de détermination du régime alimentaire d'animaux herbivores à l'aide de phytolithes siliceux des Graminées et des Cypéracées. Description, après étude en microscopie électronique à balayage, des principaux types de phytolithes rencontrées, C. R. Acad. Sci. Paris, sér. III 281 (1975) 839–842.

[42] McNaughton S.J., Tarrants J.L., McNaughton M.M., Davis R.H., Silica as a defense against herbivory and a growth promotor in African grasses, Ecology 66 (1985) 528–535.

[43] Coll M., Bottrell D.G., Effects of nonhost plants on an insect herbivore in diverse habitats, Ecology 75 (1994) 723–731.

[44] Schoonhoven L.M., Jermy T., van Loon J.J.A., Insect-plant biology. From physiology to evolution, London, 1998.

[45] Chopard L., Orthoptéroïdes, collection Faune de France, 56, Paris, 1951.

[46] Ritchie M.E., Nitrogen limitation and trophic vs. abiotic influences on insect herbivores in a temperate grassland, Ecology 81 (2000) 1601–1612.

[47] Murcia C., Edge effects in fragmented forests: implications for conservation, Trends Ecol. Evol. 10 (1995) 58-62.

[48] Meiners S.J., Handel S.N., Pickett S.T.A., Tree seedling establishment under insect herbivory: edge effects and inter-annual variation, Plant Ecol. 151 (2000) 161–170.

[49] Blandin P., Celerier M.L., Les araignées des savanes de Lamto (Côte d'Ivoire). Organisation des peuplements, bilans énergétiques, place dans l'écosystème, Publ. Lab. Zool. E.N.S. 21 (1981) 1–586.

[50] Oedekoven M.A., Joern A., Plant quality and spider predation affects grasshoppers (*Acrididae*): food-quality-dependent compensatory mortality, Ecology 81 (2000) 66–77.

[51] Thiollay J.M., Besoins alimentaires quantitatifs de quelques oiseaux tropicaux, La Terre et la Vie 32 (1976) 89–135.

[52] Austin M.P., Williams O.B., Belbin L., Grassland dynamics under sheep grazing in an Australian Mediterranean type climate, Vegetatio 47 (1981) 201–211.

[53] Collins S., Interaction of disturbances in tallgrass prairie : a field experiment, Ecology 68 (1987) 1243–1250.

[54] Rousset O., Lepart J., Positive and negative interactions at different life stages of a colonizing species (*Quercus humilis*), J. Ecol. 88 (2000) 401–412.

[55] Oksanen L., Ranta E., Plant strategies along mountain vegetation gradients: a test of two theories, J. Veg. Sci. 3 (1992) 175–186.

[56] Pykes D.A., Demographic responses of *Bromus tectorum* and seedlings of *Agropyron spicatum* to grazing by small mammals: occurrence and severity of grazing, J. Ecol. 74 (1986) 739–754.

[57] Boyd R.S., Microdistribution of the beach plant *Cakile maritima* (*Brassicaceae*) as influenced by a rodent herbivore, Am. J. Bot. 75 (1988) 1540–1548.

[58] Prins A.H., Nell H.W., Positive and negative effects of herbivory on the population dynamics of *Senecio jacobea* L. and *Cynoglossum officinale* L, Oecologia 83 (1990) 325–332.

[59] Rodriguez M.A., Brown V.K., Plant competition and slug herbivory : effects on the yield and biomass allocation pattern of *Poa annua* L, Acta Oecol. 19 (1998) 37–46.

[60] Escarré J., Lepart J., Sans X., Sentuc J.J., Gorse V., Effects of herbivory on the growth and reproduction of *Picris hieracioides* in the Mediterranean region, J. Veg. Sci. 10 (1999) 101–110.

[61] Parmesan C., Unexpected density-dependant effects of herbivory in a wild population of the annual *Collinsia torreyi*, J. Ecol. 88 (2000) 392–400.

[62] Huisman J., Olff H., Competition and facilitation in multispecies plant-herbivore systems of productive environments, Ecol. Lett. 1 (1998) 25–29.

[63] Ellison A.M., Effects of competition, disturbance, and herbivory on *Salicornia europea*, Ecology 68 (1987) 576–586.

# The relative importance of competition for two dominant grass species as affected by environmental manipulations in the field<sup>1</sup>

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Abstract: We examined how shade, drought and disturbance influenced the intensity and relative importance of competition experienced by Bromus erectus and Brachypodium pinnatum, the two dominant species of a calcareous grassland. Competition was intense in all treatments for both species, but its importance differed substantially. Competition was not important for Brachypodium when the drought was severe. Shade had a strong positive effect on Brachypodium by decreasing water stress. Bromus was more tolerant of drought and disturbance than Brachypodium, but was more inhibited by competition, which was important in all environmental conditions. Differences in the relative importance of Bromus in conditions of high stress and disturbance and its exclusion in mesic grasslands. We suggest that the debate on how competition varies along productivity gradients may be due to the focus on competition intensity, which is highly dependant on particular target species and study systems, rather than on the relative importance of competition in different conditions. Keywords: Brachypodium, Bromus erectus, competition, drought, mowing, shade.

*Résumé*: Nous avons mesuré les variations d'intensité et d'importance relative de la compétition subies par *Bromus* erectus et Brachypodium pinnatum dans une pelouse calcicole à haut et bas niveaux d'ombrage, de sécheresse et de perturbation. La compétition était intense dans tous les traitements, mais son importance différait selon l'espèce. La compétition ne s'est pas avérée importante pour le brachypode dans les lots de sécheresse intense. Par contre, l'ombrage a eu un fort effet positif sur le brachypode en modifiant les disponibilités en eau et en nutriments. Le brome s'est montré plus tolérant à la sécheresse et à la perturbation que le brachypode, mais il était fortement affecté par la compétition. Les différences d'importance relative de la compétition pour le brome et le brachypode et les effets de l'environnement permettent d'expliquer la dominance du brome en conditions de contrainte hydrique et de forte perturbation, ainsi que son exclusion des prairies mésiques. Nous pensons que les résultats des études expérimentales concernant les variations de compétition le long de gradients de productivité sont difficiles à interpréter, car jusqu'à ce jour, les auteurs se sont surtout concentrés sur l'intensité de la compétition, fortement dépendante des espèces cibles et des systèmes d'étude, plutôt que sur l'importance de la compétition.

Mots-clés : Brachypodium pinnatum, Bromus erectus, compétition, fauche, ombrage, sécheresse.

Nomenclature: Fournier, 1990.

#### Introduction

Interspecific competition has been a focal interaction in plant community ecology for decades, but the conditions in which competition is significant are still debated (Grime, 1974; Welden & Slauson, 1986; Tilman, 1987; Thompson & Grime, 1988; Goldberg & Barton, 1992; Goldberg & Novoplansky, 1997; Brooker & Callaghan, 1998; Goldberg *et al.*, 1999). There are two primary models for how competition varies in intensity or importance along environmental gradients. A number of researchers argue that total competition is intense and important in productive communities only and that it decreases when abiotic stress increases (Grime, 1979; Keddy, 1989). Other researchers argue that total competition is intense and important whatever the kind of environment or productivity level: total competition includes aboveground

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and underground components respectively driven by light and nutrient resource availability (Newman, 1973; Tilman, 1988). Taylor, Aarssen, and Loehle (1990) proposed a third model, in which competition is also independent of productivity, but driven by the ratio of resource supply to demand, which in turn depends on the rate of disturbance.

There have been two general experimental approaches used to evaluate the dynamic (Grime's model) versus stable (Tilman's model) nature of competition, although the two cannot be completely separated. One approach has been to change the productivity or biomass of the communities in which plant species compete, and the second has been to experimentally manipulate the abiotic conditions in which plant species compete. In either case, the fundamental goal of these studies is to know whether or not competition intensity is similar over a wide range of conditions (stable) or variable over a wide range of conditions (dynamic). As observed by Goldberg and Barton

(1992), results from experiments that use natural gradients of productivity are generally consistent with Grime's hypothesis that competition intensity is dynamic, i.e., positively correlated with productivity and decreasing with increasing abiotic stress (Gurevitch, 1986; Wilson & Keddy, 1986; Reader & Best, 1989; Pennings & Callaway, 1992; Twolan-Strutt & Keddy, 1996; Gerdol et al., 2000; Pugnaire & Luque, 2001; Choler, Michalet & Callaway, 2001; Callaway et al., 2002), whereas results from experiments using manipulations of specific abiotic conditions are generally consistent with the Tilman model: total competition intensity is similar across gradients of stress and productivity (Wilson & Shay, 1990; Di Tommasso & Aarssen, 1991; Wilson & Tilman, 1991; Wilson & Tilman, 1995; Peltzer, Wilson & Gerry, 1998; Cahill, 1999; Brown & Archer, 1999).

Not all experimental studies fall neatly into one of these two categories. Reader et al. (1994) and Belcher, Keddy, and Twolan-Strutt (1995) found no increase in competition intensity along natural gradients of productivity, whereas Kadmon (1995) tested both approaches (natural versus experimental gradients) within the same system along a gradient of moisture availability and found an increase in competition intensity with both approaches. Other experimental manipulations of water have found the opposite result. Novoplansky and Goldberg (2001) found no variation in competition intensity with increasing water availability in a glass house experiment for three grass species, and Davis, Wrage, and Reich (1998) found a decrease of competition intensity with increasing water availability for tree seedlings competing with grass species. These contrasted results may largely be dependent on the competitive ability of particular species, which may differ along productivity gradients (Mahmoud & Grime, 1976; Wilson & Keddy, 1986; Dutoit et al., 2001). Therefore, competition intensity between species may decrease or remain the same across productivity gradients because of changes in the identity of target species (Goldberg et al., 1999), but the relative importance of competition for structuring communities is more likely to increase with productivity across the same gradient because the relative importance of abiotic stress for target species is likely to decrease along the same gradient (Grime, 1974). The distinction between the intensity (i.e.,the decrease in plant growth or survival due to neighbours) and the importance of competition (i.e., the relative part of the effects of competition compared to the effects of other processes such as stress and disturbance) has been emphasized by Welden and Slauson (1986) and other authors have argued that this subtle distinction may help to resolve the debate about competition, productivity, and environmental stress (Goldberg & Novoplansky, 1997; Brooker & Callaghan, 1998).

1996-1994

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To our knowledge, there have been no experimental field studies designed to quantify both the intensity of competition and the effect of the abiotic environment with the ultimate goal to measure the relative importance of competition. Here, we have conducted experiments in which we have altered the trajectories of a natural plant community by manipulating environmental conditions. Our design allowed us to measure the intensity of competition by comparing the effects of neighbour removal and the importance of competition in different environments by comparing the effects of environmental treatments on target individuals without neighbours.

## Methods

# STUDY SITE AND TARGET SPECIES

The experiment was performed in Saint-Nizier d'Uriage (700 m a.s.l.,  $45^{\circ}$  10' N,  $5^{\circ}$  50' E) in the foothills of the French Alps, about 15 km east of Grenoble. The field site, "Liche Petet", has a south-southwest exposure with a slope of 25°. The soil is a 30-cm-deep Inceptisol (38% sand, 35% silt, 20% clay, and 7% organic matter) and is underlain by a 1-m-deep calcareous glacial deposit (Würm) that covers Jurassic calcareous shale. This grassland has been extensively grazed by sheep and cattle for decades and is dominated by *Bromus erectus* (54% cover) and *Brachypodium pinnatum* (15%). At Liche Petet, as in many of these mesoxeric grasslands, shrubs and trees (*Rosa* spp., *Fraxinus excelsior, Juniperus communis*, and *Pinus sylvestris*; currently <5% cover) are encroaching and creating shade.

Bromus erectus and Brachypodium pinnatum are both perennial grasses, but Bromus is cespitose, whereas Brachypodium is rhizomatous and clonal (de Kroon, Hara & Kwant, 1992). Both species are considered as stress-tolerant competitors (Grime, 1979). Bromus occurs mainly in open, xeric habitats and in grazed or mowed grasslands, whereas Brachypodium occurs in more mesic grasslands and increases in abundance in intermediate shaded habitats (forest edges) and abandoned fields (Wilczek, Beauvert & Dutoit, 1928; Royer, 1987; Barbaro & Cozic, 1998).

### EXPERIMENTAL DESIGN

We designed our experiment to mimic shade (caused by shrub and tree encroachment), soil moisture (caused by substrate and climate variation), and disturbance (caused by hay mowing) in a fully factorial design of eight  $32\text{-m}^2$  plots: control (C), shade (S), drought (D), mowing (M), shade and drought (SD), shade and mowing (SM), drought and mowing (DM), and shade, drought, and mowing (SDM). One-metre buffer areas separated each plot.

In the foothills around Grenoble, as in many other places in the Alps, the landscape is fragmented into a great number of small units, due to the great heterogeneity of land use and ecological conditions. These differences between the grasslands made it difficult for us to find two Mesobromion-type grasslands that were dominated by Bromus erectus and extensively grazed, i.e., to replicate the site. The Liche Petet grassland is typical of these small fragments (100 m  $\times$  80 m), and placing a large experimental unit (30 m  $\times$  30 m) within the fragment did not permit replication while avoiding shading effects from isolated and border trees and obvious topographic heterogeneousness. We followed Oksanen (2001) and emphasized scale effect over experimental replication. Rather than substantially decreasing the size of experimental plots to replicate treatment combinations within

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# CORCKET ET AL.: RELATIVE IMPORTANCE OF COMPETITION

the Liche Petet grassland, we opted for a technically pseudoreplicated experimental design in order to have the ecologically realistic benefit of having an area as big as feasible at the experimental site. The main reason for this decision was to allow us to account for the ecosystemscale effects of our treatments, such as nutrient cycling, soil moisture, and dynamics of small herbivores (Corcket et al., 2002). Because our experiment is pseudoreplicated, we carefully chose an area with homogeneous species composition, exposure, microtopography, and substrate, and we compared target plant size, total biomass, biomass of Bromus, biomass of Brachypodium, and soil depth in all eight plots prior to applying the treatments. Furthermore, to test whether growth trajectories of the targets were spatially homogeneous at Liche Petet, we conducted an additional experiment during the 2001 growing season. New individuals of Bromus erectus and Brachypodium pinnatum (grown from local seeds in a greenhouse) were transplanted at the same life stage (3-4 leaves) in four 16-m<sup>2</sup> control plots randomly distributed throughout the grassland. The number of leaves was counted on each of the targets with or without neighbours (8 replicates per plot) at the beginning of the experiment, in early May, and at the end of the experiment, in late September.

Shade was applied to the four plots on the east side of the experimental site using a  $350\text{-m}^2 50\%$  shade cloth fixed permanently at 1.70 m directly above the plots and extended to 50 cm above the ground on the sides to avoid edge effects in the morning and evening. The net was installed each year in late April and removed in late October to mimic natural shading by the deciduous canopy species invading the grassland.

Soil moisture in the four  $32-m^2$  plots in the lower half of the experimental site was reduced below normal in two ways. First,  $15\text{-m} \times 13\text{-m}$  transparent plastic sheets were manually extended over the plots before (or soon after the beginning of) every precipitation event between late April and late June of 1998 and early May and late September of 1999. The plastic sheets were pulled back immediately after each precipitation event to avoid any greenhouse effect. Based on precipitation data from a nearby weather station, we estimate that >90% and > 70% of the rainfall during the experimental drought was intercepted in 1998 and 1999, respectively. The plastic sheets were removed in late June in 1998 because of an exceptionally dry spring preceding the experimental treatment. The transparency of the plastic cover allowed almost complete light transmission.

Second, overland flow into the dried plots was reduced by digging 1-m-deep and 0.5-m-wide trenches around the upper ends of the plots. Trenching was conducted in the four plots situated down slope so as not to affect soil moisture in the four plots in which soil moisture was not reduced and to establish a conservative dry treatment on the part of the slope where the soils were likely to be the deepest.

Traditional farming for hay in these meadows was simulated by cutting vegetation in plots with a lawn mower. In the first year, we mowed four plots in late

April 1998 to mimic an early mowing. In the second year, we mimicked a late mowing in the same plots on 15 July 1999. Irregular mowing is common in these non-fertile grasslands (Thierry Vianey-Liaud, local Liche Petet farmer, pers. comm.). Mowed biomass was removed from experimental plots.

We sampled 250 g of soil at 10-15 cm depth, with three replications per plot, to determine soil moisture in each of the eight plots. Samples were collected in June 1998, June 1999, and September 1999. Soil moisture was determined after drying samples at 105°C for 72 h. Available forms of nitrogen (N-NH4<sup>+</sup> and N-NO3<sup>-</sup>) were determined in each of the eight plots using three replicates of the soil samples in June 1999 and September 1999. After storage in the field at 4°C, extractions of N-NO3<sup>-</sup> (in water) and N-NH4+ (in 1 M KCl) were performed immediately in the lab and then stored at -18°C (Wheatley, McDonald & McSmith, 1989). N-NO3- was determined by ion chromatography (Dionex 4500i, Dionex Corporation, Sunnyvale, California), and N-NH4<sup>+</sup> was determined with the blue indophenol method (Dorich & Nelson, 1983). Nitrogen content was expressed in mg of  $N-NH_4^+$  and  $N-NO_3^-$  in 1 kg of dried soil.

In each plot, 24 target individuals of the two experimental species were chosen in late April 1998, such that (i) targets were scattered throughout the entire plot, (ii) targets were surrounded almost exclusively by individuals of the other species, (iii) targets did not have obvious connections with other ramets of the same species, and (iv) targets were in a similar morphological stage of development (i.e., the number of leaves and height were comparable and there were no reproductive spikelets). Because Bromus erectus is a cespitose species, individual targets were easily identified for permanent tagging. Brachypodium pinnatum is a rhizomatous clonal species, but the compactness of the clones (de Kroon, Hara & Kwant, 1992) and the tendency of individuals to recruit from seed (Schlaepfer, 1997) allowed us to select targets that were isolated and not connected to other ramets. The target individuals may therefore be considered as independent. All targets were marked with plastic twist ties loosely around the base, and growth responses of these targets were followed for two years.

To measure competition intensity in each plot and for each species, in late April 1998 we clipped all aboveground biomass of neighbouring plants at ground level and in a radius of 15 cm around 12 randomly chosen target individuals of the original 24 targets for each species. Because we used *in situ* targets instead of transplants, we only removed aboveground biomass in our experiments, without direct manipulation of belowground biomass.

All regrowth of neighbouring plants was cut back every 3-4 weeks. We counted the number of green leaves from late April 1998 to late October 1999. The growth of target individuals was assessed by the change in leaf number during the course of the experiment, calculated as the difference between leaf number at the end and at the beginning of the experiment.

We did not harvest target species at the end of the experiment because target plant roots could not be sepa-

rated from neighbouring roots in the field; however, we measured the relationship between leaf number and total biomass for both species in a greenhouse experiment. Seeds collected at the study area were planted in pots containing a mixture of 1/4 field soil and 3/4 sand to facilitate root extraction from the soil of the pots. Plants were grown in a complete factorial experiment with high and low soil moisture, full sunlight, and 50% shade, with 15 replicates per species and treatment combination. For each species, after three months, the number of leaves was highly related to total plant biomass ( $r_{Bromus} = 0.86$ , p < 0.001;  $r_{Brachypodium} = 0.79$ , p < 0.001).

To analyze the variation in competition intensity along environmental treatments, we calculated the Relative Neighbour Effect:  $RNE = [P_N - P_+N]/x$ , where  $P_N$  is the performance of plants without neighbours,  $P_+N$  the performance of plants with neighbours, and x the greater absolute value between  $P_{-N}$  and  $P_{+N}$  (Markham & Chanway, 1996). To evaluate the effect of our environmental treatments on the potential growth of our target species, we calculated an index of Relative Environmental Constraint Intensity: RECI= $[P_C - P_T]/y$ , where  $P_C$  is the performance of plants without neighbours in the control plot, PT the performance of plants without neighbours in the treated plot, and y the greatest absolute performance among  $P_C$  and  $P_T$ . The principle of RECI is very similar to that of RNE. When RECI was positive the treatment had a negative effect on target-species growth; when RECI was negative the treatment had a positive effect on targetplant growth. The use of target performances without neighbours rather than with neighbours in the calculation of RECI allows the metric to be independent of competition. Afterwards, we are able to compare the relative importance of plant interactions (RNE) or environmental constraints (RECI) on potential performances (i.e., with low level of plant interactions [without neighbours] and low level of stress or disturbance [control plot]) of target species.

# STATISTICAL ANALYSES

Paired *t*-test analyses were performed on the mean value of the three replicates per plot of soil moisture, soil content in N-NH<sub>4</sub><sup>+</sup>, and soil content in N-NO<sub>3</sub><sup>-</sup>. The mean value of an experimental plot was compared with the associated control plot for each treatment (*i.e.*, for shade effect, C versus S plots, D versus SD plots, M versus SM plots, DM versus SDM plots).

Statistical analyses on survival and change in leaf number were performed as six different models, crossing the two species (*Bromus* and *Brachypodium*) and the three environmental manipulations (shade, drought, mowing). Differences in survival for each target species were analyzed with logistic regressions using neighbour and environmental treatment (shade, drought, or mowing) as effects. Change in leaf number for *Bromus* and for *Brachypodium* in 1999 were analyzed using three-way ANOVAs crossing block, environmental manipulation, and neighbour as fixed factors. For each environmental treatment (shade, drought, mowing), four blocks were distinguished, each one including one control and one treated plot (*i.e.*, in the shade model, block 1 included C versus S plots, block 2 included M versus SM plots,

block 3 included D versus SD plots, and block 4 included DM versus SDM plots). One aim of replicating experimental treatments in different blocks was to ensure that the responses of plants were due to the treatment effects and were not disturbed by spatial effects. The variance due to the blocks was considerably overestimated: our blocks included not only spatial heterogeneity, but also strong effects of the other crossing factors included in the fully factorial design. Therefore, when one of our experimental treatments or interactions was significant in spite of the overestimated block effect, that experimental treatment or interaction was very strong. Use of the block factor was just a way to test the strength of our experimental treatments; therefore, we only took into account treatments as single effects or first-order interactions, excluding those with a block effect. Our statistical models were conservative because they tended to minimize the effects of experimental manipulations. Analyses for change in leaf number were performed only on individuals that survived to the end of the experiment.

Differences in leaf numbers for either grass species between the eight plots at the beginning of the experiment were tested using one-way ANOVAs. We performed Mann-Whitney tests to analyze for differences in total biomass, biomass of *Bromus*, and biomass of *Brachypodium* between the eight plots prior to the experiment. Differences in leaf number between the four control plots at the end of the 2001 additional experiment were analyzed using a three-way ANOVA (species × neighbour × plot), with plot as a random factor.

In order to analyze variation in competition intensity along environmental treatments, we used regressions, plotting means of RCI of each species per plot against means of RECI in the same plot. Variation in the importance of competition for each species per plot was analyzed by drawing a diagonal line on the previous graph, separating in the upper left part of the graph the plots where the importance of competition overrides the importance of the environmental constraint induced by the treatment and in the lower right part of the graph the plots where the importance of the environmental constraint overrides the importance of competition.

#### Results

#### HOMOGENEITY OF THE FIELD EXPERIMENT

At the beginning of the experiment, there were no significant differences in leaf number for either grass species among the eight plots (one-way ANOVA,  $p \ge 0.836$  in all cases). Mann-Whitney tests on total biomass, biomass of *Bromus* and biomass of *Brachypodium* showed no significant differences between the eight plots prior to treatments ( $p \ge 0.114$  for all comparisons). Soil and glacial deposit depth substantially exceeded 1 m on the eight plots, and grass roots extended no more than 60 cm in depth; therefore, the plots did not differ in available substrate volume. Furthermore, the growth trajectories of the transplanted *Bromus* and *Brachypodium* with or without neighbours in the 2001 experiment were not spatially dependant; there were no significant block effects, whereas there were significant effects of both species and neighbour

treatments (respectively, F=25.4, p=0.015 and F=23.5, p=0.013). These results show that the treatments were initiated in highly homogeneous conditions.

#### ABIOTIC CHANGES DUE TO EXPERIMENTAL TREATMENTS

Among the three treatments, shade had by far the strongest effect on the abiotic environment and altered all of the abiotic variables that we measured. Shade increased soil moisture by 71% in the early summer of 1998 (t=5.8, p=0.010), N-NO<sub>3</sub><sup>-</sup> by 90% in June 1999 (t=7.7, p=0.005), and N-NH<sub>4</sub><sup>+</sup> by 20% in September 1999 (t=3.8, p=0.031). The drought treatment reduced soil moisture by 60% in June 1998 and 49% in September 1999 (respectively t=-28.3, p<0.001 and t=-27.9, p<0.001). N-NH<sub>4</sub><sup>+</sup> and N-NO<sub>3</sub><sup>-</sup> were not significantly affected (t>-1.8, p>0.150). Mowing did not alter soil moisture or any other edaphic variable, with the exception of N-NO<sub>3</sub><sup>-</sup>, which increased by 20% in June 1999 (t=3.4, p=0.042).

# SURVIVAL AND CHANGE IN LEAF NUMBER

Among the four treatments, the removal of neighbours had the most significant effect, enhancing leaf production of both species (Table I; Figure 1). In contrast, the removal of neighbours did not affect survival of each species (r=0.000, p > 0.400).

Shade increased the survival of *Brachypodium* (78.7% versus 88.2%, r=0.184, p=0.004) but did not significantly affect the survival of *Bromus* (r=0.000, p=0.997). Shade enhanced leaf production of *Brachypodium*, in particular when neighbours were removed (shade × neighbour interaction significant; Table Ia; Figure 1a). The effect of shade on *Bromus* leaf number was more complex; there was no significant overall effect, but shade decreased leaf number for target individuals with neighbours, whereas shade increased leaf number for target individuals without neighbours (shade × neighbour interaction significant; Table Ia; Figure 1a).

Drought decreased the survival of *Brachypodium* (85.9% versus 81.2%, r=-0.123, p=0.030), but did not significantly affect the survival of *Bromus* (r=0.000, p=0.395). Drought decreased leaf production for both species, in particular without neighbours (drought × neighbour interaction significant for both species; Table Ib; Figure 1b).

Mowing did not significantly alter survival of both *Brachypodium* and *Bromus*. Mowing had no overall effect on the leaf productivity of *Brachypodium* but it decreased leaf production without neighbours and increased leaf production with neighbours (mowing  $\times$  neighbour interaction significant; Table Ic; Figure 1c). In contrast, mowing significantly increased leaf productivity of *Bromus* with and without neighbours (Table Ic; Figure 1c).

#### RCI, RECI, AND IMPORTANCE OF COMPETITION

For the two species, RCI increased significantly when RECI decreased. The relation was polynomial for *Bromus* (r=-0.916, p=0.010; Figure 2) but linear for *Brachypodium* (r=-0.879, p=0.004; Figure 2). For *Bromus*, the importance of competition overrode the importance of the

environment in all eight plots, and only the shade-drought treatment negatively affected leaf-number variation without neighbours. Competition intensity remained high in this treatment, which may explain this polynomial function. For *Brachypodium*, the importance of competition overrode the importance of the environment in six of the eight plots, and competition intensity decreased strongly with increasing stress and disturbance in the two unshaded and drought plots, which rendered competition relatively unimportant in these plots. Both the intensity and importance of competition increased strongly for *Brachypodium* in the four shaded plots, but for *Bromus* these components of competition increased with shade only when applied without drought.

# Discussion

The removal of neighbours had a strong positive effect on the growth of both species. Previous experimental studies in calcareous grassland communities have shown that competition may explain the occurrence of rare annual species in gaps created by animal disturbance (Mitchley & Grubb, 1986; Ryser, 1990; Bonis, Grubb & Coomes, 1997; McLellan, Law & Fitter, 1997). Our results demonstrate the significance of competition among dominant species in structuring calcareous grassland communities. More importantly, the effect of interspecific competition differed substantially when we manipulated shade, drought, and disturbance. Competition intensity increased for each species when the negative effect of the environment decreased, which led to a strong increase in the importance of competition in the plots where the environment was favourable and a decrease in the importance of competition in the plots where the environment was harsh. This result is consistent with the model of Grime (1974). However, Bromus and Brachypodium differed in their response to environmental manipulations and competition. Bromus was more affected by competition over all environmental conditions, whereas Brachypodium was affected the least by competition in the harshest conditions, i.e., the driest plots. Shade had a strong effect on Brachypodium, decreasing the negative effect of the environment and increasing competition intensity. One important but unexpected result of this experiment was the large change in ecosystem processes induced by the shade treatment. Soil moisture and available forms of nitrogen increased in the shade treatment. The strong variation in soil resources and, in particular, in water availability induced by our treatments may explain why competition was highly dynamic, supporting Grime's model, rather than stable across environments, as found in other studies in which only nutrient availability was manipulated, which generally support Tilman's model.

### DIFFERENCES IN LIFE-HISTORY STRATEGIES

The positive growth response of *Bromus* to mowing corresponded with this species' ability to colonize disturbed habitats (Grime, 1979; Tilman, 1990; Lavorel, Rochette & Lebreton, 1999). *Brachypodium* did not show this response to disturbance, as also found by Bobbink and Willems (1991), Schlaepfer (1997), and Barbaro and

		Brachy	podium					
	df	MS	F	р	df	MS	F	р
a.	2	2 609	6.1	< 0.001	3	1 171	7.3	< 0.001
Block	3	3,508	25.2	< 0.001	1	214	13	0 249
Shade	1	14,499	44.2	< 0.001	1	0 057	67.4	~0.001
Neighbour	1	25,454	44.5	< 0.001	1	605	43	0.001
Shade × Neighbour	1	7,796	13.5	< 0.001	2	513	3.7	0.025
$Block \times Shade$	3	1,977	3.4	0.019	2	515	3.7	0.023
Block × Neighbour	3	3,006	5.2	0.002	2	100	1.2	0.012
Block $\times$ Shade $\times$ Neighbour	3	1,332	2.3	0.078	.) 141	199	1.2	0.295
Error	124	574			141	159		
b.						1 000		0.001
Block	3	6,463	11.2	< 0.001	3	1,002	0.2	< 0.001
Drought	1	4,813	8.3	0.004	1	921	5.7	0.018
Neighbour	1	25,454	44.3	< 0.001	1	9,957	62.4	< 0.001
Drought $\times$ Neighbour	1	3,653	6.3	0.013	1	1,004	6.3	0.013
Block × Drought	3	2,430	4.2	0.007	3	451	2.8	0.041
Block × Neighbour	3	4,939	8.6	< 0.001	3	461	2.8	0.038
$Block \times Drought \times Neighbour$	3	1,029	1.7	0.152	3	274	1.7	0.166
Error	124	574			141	159		
с.				.0.001	2	504	36	6 61 <i>4</i>
Block	3	6,611	11.5	< 0.001	5	2 004	12.5	~0.001
Mowing	1	949	1.6	0.201	1	2,004	12.5	< 0.001
Neighbour	1	25,454	44.3	< 0.001	1	9,957	02.4	< 0.001
Mowing $\times$ Neighbour	1	2,336	4.0	0.046	1	512	3.2	0.075
Block $\times$ Mowing	3	2,213	3.8	0.011	3	455	2.8	0.039
Block $\times$ Neighbour	3	3,817	6.6	< 0.001	3	697	4.5	0.006
Block × Mowing × Neighbour	3	1,445	2.5	0.061	3	146	0.9	0.434
Error	124	574			159			

TABLE I. Three-way ANOVA of change in leaf number for *Brachypodium pinnatum* and *Bromus erectus* (in bold, significance at p-level <0.05); a: shade model; b: drought model; c: mowing model.

Cozic (1998). Survival of Bromus was not affected by drought, although its leaf production was, which suggests that this species is relatively tolerant to dry conditions (Grime & Curtis, 1976; Grime, 1979; Thompson & Grime, 1988). Our results on the ability of Bromus to tolerate both stress and disturbance are consistent with the findings of Buckland and Grime (2000), who argued that dominance in unfertile grasslands is driven by the ability of stress-tolerant species to resist disturbance. In contrast, the decrease in survival and in leaf-number production for Brachypodium without neighbours in drought plots indicates a low tolerance to water stress. Responses to the improvement in soil resources induced by shade were very different for each species when neighbours were removed, with no effect of removal for Bromus but a strong increase in growth for Brachypodium (Figure 1a). The ability to allocate resources to leaf production in fertile environments characterizes species with competitive strategies (Grime, 1979; Ryser & Notz, 1996). These differences demonstrate a generally higher competitive ability for Brachypodium than Bromus. Our results correspond with studies that have shown that species from mesic and fertile environments generally have higher competitive abilities than species that are dominant in drier and more nutrient-poor sites (Mahmoud & Grime, 1976; Wilson & Keddy, 1986), but not with studies that have shown a reversal in competitive ability along environmental gradients (McGraw & Chapin, 1989; Aerts, 1990; Novoplansky & Goldberg, 2001). The competition intensity experienced by Brachypodium in the fertile shaded plots was very high, which may seem inconsistent with Brachypodium's high competitive ability. This may be

explained by strong intraspecific competition, which is important in compact clones of *Brachypodium* (de Kroon, Hara & Kwant, 1992), or by increasing interspecific competition with other grass species, such as *Dactylis glomerata* and *Arrhenatherum elatius*, which also increases in abundance as soil resources, and in particular nitrates, increase (Corcket, 2001).

VARIATION IN COMPETITION INTENSITY AND IMPORTANCE WITH SPECIES AND TREATMENTS

The species with the highest competitive ability, Brachypodium, also had the highest variation in intensity and importance of competition between environmental treatments. Competition intensity and importance were high in the shaded plots and low in the unshaded dried plots. This result, consistent with Grime's model, may have been due to the strong differences in moisture availability that developed in our experiment and to the low tolerance of water stress of Brachypodium. Goldberg and Barton (1992) found that most experimental studies using natural gradients of productivity demonstrated an increase in competition intensity with decreasing environmental stress (Gurevitch, 1986; Wilson & Keddy, 1986; Reader & Best, 1989; Pennings & Callaway, 1992; Kadmon, 1995; Twolan-Strutt & Keddy, 1996; Gerdol et al., 2000; Pugnaire & Luque, 2001; Choler, Michalet & Callaway, 2001; Callaway et al., 2002). Natural gradients generally involve topographic gradients along which variation in productivity is mainly driven by variation in moisture availability (Goldberg & Novoplansky, 1997). As in our system, variation in nutrient availability may also occur at the wet end of these gradients. Goldberg and



FIGURE 1. Means for change in leaf number for *Bromus erectus* and *Brachypodium pinnatum* with and without neighbours in the treated and control plots. Bars show standard error; a) shade model; b) drought model; c) mowing model.

Novoplansky (1997) suggested that results from gradients of water availability should be consistent with Grime's model, arguing that competition becomes unimportant in dry sites, relative to environmental constraints, because pulses of the limiting resource are less frequent in dry environments than in wet fertile conditions. Our experimental design allowed us to measure both intensity and importance of competition, and the results we observed for *Brachypodium* supported Goldberg and Novoplansky's hypothesis. Exceptions to the general pattern of increasing competition intensity along natural gradients have been reported in studies using either a narrow range of biomass



FIGURE 2. Regressions between means of RNE and RECI values in the eight experimental plots for *Bromus erectus* and *Brachypodium pinnatum*. Diagonal lines separate in the left upper part of each graph the plots where the relative importance of competition overrides the importance of the environment.

variation along the gradient (Reader et al., 1994) or a small portion of a non-linear gradient of increasing productivity or biomass (Belcher, Keddy & Twolan-Strutt, 1995; Foster, 1999). In our experiment, competition was always more important than the environment for the stress-tolerant species *Bromus*, even in stressful environments; competition intensity did not correlate linearly with environmental constraints, and in the most environmentally constrained part of the gradient, variation in competition intensity was weak, which is consistent with Belcher, Keddy, and Twolan-Strutt (1995). Species-specific life-history strategies will affect response to competition, and stress tolerant species such as *Bromus* are likely to experience high competition intensity even in stressful environments.

Goldberg and Barton (1992) have shown that most of the studies using experimentally induced gradients of productivity found no increase in competition intensity with increasing productivity (Wilson & Shay, 1990; Di Tommasso & Aarssen, 1991; Wilson & Tilman, 1991;

Wilson & Tilman, 1995; Peltzer, Wilson & Gerry, 1998; Cahill, 1999). Goldberg and Novoplansky (1997) argued that this may be due to the fact that authors improved the productivity of their communities by manipulating only nutrients in systems that otherwise were not unusually stressful. Most of the cited studies were conducted in the tall-grass prairie or old fields of northeast America where water availability is sufficient and nutrient availability is the primary limiting factor for productivity (Tilman, 1988). Low nutrient availability combined with high water availability may occur due to the sandy texture of the soils (72% sand at Cedar Creek Natural History Area; Tilman & Wedin, 1991) and the summer rainfall regime of northeast America. In such systems there may only be competitors, not stress-tolerators, according to Grime's classification (1974). Therefore, competition may be high even at the infertile end of these gradients, and nutrient addition cannot increase total competition intensity. Instead, competition induces a shift in the dominance of different types of good competitors (Tilman, 1988). Our results for the competitive species Brachypodium indicate that the main changes in competition intensity occurred at the dry end of the gradient, not at the wet end, where competition was always intense and important (Figure 2). This result is consistent with the findings of Tilman (1988) and others working in similar environments, where physical stress is low. Among the few studies in which water availability has been manipulated, Kadmon (1995) found an increase in competition intensity with decreasing water stress, which is consistent with our experiment and Grime's model. However, Davis, Wrage, and Reich (1998) and Goldberg et al. (2001) found converse results (with tree seedlings and desert annuals, respectively), and Novoplansky and Goldberg (2001) stressed the importance of the identity of the target species and of the neighbouring vegetation. Our results also have emphasized that competition intensity is highly dependent on the identity of the species and that including measurements of the direct effect of the environment in competition studies may be fruitful to improve our understanding of the importance of the mechanism of competition in determining community structure.

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### Literature cited

- Aerts, R., 1990. Nutrient use efficiency in evergreen and deciduous species from heathlands. Oecologia, 84: 391-397.
- Barbaro, L. & P. Cozic, 1998. Organisation agro-écologique des pelouses et landes calcicoles du Parc Naturel Régional du Vercors. Écologie, 29: 443-457.

- Belcher, J. W., P. A. Keddy & L. Twolan-Strutt, 1995. Root and shoot competition intensity along a soil depth gradient. Journal of Ecology, 83: 673-682.
- Bobbink, R. & J. H. Willems, 1991. Impact of different cutting regimes on the performance of *Brachypodium pinnatum* in Dutch chalk grassland. Biological Conservation, 56: 1-21.
- Bonis, A., P. J. Grubb & D. A. Coomes, 1997. Requirements of gap-demanding species in chalk grassland: Reduction of root competition versus nutrient-enrichment by animals. Journal of Ecology, 85: 625-633.
- Brooker, R. W. & T. V. Callaghan, 1998. The balance between positive and negative plant interactions and its relationship to environmental gradients: A model. Oikos, 81: 196-207.
- Brown, J. R. & S. Archer, 1999. Shrub invasion of grassland: Recruitment is continuous and not regulated by herbaceous biomass or density. Ecology, 80: 2385-2396.
- Buckland, S. M. & J. P. Grime, 2000. The effects of trophic structure and soil fertility on the assembly of plant communities: A microcosm experiment. Oikos, 91: 336-352.
- Cahill, J. F., 1999. Fertilization effects on interactions between above and belowground competition in an old field. Ecology, 80: 466-480.
- Callaway, R. M., R. W. Brooker, P. Choler, Z. Kikvidze, C. J. Lortie, R. Michalet, L. Paolini, F. I. Pugnaire, B. Newingham, E. T. Aschehoug, C. Armas, D. Kikodze & B. J. Cook, 2002. Positive interactions among alpine plants increase with stress. Nature, 417: 844-848.
- Choler, P., R. Michalet & R. M. Callaway, 2001. Competition and facilitation on gradients in alpine communities. Ecology, 82: 3295-3308.
- Corcket, E., 2001. Approche expérimentale de la compétition entre graminées dominantes et de l'herbivorie par les Orthoptères dans une pelouse sèche collinéenne. Thèse de 3<sup>e</sup> cycle, Université Joseph Fourier, Grenoble.
- Corcket, E., I. Chintauen-Marquier, R. M. Callaway & R. Michalet, 2002. Sélectivité et variations environnementales de l'herbivorie par les Orthoptères. Comptes Rendus Biologies, 325: 155-164.
- Davis, M. A., K. J. Wrage & P. B. Reich, 1998. Competition between tree seedlings and herbaceous vegetation: Support for a theory of resource supply and demand. Journal of Ecology, 86: 652-661.
- de Kroon, H., T. Hara & R. Kwant, 1992. Size hierarchies of shoots and clones in clonal herb monocultures: Do clonal and non-clonal plants compete differently? Oikos, 63: 410-419.
- Di Tommasso, A. & L. W. Aarssen, 1991. Effect of nutrient level on competition intensity in the field for three coexisting grass species. Journal of Vegetation Science, 2: 513-522.
- Dorich, R. A. & D. W. Nelson, 1983. Ammonium measurement. Soil Science Society of America Journal, 47: 833-836.
- Dutoit, T., E. Gerbaud, J. M. Ourcival, M. Roux & D. Alard, 2001. Recherche prospective sur la dualité entre caractéristiques morphologiques et capacités de compétition entre les végétaux: le cas des espèces adventices et du blé. Comptes Rendus de l'Académie des Sciences, Sciences de la vie, 324: 261-272.
- Foster, B. L., 1999. Establishment, competition and the distribution of native grasses among Michigan old-fields. Journal of Ecology, 87: 476-489.
- Fournier, P., 1990. Les quatre flores de France. Lechevalier, Paris, France.
- Gerdol, R., L. Brancaleoni, M. Menghini & R. Marchesini, 2000. Response of dwarf shrubs to neighbour removal and nutrient addition and their influence on community structure in a subalpine heath. Journal of Ecology, 88: 256-266.

- Goldberg, D. E. & A. M. Barton, 1992. Patterns and consequences of interspecific competition in natural communities: A review of field experiments with plants. American Naturalist, 139: 771-801.
- Goldberg, D. & A. Novoplansky, 1997. On the relative importance of competition in unproductive environments. Journal of Ecology, 85: 409-418.
- Goldberg, D. E., T. Rajaniemi, J. Gurevitch & A. Stewart-Oaten, 1999. Empirical approaches to quantifying interaction intensity: Competition and facilitation along productivity gradients. Ecology, 80: 1118-1131.
- Goldberg, D. E., R. Turkington, L. Olsvig-Whittaker & A. R. Dyer, 2001. Density dependence in an annual plant community: Variation among life history stages. Ecological Monographs, 71: 423-446.
- Grime, J. P., 1974. Vegetation classification by reference to strategies. Nature, 250: 26-31.
- Grime, J. P., 1979. Plant Strategies and Vegetation Processes. J. Wiley & Sons, Chichester.
- Grime, J. P. & A. V. Curtis, 1976. The interaction of drought and mineral nutrient stress in calcareous grassland. Journal of Ecology, 64: 976-998.
- Gurevitch, J., 1986. Competition and the local distribution of the grass *Stipa neomexicana*. Ecology, 67: 46-57.
- Kadmon, R., 1995. Plant competition along soil moisture gradients: A field experiment with the desert annual *Stipa capen*sis. Journal of Ecology, 83: 253-262.
- Keddy, P. A., 1989. Competitive hierarchies and centrifugal organization in plant communities. Pages 265-290 in D. T. J. Grace (ed.). Perspectives on Plant Competition. San Diego, California.
- Lavorel, S., C. Rochette & J. D. Lebreton, 1999. Functional groups for response to disturbance in Mediterranean old fields. Oikos, 84: 480-498.
- Mahmoud, A. & J. P. Grime, 1976. An analysis of competitive ability in three perennial grasses. New Phytologist, 77: 431-435.
- Markham, J. H. & C. P. Chanway, 1996. Measuring plant neighbour effects. Functional Ecology, 10: 548-549.
- McGraw, J. B. & F. S. Chapin III, 1989. Competitive ability and adaptation to fertile and infertile soils in two *Eriophorum* species. Ecology, 70: 736-749.
- McLellan, A. J., R. Law & A. H. Fitter, 1997. Response of calcareous grassland plant species to diffuse competition: Results from a removal experiment. Journal of Ecology, 85: 479-490.
- Mitchley, J. & P. J. Grubb, 1986. Control of relative abundance of perennials in chalk grassland in southern England.
  I. Constancy of rank order and results of pot- and field-experiments on the role of interference. Journal of Ecology, 74: 1139-1166.
- Newman, E. I., 1973. Competition and diversity in herbaceous vegetation. Nature, 244: 310-311.
- Novoplansky, A. & D. E. Goldberg, 2001. Effects of water pulsing on individual performance and competitive hierarchies in plants. Journal of Vegetation Science, 12: 199-208.
- Oksanen, L., 2001. Logic of experiments in ecology: Is pseudoreplication a pseudoissue? Oikos, 94: 27-38.
- Peltzer, D. A., S. D. Wilson & A. K. Gerry, 1998. Competition intensity along a productivity gradient in a lowdiversity grassland. American Naturalist, 151: 465-476.
- Pennings, S. C. & R. M. Callaway, 1992. Salt marsh plant zonation: The relative importance of competition and physical factors. Ecology, 73: 681-690.
- Pugnaire, F. I. & M. T. Luque, 2001. Changes in plant interactions along a gradient of environmental stress. Oikos, 93: 42-49.

- Reader, R. J. & B. J. Best, 1989. Variation in competition along an environmental gradient: *Hieracium floribundum* in an abandoned pasture. Journal of Ecology, 77: 673-684.
- Reader, R. J., S. D. Wilson, D. Tilman, E. C. Morris, J. B. Grace, J. B. McGraw, H. Olff, R. Turkington, E. Klein, Y. Leun, B. Shipley, R. Van Hulst, M. E. Johansson, C. Nilsson, J. Gurevitch, K. Grigulis & B. E. Beisner, 1994. Plant competition in relation to neighbor biomass: An intercontinental study with *Poa pratensis*. Ecology, 75: 1753-1760.
- Royer, J. M., 1987. Les pelouses des *Festuco-Brometea*. D'un exemple régional à une vision eurosibérienne: étude phytosociologique et phytogéographique. Thèse d'état, Université de Franche-Comté, Besançon.
- Ryser, P., 1990. Influence of gaps and neighbouring plants on seedling establishment in limestone grassland. Experimental field studies in northern Switzerland. Veröffentlichungen des Geobotanischen Institutes der ETH, No. 104, Stiftung Rübel, Zürich.
- Ryser, P. & R. Notz, 1996. Competitive ability of three ecologically contrasting grass species at low nutrient supply in relation to their maximal relative growth rate and tissue density. Bulletin of the Geobotanical Institute ETH, 62: 3-12.
- Schlaepfer, F., 1997. Influence of management on cover and seed production of *Brachypodium pinnatum* (L.) Beauv. in calcareous grassland. Bulletin of the Geobotanical Institute ETH, 63: 3-10.
- Taylor, D. R., L. W. Aarssen & C. Loehle, 1990. On the relationship between r/K selection and environmental carrying capacity: A new habitat templet for plant life-history strategies. Oikos, 58: 239-250.
- Thompson, K. & J. P. Grime, 1988. Competition reconsidered: a reply to Tilman. Functional Ecology, 114-116.
- Tilman, D., 1987. On the meaning of competition and the mechanisms of competitive superiority. Functional Ecology, 1: 304-315.
- Tilman, D., 1988. Plant Strategies and the Dynamic and Structure of Plant Communities. Princeton University Press, Princeton, New Jersey.
- Tilman, D., 1990. Constraints and tradeoffs: Toward a predictive theory of competition and succession. Oikos, 58: 3-15.
- Tilman, D. & D. Wedin, 1991. Dynamics of nitrogen competition between successional grasses. Ecology, 72: 1038-1049.
- Twolan-Strutt, L. & P. A. Keddy, 1996. Above- and belowground competition intensity in two contrasting wetland plant communities. Ecology, 77: 259-270.
- Welden, C. W. & W. L. Slauson, 1986. The intensity of competition versus its importance: An overlooked distinction and some applications. Quantitative Review of Biology, 61: 23-44.
- Wheatley, R. E., R. McDonald & A. McSmith, 1989. Extraction of nitrogen from soils. Biology and Fertility of Soils, 8: 189-190.
- Wilczek, E., G. Beauvert & D. Dutoit, 1928. Le comportement écologique du *Bromus erectus* L. Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich, 73: 469-508.
- Wilson, S. D. & P. A. Keddy, 1986. Species competitive ability and position along a natural stress/disturbance gradient. Ecology, 67: 1236-1242.
- Wilson, S. D. & J. M. Shay, 1990. Competition, fire, and nutrients in a mixed-grass prairie. Ecology, 71: 1959-1967.
- Wilson, S. D. & D. Tilman, 1991. Components of plant competition along an experimental gradient of nitrogen availability. Ecology, 72: 1050-1065.
- Wilson, S. D. & D. Tilman, 1995. Competitive responses of eight old-field plant species in four environments. Ecology, 76: 1169-1180.



Société botanique de France



# Croissance compensatoire et stimulation de croissance chez *Elytrigia juncea* soumis à différents régimes de défoliation

# Growth stimulation and compensatory growth on *Elytrigia juncea* experiencing different defoliation regimes

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**Résumé:** La compensation de la croissance chez les plantes soumises à une défoliation (herbivorie, fauche) reste un phénomène controversé, dont la mise en évidence est problématique et les modalités d'occurrence mal connues. Nous avons appliqué expérimentalement différentes combinaisons d'intensité et de fréquence de défoliations sur *Elytrigia juncea*, une Poacée dominante des dunes embryonnaires atlantiques. Les biomasses finales ainsi que les taux de croissance relatifs en hauteur et en nombre de feuilles ont été quantifiés. Une importante stimulation de croissance en hauteur a été mise en évidence dans les deux semaines suivant la coupe de plus forte intensité. Bien que cette stimulation ait été observée pendant toute la durée de l'expérimentation, aucune surcompensation finale de croissance n'a été relevée. Une compensation de croissance a été mise en évidence lorsque les tissus défoliés au cours de l'expérimentation étaient pris en compte.

Mots clés: croissance compensatoire; dune; Elytrigia juncea; herbivorie; perturbation

**Abstract:** Compensatory growth for defoliated plants is an issue still debated in literature, due to methodological weakness and lack of knowledge about its occurrence. In a green-house experiment, we applied three types of defoliation on *Elytrigia juncea*, a Poaceae species dominating costal foredunes. Responses to the different combinations of intensity and frequency of defoliation were assessed by measurements of final biomass of target species. Relative growth rates in height and number of leaves were also calculated, enable us to assess more accurately compensatory growth. An important growth stimulation was pointed out two weeks after a severe cut, and was still visible over the course of the experimentation without being able to induce overcompensation of growth. The occurrence of compensatory growth was pointed out only when plant tissues removed by cutting were added.

Keywords: compensatory growth; disturbance; dune; Elytrigia juncea; herbivory

# Introduction

La défoliation des plantes constitue une source de perturbation majeure dans les communautés végétales. Elle est généralement appréhendée aux niveaux théorique et expérimental *via* l'action des herbivores, sauvages ou domestiques, qui consomment des feuilles et parfois aussi des tiges d'espèces herbacées ou ligneuses. Une autre cause majeure de défoliation dans les écosystèmes est la gestion mécanique des milieux, en particulier la gestion par la fauche des milieux herbacés. Ces régimes de défoliations sont très variables en intensité et en fréquence du fait de la dynamique des populations d'herbivores et des spécificités de gestion par l'homme.

Les conséquences de la défoliation pour les plantes ont généralement été considérées comme négatives, accréditant l'idée par exemple que les herbivores ne

suggéré une relativisation des effets négatifs de l'herbivorie, voire une stimulation de la croissance suite à l'herbivorie. végétale Cette « croissance compensatoire» a été et reste encore fort débattue, car insuffisamment caractérisée au niveau expérimental (Belsky 1986, 1987; Trumble, Kolodny-Hirsch & Ting 1993) bien que des cas aient été documentés (Paige & Whittam 1987). Les mécanismes physiologiques de tolérance des plantes à la défoliation sont pourtant bien identifiés (Belsky 1986; Trumble, Kolodny-Hirsch & Ting 1993; Tiffin, 2000): activation de méristèmes dormants, augmentation de l'efficacité photosynthétique des tissus restants, migration accrue de photosynthétats dans les nouvelles feuilles, réallocation de ressources vers l'appareil

puissent être que des antagonistes pour les plantes. Cependant, certains auteurs (voir McNaughton 1983) ont

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végétatif aérien, perte de tissus âgés photosynthétiquement moins actifs, augmentation de l'intensité lumineuse dans les tissus bas, perte de la dominance apicale, augmentation de l'efficacité d'utilisation en eau par la réduction des surfaces de transpiration. Certains auteurs suggèrent que la meilleure tolérance à la défoliation ne correspond pas à une croissance compensatoire maximale, mais plutôt sub-maximale (Tiffin 2000).

La notion de croissance compensatoire inclut en fait plusieurs types de réponses à la défoliation. Une «sous-compensation» indique que la biomasse totale des plantes défoliées est inférieure à celle des plantes témoins non défoliées. On observe dans ce cas soit une «compensation partielle» si la croissance de l'espèce défoliée a été stimulée et engendre un rattrapage partiel de biomasse après défoliation, soit des effets négatifs de la défoliation sur la plante si la croissance de l'espèce défoliée diminue après la coupe ou si la plante meurt après la défoliation. Une «surcompensation» représente un effet positif de la défoliation (Belsky 1986). Bien qu'âprement discutée, la notion de surcompensation est séduisante, notamment au niveau évolutif, car elle suggère que les interactions plantes-herbivores puissent être mutualistes et non plus antagonistes (Agrawal 2000).

Ces de croissance compensatoire sont types théoriquement calculés en comparant la biomasse finale du témoin non défolié (BF<sub>Tem</sub>) avec la biomasse finale du plant défolié (BF<sub>Déf</sub>) à laquelle on rajoute la biomasse qui a été supprimée par défoliation (B<sub>Sup</sub>; Belsky 1986). Ainsi, si  $(BF_{Déf}+B_{Sup})/BF_{Tém} > 1$ , alors on observe une surcompensation et si (BF<sub>Déf</sub>+B<sub>Sup</sub>)/BF<sub>Tém</sub> < 1 on observe une sous-compensation de croissance (Belsky 1986). En pratique, la croissance compensatoire est fréquemment déterminée en comparant les biomasses finales des deux modalités, sans tenir compte de la biomasse défoliée, ce qui engendre une sous-estimation de l'ampleur de la croissance compensatoire. Cette pratique est inévitable lorsque la défoliation n'est pas directement contrôlée par l'expérimentateur mais qu'elle résulte par exemple du prélèvement par un herbivore. Cependant, même dans les expérimentations en conditions contrôlées, la biomasse défoliée est souvent omise (Callaway et al. 2001; Callaway, Kim & Mahall 2006; Moser & Schütz 2006). Les calculs de croissance compensatoire peuvent porter sur d'autres variables que les biomasses, comme la hauteur, le nombre de feuilles, des caractéristiques foliaires et de l'architecture des plantes (Trumble, Kolodny-Hirsch & Ting 1993; Gonzalez-Teuber & Gianoli 2007), ou encore le nombre d'inflorescences ou de fruits qui renseignent sur les conséquences en terme de valeur adaptative (Parra-Tabla, Rico-Gray & Carbajal 2004). Supportant l'idée que la tolérance des plantes à la défoliation est directement liée à la maximisation de leur taux de croissance (Alward & Joern 1993), nous nous proposons à travers ce travail de développer une méthode originale permettant d'appréhender la croissance compensatoire par le calcul des taux de croissance des espèces et par la prise en compte de leur variation temporelle.

Les milieux dunaires littoraux sont caractérisés par une de perturbations physiques et biotiques variété susceptibles d'engendrer des pertes de surface foliaire. La dune embryonnaire, qui constitue le premier cordon dunaire à partir de l'océan, est particulièrement exposée à l'ensablement (Forey et al. 2008). Cet ensablement provoque un recouvrement du système aérien de la végétation et donc une diminution de la surface foliaire des plantes sans pour autant qu'il y ait d'exportation de biomasse. La persistance des feuilles, même enterrées, pourrait alors favoriser la croissance compensatoire en réallouant les nutriments des feuilles enfouies vers les feuilles non enfouies (Bach 2000; Gilbert & Ripley 2008). Par ailleurs, l'herbivorie est un phénomène important dans les dunes (Zeevalking & Fresco 1977; Harris & Davy 1986; Wallage-Drees & Michielsen 1989). Celle-ci engendre une exportation de biomasse par défoliation, dont les conséquences sur la végétation sont incertaines. Les principaux herbivores concernés sont les lagomorphes, et notamment le lapin Orictolagus cuniculus (L.) (Zeevalking & Fresco 1977; Harris & Davy 1986; Wallage-Drees & Michielsen 1989). Les chevreuils (Capreolus capreolus (L.)) peuvent également avoir un effet non négligeable : dans la forêt landaise contiguë aux dunes aquitaines, ils consomment les Poacées de manière fréquente mais en faible quantité du fait du manque relatif d'appétence de celles-ci. Cependant, ils montrent une adaptation potentiellement importante de leur régime alimentaire en fonction de la ressource, et les Poacées peuvent représenter localement jusqu'à 80% du poids de leur contenu stomacal (Maizeret & Tran Manh Sung 1984).

L'objectif de cette étude est de tester l'impact de différents régimes de défoliation sur la croissance du chiendent à feuilles de jonc (*Elytrigia juncea* (L.) Nevski = *Elymus farctus* 

(Viv.) Runemark ex Melderis subsp. boreoatlanticus (Simonet & Guinochet) Melderis). Cette Poacée clonale et rhizomateuse, dominante dans les dunes embryonnaires européennes atlantiques des Ammophiletalia arenariae (Macedo et al. 2010), appartient au groupe fonctionnel des plantes dunaires de haute stature, avec des organes souterrains développés (García-Mora, Gallego-Fernández & García-Novo 1999). Elle est adaptée au stress, mais se distingue surtout par sa résistance à la perturbation (Macedo et al. 2010). E. juncea peut subir des dégâts importants par herbivorie dans les milieux naturels (Harris & Davy 1986). Nous testerons plus particulièrement dans cette étude 1°) l'occurrence de la surcompensation suite à la défoliation d'E. juncea, 2°) les effets différentiels de l'intensité et de fréquence de défoliation, ainsi que 3°) l'évolution temporelle de la croissance compensatoire grâce aux calculs des taux de croissance relatifs.

# Matériel et méthodes

## Matériel végétal et mise en culture

Des rhizomes d'*E. juncea* ont été prélevés en avril 2005 dans les dunes sableuses embryonnaires atlantiques du
nord de la presqu'île de Lège-Cap Ferret (44°44'N, 01° 14'W, Crohot noir, Gironde, Aquitaine, France). Ces rhizomes ont ensuite été conditionnés en fragments non feuillés de 2 cm environ portant un bourgeon végétatif et un faisceau racinaire. Ces fragments ont ensuite été placés dans des pots cubiques de 11 cm de côté, remplis d'un mélange de 7 volumes de sable prélevé sur les dunes du Crohot noir pour un volume de terreau. Pendant deux mois, les pots ont été placés en salle de germination à température ambiante (15-20 °C) et arrosés à raison de deux à trois fois par semaine sans aucun traitement de fertilisation.

# Traitements expérimentaux

L'expérimentation en conditions contrôlées a été réalisée dans les serres climatisées de l'École nationale d'ingénieurs des travaux agricoles de Bordeaux (Bordeaux Sciences Agro, Gradignan, Gironde, France), à une température d'environ 18 °C et une hygrométrie supérieure à 60%. La défoliation par les herbivores vertébrés a été simulée en juin et juillet 2005, par une coupe (aux ciseaux) de l'appareil végétatif aérien. Quatre traitements ont été appliqués : un témoin non défolié (T), une coupe modérée à 6 cm de hauteur (1C6), une coupe sévère à 2 cm de hauteur (1C2) et deux coupes à 6 cm de hauteur et à 12 jours d'intervalle (2C6). Les traitements expérimentaux ont été appliqués sur vingt individus par modalité de coupe, et sur quinze individus en condition témoin. Les 75 pots ont été mélangés aléatoirement sur les plateaux de la serre.

# Mesures, pesées et analyses

La hauteur (H) et le nombre de feuilles (F) ont été mesurés juste avant la première défoliation (H0, F0 : le 10 juin), 12 jours après la première défoliation (H2, F2 : le 22 juin, juste avant la seconde défoliation), 12 jours après la seconde défoliation (H4, F4 : le 4 juillet) et enfin le 25 juillet 2005 (H5, F5; Figure 1). Les hauteurs H1 et H3 correspondent respectivement aux hauteurs immédiatement après la première et la seconde défoliation,



Figure 1. Calendrier d'application des traitements expérimentaux (C : coupes aux ciseaux), des mesures effectuées (hauteurs H et nombre de feuilles F) et des périodes retenues pour le calcul des taux de croissance relatifs (TCR).

Figure 1. Schedule of experimental treatments (C: scissors cutting), measurements (height H and leaf numbers F), and periods used to the calculation of relative growth rates (TCR).

soit 2 ou 6 cm pour les individus défoliés en fonction de la sévérité du traitement, voire exceptionnellement aux hauteurs H0 et H2 pour les rares individus qui devaient être défoliés et avaient une taille inférieure à la hauteur de coupe. La hauteur a été mesurée en étirant les feuilles vers le haut et en mesurant la distance entre le collet de la plante et la pointe foliaire la plus haute. Les nombres de feuilles F1 et F3 restent généralement égaux à ceux de F0 et F2. La hauteur finale globale (H<sub>Glob</sub>) correspond à la hauteur H5 à laquelle ont été rajoutées les hauteurs défoliées; H<sub>Glob</sub> correspond donc à la hauteur globale de biomasse ayant été produite par la plante au cours de l'expérimentation, et pas simplement à la hauteur observée en fin d'expérimentation. Nous n'avons pas calculé de nombre de feuilles finales globales, puisque les coupes engendraient rarement des différences de nombre de feuilles.

Les taux de croissance relatifs en hauteur (TCR<sub>H</sub>) ou en nombre de feuilles (TCR<sub>F</sub>) des phytomètres ont été calculés. Ils représentent la différence entre mesure finale et mesure initiale rapportée à la mesure initiale (pour la hauteur, TCR<sub>HF-i</sub> = [H<sub>fin</sub>-H<sub>ini</sub>]/H<sub>ini</sub>). Les taux de croissance relatifs ont été calculés sur toute la durée de l'expérimentation (TCR<sub>H5-0</sub>, TCR<sub>F5-0</sub>), entre la première coupe et la fin de l'expérimentation (TCR<sub>H5-1</sub>, TCR<sub>F5-1</sub>) et pour des périodes de douze jours immédiatement après chaque défoliation (TCR<sub>H2-1</sub>, TCR<sub>F2-1</sub> et TCR<sub>H4-3</sub>, TCR<sub>F4-3</sub>; Figure 1).

Les TCR permettent d'estimer la stimulation de croissance lorsqu'ils sont calculés sur une période suivant immédiatement la défoliation (stimulation globale pour la période 5-1, suite à la première défoliation pour la période 2-1 et suite à la seconde défoliation pour la période 4-3). Les TCR permettent d'estimer la croissance compensatoire (CC) lorsqu'ils sont calculés sur des périodes incluant la mesure avant défoliation (CC globale pour la période 5-0, suite à la première défoliation pour la période 2-0 et suite à la seconde défoliation pour la période 2-0 et suite à la seconde défoliation pour la période 4-2).

Les biomasses aériennes et souterraines de chacune des 75 plantes ont été déterminées le 29 juillet 2005, après passage des échantillons végétaux à l'étuve (65 °C pendant 72 heures).

Les données ont été analysées sous «R» (version 2.6.1, R Development Core Team 2007) par analyse de variance à une voie (fonction «aov»), avec comme unique facteur le régime de défoliation. Lorsque l'effet du facteur «régime de défoliation» était significatif, des tests de Tukey ont été effectués afin d'identifier les différences entre les quatre modalités expérimentales. Les relations existant entre nos variables non destructives (hauteur, nombre de feuilles) et la biomasse (aérienne, racinaire et totale) ont été évaluées en effectuant des corrélations linéaires entre ces variables prises au temps final (fonctions «cor» et «cor.test», coefficient de Pearson).

# Résultats

#### **Biomasses** finales

À la fin de l'expérimentation, la biomasse totale (BIOTOT) est significativement plus élevée dans la

modalité témoin que dans les modalités défoliées. Ceci est dû aux variations de biomasses aériennes (BIOAER) consécutives aux défoliations, puisque les biomasses souterraines (BIOSOU) ne varient pas significativement (Tableau 1 ; Figure 2).

#### Hauteurs

Les hauteurs finales brutes (H5) des modalités de défoliation sévères en intensité (1C2) ou en fréquence (2C6) sont significativement inférieures aux modalités non défoliées (T) ou à défoliation modérée (1C6). Par contre,

plus aucune différence significative n'est décelable lorsque l'on inclut les parties défoliées ( $H_{glob}$ ; Tableau 1; Figure 3).

Les taux de croissance relatifs en hauteur varient de manière importante en fonction de la période considérée et en fonction de la prise en compte ou non des parties défoliées. Sur toute la période de l'expérimentation, en incluant les mesures avant traitements expérimentaux (période 5-0), les  $TCR_{H5-0}$ des individus témoins non défoliés (T) sont supérieurs à ceux des individus défoliés deux fois (2C6) mais ne diffèrent pas des individus défoliés une seule fois

Tableau 1. Analyses de variances à un facteur (défoliation) effectuées sur les biomasses totales (BIOTOT), aériennes (BIOAER), souterraines (BIOSOU), les hauteurs brutes en fin d'expérimentation (H5) ou en tenant compte des parties défoliées (H<sub>glob</sub>), les taux de croissance relatifs en hauteur (TCR<sub>H</sub>), les nombre de feuilles en fin d'expérimentation (F5), les taux de croissance relatifs en nombre de feuilles (TCR<sub>F</sub>). N.s. : non significatif au seuil de 5%; \*: 0,01  $\le p \le 0,05$ ; \*\*: 0,001  $\le p < 0,01$ ; \*\*\*: p < 0,001. Table 1. One ways analysis of variance performed on total (BIOTOT), aboveground (BIOAER), belowground (BIOSOU) biomasses, gross heights at the end of the experiment (H5) or height taking into account defoliated tissues (H<sub>glob</sub>), height relative growth rates (TCR<sub>H</sub>), leaf numbers at the end of the experiment (F5), leaf numbers at the end of the experiment (F5), leaf numbers at the end of the experiment (F5), leaf numbers at the end of the experiment (F5), leaf numbers at the end of the experiment (F5), leaf numbers at the end of the experiment (F5), leaf numbers at the end of the experiment (F5), leaf numbers at the end of the experiment (F5), leaf numbers at the end of the experiment (F5), leaf numbers at the end of the experiment (F5), leaf numbers end to the experiment (F5), leaf numbers end to the experiment (F5), leaf numbers end to the experiment (F5), leaf numbers at the end of the experiment (F5), leaf numbers at the end of the experiment (F5), leaf numbers end to the experiment (F5), leaf numbers end

Variables	d.l.	Carrés moyens	F	р	
Biomasses					
BIOTOT	3	12210,0	4,41	0,007	**
BIOAER	3	5940,0	12,80	<0,001	***
BIOSOU	3	1656,0	1,05	0,375	n.s.
Hauteur					
H5	3	534,5	8,4	<0,001	***
H <sub>Glob</sub>	3	185,1	1,02	0,390	n.s.
TCR Hauteurs					
TCR <sub>H5-0</sub>	3	12,4	3,33	0,024	*
TCR <sub>HGlob-0</sub>	3	4,8	1,43	0,241	n.s.
TCR <sub>H5-1</sub>	3	119,7	15,16	<0,001	***
TCR <sub>H2-1</sub>	3	28,2	8,90	<0,001	***
TCR <sub>H4-3</sub>	3	0,1	0,81	0,492	n.s.
Nombre de feuilles					
F5	3	11,5	8,35	<0,001	***
TCR Nombres de feuilles					
TCR <sub>F5-0</sub>	3	2,3	4,27	0,008	**
TCR <sub>F5-1</sub>	3	2,3	4,27	0,008	**
TCR <sub>F2-1</sub>	3	2,5	9,31	<0,001	***
TCR <sub>F4-3</sub>	3	0,3	1,26	0,295	n.s.



Figure 2. Biomasses totales et aériennes à la fin de l'expérimentation en fonction des modalités expérimentales (T: témoin sans défoliations, 1C2: une coupe à 2 cm de hauteur, 1C6: une coupe à 6 cm de hauteur, 2C6: deux coupes à 6 cm de hauteur)  $\pm$  erreur standard.

Figure 2. Total and aboveground biomasses at the end of the experiment according to experimental treatments (T : control without any defoliation, 1C2 : one cut at 2 cm height, 1C6 : one cut at 6 cm height, 2C6 : two cuts at 6 cm height)  $\pm$  standard error.



Figure 3. Hauteurs en fin d'expérimentation, brute (H5) ou en tenant compte des parties défoliées (hauteur globale  $H_{glob}$ ; T: témoin sans défoliations, 1C2: une coupe à 2 cm de hauteur, 1C6: une coupe à 6 cm de hauteur, 2C6: deux coupes à 6 cm de hauteur)  $\pm$  erreur standard.

Figure 3. Gross height at the end of the experiment (H5) or height taking into account defoliated tissues (global height  $H_{glob}$ ; T: control without any defoliation, 1C2: one cut at 2 cm height, 1C6: one cut at 6 cm height, 2C6: two cuts at 6 cm height)  $\pm$  standard error.



Figure 4. Taux de croissance relatifs en hauteur sur toute la durée de l'expérimentation prenant en compte les hauteurs brutes (5-0) ou les parties défoliées (glob-0), entre la fin de l'expérimentation et après la première coupe (5-1), ou lors de la première période de repousse de 12 jours (2-1). T: témoin sans défoliations, 1C2: une coupe à 2 cm de hauteur, 1C6: une coupe à 6 cm de hauteur, 2C6: deux coupes à 6 cm de hauteur;  $\pm$  erreur standard.

Figure 4. Height gross relative growth rates along the whole experiment taking into account gross height (5-0) or defoliated tissues (glob-0), between the end of the experiment and after the first cut (5-1), or during the first 12-days period of regrowth (2-1). T : control without any defoliation, 1C2 : one cut at 2 cm height, 1C6 : one cut at 6 cm height, 2C6 : two cuts at 6 cm height)  $\pm$  standard error.

(1C2, 1C6; Tableau 1; Figure 4). Ces résultats suggèrent une compensation de croissance pour les modalités 1C2 et 1C6 et une compensation partielle pour 2C6. Si l'on tient compte de la hauteur des parties défoliées (hauteurs globales), il n'existe plus de différence significative entre les TCR<sub>Hglob-0</sub> des diverses modalités de défoliation, suggérant une croissance compensatoire quels que soit le régime et l'intensité de défoliations subies (Tableau 1; Figure 4). Si l'on considère par contre la période immédiatement après la première défoliation (TCR<sub>H5-1</sub>), la modalité défoliation sévère en intensité (1C2) montre un TCR<sub>H</sub> significativement plus important que toutes les autres modalités (Tableau 1; Figure 4). Ceci reflète un TCR<sub>H</sub> particulièrement élevé pour 1C2, donc une stimulation de croissance qui a en fait lieu pendant la douzaine de jours suivant la première défoliation (TCR<sub>H2-1</sub>) et dont les effets perdurent jusqu'à la fin de l'expérimentation (Tableau 1; Figure 4). Pendant la seconde douzaine de jours de croissance (TCR<sub>H4-3</sub>), il n'y a plus de différences significatives de TCR<sub>H</sub> entre les différentes modalités de défoliation (Tableau 1).

#### Nombre de feuilles

Le nombre de feuilles d'*E. juncea* en fin d'expérimentation (F5) est significativement plus faible pour le traitement d'une coupe à 2 cm (1C2) que pour tous les autres traitements (Tableau 1; Figure 5).

Les taux de croissance relatifs foliaires  $(TCR_F)$ réagissent de manières très différentes par rapport aux TCR<sub>H</sub>. Les TCR<sub>F</sub> des individus témoins sont significativement supérieurs aux TCR<sub>F</sub> des modalités défoliées quelle que soit la période considérée (TCR<sub>F5-0</sub>, TCR<sub>F5-1</sub>, TCR<sub>F2-1</sub>; Tableau 1 ; Figure 5), à l'exception de la seconde douzaine de jours (TCR<sub>F4-3</sub>) pour laquelle, à l'instar des TCR<sub>H4-3</sub>, aucune différence significative n'est visible (Tableau 1).

# *Corrélations entre biomasses et variables non destructives*

La hauteur des plantes au temps final est très significativement corrélée avec les biomasses aérienne et totale (r > 0,62; p < 0,0001) et dans une moindre mesure avec la biomasse racinaire (r = 0,37; p =



Figure 5. Nombre de feuilles en fin d'expérimentation (F5), taux de croissance relatifs pour les nombres de feuilles sur la durée totale de l'expérimentation (5-0), entre la fin de l'expérimentation et après la première coupe (5-1), ou lors de la première période de repousse de 12 jours (2-1). T: témoin sans défoliations, 1C2: une coupe à 2 cm de hauteur, 1C6: une coupe à 6 cm de hauteur, 2C6: deux coupes à 6 cm de hauteur;  $\pm$  erreur standard.

Figure 5. Leaf numbers at the end of the experiment (F5), leaf numbers relative growth rates along the whole experiment (5-0), between the end of the experiment and after the first cut (5-1), or during the first 12-days period of regrowth (2-1). T : control without any defoliation, 1C2 : one cut at 2 cm height, 1C6 : one cut at 6 cm height, 2C6 : two cuts at 6 cm height)  $\pm$  standard error.

0,0011). Le nombre de feuilles est lui aussi significativement corrélé avec les biomasses aérienne et totale (r > 0,39; p < 0,0005); sa corrélation à la biomasse racinaire est marginalement significative (r = 0,21; p = 0,0678).

# Discussion

La réponse d'*E. juncea* à la défoliation dépend donc à la fois des fréquences et intensités des défoliations appliquées, de la période temporelle par rapport à la défoliation et des types de variables biologiques mesurées.

# Effet des régimes de défoliation

Il existe théoriquement un optimum de défoliation pour lequel la production de biomasse à l'échelle de la communauté végétale est maximale. Cette théorie de l'optimisation par le pâturage correspond à des niveaux d'herbivorie intermédiaires et l'optimisation de production végétale est difficile à atteindre à des intensités ou des fréquences de défoliations importantes (Hilbert et al. 1981). De manière convergente, plusieurs résultats prédisent une compensation maximale de la croissance pour des niveaux de perturbation relativement faible (McNaughton 1983; Zhao, Chen & Lin 2008). Même s'il est difficile d'interclasser formellement la sévérité de nos traitements de défoliation (une coupe à 2 cm est-elle plus sévère que deux coupes à 6 cm?), la modalité «une coupe à 6 cm» (1C6) paraît être la plus modérée. Or, aucun de nos résultats ne suggère de compensation de croissance plus élevée pour cette modalité. Nos traitements de défoliation peuvent apparaître comme relativement faibles : une expérience de terrain sur une Poacée stolonifère (Puccinellia phryganodes) montre une augmentation de biomasse aérienne après trois et quatre défoliations successives, chacune séparée de douze jours (Hik, Sadul & Jefferies 1991).

L'influence respective de la fréquence et de l'intensité de défoliation sur des espèces cibles isolées en pots est peu testée dans la littérature. Nos résultats indiquent que la coupe la plus sévère en intensité (à 2 cm) déclenche une vigoureuse stimulation de la croissance en hauteur. Des coupes répétées (deux fois par an, correspondant à la modalité 2C6 de notre expérimentation) altèrent les potentialités de réponses à la défoliation, mais cet effet semble être dépendant de l'espèce et ne semble pas survenir chez les Dicotylédones et les Cypéracées (Moser & Schütz 2006).

# Importance de la période de défoliation

L'importance de la croissance compensatoire est fortement sous-estimée par l'absence de prise en compte du facteur temps, notamment dans les milieux naturels (Paige 1999). La date de défoliation peut avoir des conséquences très différentes selon le stade phénologique auquel elle intervient, et même selon l'année (Lennartsson, Nilsson & Tuomi 1998) ou le stade de développement de la plante (Trumble, Kolodny-Hirsch & Ting 1993). Une différence d'une à trois semaines dans la date de défoliation peut affecter de manière très sensible la réponse des plantes (Paige 1999), une défoliation précoce dans la saison étant plus favorable aux croissances compensatoires qu'une défoliation tardive (Maschinski & Whitham 1989; McIntire & Hik 2002).

Dans notre cas, la stimulation de croissance que nous avons observée est relativement ponctuelle, puisqu'elle concerne uniquement la première douzaine de jours après l'évènement de défoliation de forte intensité (1C2). Les réponses à la défoliation sont donc très rapides et s'estompent au cours du temps, suggérant que la stimulation de croissance est un phénomène fugace et inductible à la première défoliation, à condition que celle-ci soit suffisamment intense (voir également Parra-Tabla, Rico-Gray & Carbajal 2004).

#### Influence des variables biologiques mesurées

Les réponses aux modalités de défoliations sont très différentes selon que l'on considère la hauteur de l'appareil végétatif ou le nombre de feuilles.

Pour les graminoïdes, les réponses des différentes variables foliaires semblent être fortement influencées par l'histoire de pâturage. Ainsi, dans les communautés de pelouses alpines, la mortalité foliaire de *Kobresia myosuroides* dépend spécifiquement de la récurrence et la prédictibilité du pâturage, alors que la longueur de ses feuilles dépend à la fois des évènements de défoliation de l'année et de l'histoire à plus long terme de pâturage (McIntire & Hik 2002). Nos résultats montrent également une meilleure réactivité de la longueur foliaire d'*E. juncea* aux évènements récents de défoliation, c'est-à-dire à nos défoliations expérimentales, par rapport aux nombres de feuilles.

Les individus cibles défoliés ne compensent pas leur perte de biomasse due à la défoliation par rapport aux individus témoins. Cette différence de biomasse totale reflète en fait des différences de biomasses aériennes, comme cela a pu être mis en évidence dans d'autres études impliquant d'autres Poacées (Hik, Sadul & Jefferies1991; Alward & Joern 1993). La réponse de la biomasse racinaire à la défoliation est très dépendante de l'espèce considérée et des conditions environnementales (Moser & Schütz 2006). Dans notre étude, les biomasses souterraines d'E. juncea ne varient pas significativement entre les modalités de défoliation. Or, les Poacées rhizomateuses voient habituellement leur biomasse souterraine et leur quantité de réserves souterraines en hydrates de carbone diminuer après défoliation pour soutenir la croissance compensatoire (Tiffin 2000; Klimes & Klimesova 2002), bien que des exceptions soient notables (Wang et al. 2004). E. juncea est une plante clonale qui possède un rhizome particulièrement développé en conditions naturelles, jusqu'à plusieurs mètres de long. Mais son conditionnement en fragments de 2 cm a pu drastiquement amoindrir ses capacités de

mobilisation de réserves après défoliation, ce qui suggère une limitation trophique des plants défoliés dans notre expérimentation.

D'autres variables biologiques comme la floraison et la fructification sont susceptibles de montrer des compensations à la défoliation (Paige 1999), mais dans notre cas aucun phytomètre n'a fleuri.

# Occurrence de la croissance compensatoire

Aucune modalité de défoliation ne permet de retrouver une biomasse totale ou aérienne similaire à la modalité témoin. Cependant, il s'agit là de biomasses finales n'intégrant pas pour les modalités défoliées la biomasse des parties coupées. Lorsque l'on considère les taux de croissance relatifs sur toute la période de l'expérimentation, on observe une compensation partielle (sous-compensation) au niveau des nombres de feuilles. Au niveau de la longueur, il y a compensation pour les modalités à une coupe, avec notamment une surcompensation ponctuelle pour la modalité à une coupe sévère (1C2). En ce qui concerne la modalité à deux coupes, la compensation partielle qu'elle montre pour la longueur peut être sous estimée du fait de la non-prise en compte dans les calculs de la longueur défoliée lors de la seconde coupe. Par conséquent, on observe une compensation partielle de croissance chez E. juncea mais pas de surcompensation durable. Ceci traduit néanmoins une stratégie de tolérance d'E. juncea à la défoliation et non une stratégie de résistance (dont le coût est élevé pour la plante et qui ne semble pas concerner les Poacées; Strauss, Rudgers & Irwin 2002).

L'absence de surcompensation de croissance pour *E. juncea* dans notre expérimentation peut s'expliquer par l'échelle d'étude (Belsky 1987) ou par des paramètres intrinsèques aux Poacées, tels que la position basale des méristèmes foliaire. En effet, une défoliation d'arbres ou de dicotylédones herbacées à croissance monopodiale favorise la surcompensation de croissance par perte de dominance des méristèmes apicaux (Paige 1999; Huhta et al. 2000).

La croissance compensatoire dépend en outre de facteurs externes à la plante, et diminue lorsque l'intensité de compétition végétale augmente (Maschinski & Whitham 1989; Huhta et al. 2000). Par ailleurs, l'optimisation de croissance par le pâturage est maximum lorsque les apports de nutriments par déjections des herbivores sont suffisamment importants et quand l'écosystème a atteint un niveau minimal de fertilité trophique (de Mazancourt, Loreau & Abbadie 1998). Toutefois, les relations existant entre l'occurrence de la croissance compensatoire et le niveau trophique de l'environnement sont sans doute plus complexes. Ainsi, la croissance compensatoire à laquelle on peut s'attendre en milieux riches en nutriments (Maschinski & Whitham 1989) dépend fortement de l'espèce végétale considérée (Alward & Joern 1993). De plus, pour certains auteurs, l'optimisation par le pâturage s'observe dans des environnements dans lesquels les plantes sont stressées et

n'expriment pas leur maximum de potentialités de croissance (Hilbert et al. 1981). La faculté de mobiliser des ressources (nutriments, eau) pour la plante, fonction de la saisonnalité et des conditions climatiques de l'année, semble déterminante pour induire une surcompensation de croissance (Lennartsson, Nilsson & Tuomi 1998).

La part relative des facteurs intrinsèques et environnementaux pour expliquer la croissance compensatoire reste peu claire. Bien que certains modèles mettent l'accent sur l'importance des facteurs externes (Maschinski & Whitham 1989), d'autres proposent une prépondérance de la perte de dominance apicale, notamment chez les plantes monocarpiques (voir Rautio et al. 2005).

Dans notre cas, l'absence de compétition végétale, le faible niveau trophique du substrat et l'absence de dominance méristématique apicale chez E. juncea peuvent expliquer qu'aucune surcompensation de croissance n'ait été mise en évidence sur la durée totale de l'étude. De plus, les contingences expérimentales nous ont sans doute sous-estimer l'intensité de croissance conduit à compensatoire (Belsky 1987; Alward & Joern 1993). Il a été montré que les espèces clonales dunaires pouvaient être favorisées par leurs connections rhizomateuses, surtout à fort niveau de défoliation (Liu et al. 2009), bien que l'intégration clonale diminue lors de la défoliation (voir notamment pour *Elytrigia repens*, Esmaeili et al. 2009). D'autre part, pour les plantes dunaires, les sources de nutriments peuvent provenir d'une forte remobilisation interne vers les parties aériennes (Gilbert & Ripley 2008). Dans notre cas, la rupture de la clonalité que nous avons provoquée en utilisant des fragments de rhizomes pourrait fortement limiter la réallocation de ressources vers les parties aériennes (Bach 2000; Gilbert & Ripley 2008), bien que le rôle de l'intégration clonale dans la réponse à la défoliation puisse encore être sujet à caution (Wang et al. 2004).

# Implications écologiques pour E. juncea

À date équivalente, la défoliation artificielle semble mimer de façon satisfaisante un pâturage par les herbivores vertébrés, bien que certains processus de défoliation naturelle comme les dégâts mécaniques sur la plante ou la stimulation physiologique de la plante broutée ne soient pas pris en compte (Paige 1999). Nos résultats suggèrent que la défoliation par les vertébrés de jeunes *E. juncea* est susceptible d'engendrer une diminution significative de biomasse sur pied d'*E. juncea*, et ce quel que soit le régime d'herbivorie. Nous n'observons pas de surcompensation mais une compensation de la hauteur et une stimulation de la croissance suite au traitement de forte intensité.

Le niveau de défoliation infligé par les populations d'herbivores à *E. juncea* dans les dunes littorales semble extrêmement variable. Bien que des indices visuels *in situ* (traces d'abroutissement) ne permettent pas de mettre évidence un fort niveau d'abroutissement au niveau de notre zone d'étude, certaines études relatent de très fortes pressions d'herbivorie sur *E. juncea* (Harris & Davy 1986). Parmi les herbivores potentiellement présents sur les dunes, les chevreuils (Capreolus capreolus) montrent une grande sélectivité alimentaire. Leurs panses montrent fréquemment des résidus de Poacées (40 à 72% des cas) mais dans des proportions très faibles (0,6 à 4,4%) du poids moyen des contenus stomacaux (Maizeret & Tran Manh Sung 1984). L'appétence de ces Poacées est relativement faible pour les chevreuils en milieu forestier du fait d'une relativement forte teneur en matières cellulosiques et d'une faible teneur en matières azotées (Maizeret & Tran Manh Sung 1984). Les lapins (Oryctolagus cuniculus) sont par contre caractérisés par un régime alimentaire incluant une végétation à plus faible digestibilité. Leurs contenus stomacaux révèlent une forte consommation de Poacées dans les milieux dunaires, bien de que celles-ci puissent induire des problèmes déséquilibres diététiques importants (Wallage-Drees & Deinum 1986). Parmi les espèces dominantes des dunes littorales aquitaines, Е. juncea semble être particulièrement appétente : le fait que sa présence soit positivement corrélée à l'importance de la perturbation par l'ensablement et au taux de décomposition des litières (Forey et al. 2008) suggère une croissance relativement rapide dans des milieux relativement fertiles et une appétence relativement forte des tissus foliaires.

# De la stimulation de croissance à l'optimisation de défoliation

À la lumière de notre étude, les types de réponses positives des plantes à la défoliation peuvent être redéfinis et précisés au-delà de la seule notion d'intensité de croissance compensatoire (Belsky 1986). La prise en compte de variables correspondant à des échelles de temps différentes permet tout d'abord de distinguer - la stimulation de croissance de la compensation de croissance. Ainsi, les taux de croissance relatifs représentent une quantification fine des processus, puisqu'ils sont pondérés par l'état initial de l'individu (atténuant la variabilité de mesure liée à l'hétérogénéité des plantes cibles) et puisqu'ils permettent de mettre en évidence une dynamique temporelle de réponse des plantes. Les taux de croissance relatifs permettent donc d'appréhender des « stimulations de croissance » ponctuelles, qui n'auront pas forcément de répercussion à l'échelle de la durée totale de l'expérimentation. Les mesures effectuées au temps final sont par contre beaucoup plus intégratives. Elles quantifient une « compensation de croissance » sur une période relativement longue, correspondant à la durée de l'expérimentation ou de la saison de végétation. Des stimulations ponctuelles de croissance ne se traduisent donc pas nécessairement par une compensation de croissance.

Les effets positifs de la défoliation sur la végétation ont été essentiellement abordés en écologie *via* les processus d'herbivorie. À l'échelle de la communauté végétale, ceci a notamment abouti à l'hypothèse d'optimisation de pâturage (Hilbert et al. 1981; Belsky 1986). Or les sources de défoliation sont plus larges dans les écosystèmes, avec notamment la fauche qui est une perturbation majeure dans les communautés herbacées. Les mécanismes de croissance compensatoire mis en évidence peuvent aussi bien s'appliquer à des défoliations dues à l'herbivorie ou à la fauche, notamment si l'on considère les relations entre croissance compensatoire et niveaux trophiques. Nous pouvons alors intégrer ces deux types de perturbation en définissant une « optimisation de défoliation », qui engloberait à la fois l'optimisation de pâturage et les effets de la fauche.

# Conclusion

La croissance compensatoire est habituellement estimée en différence globale pour une période termes de relativement longue (en général la durée de l'expérimentation) et non en terme de taux de croissance relatif tout au long de l'expérience. Nos résultats de hauteur montrent que l'on peut avoir une croissance compensatoire à certaines périodes (TCR<sub>H5-1</sub> et TCR<sub>H2-1</sub>), voire une surcompensation temporaire, sans pour autant que les niveaux de croissance globale (H5) soient atteints. Ceci peut aussi bien s'expliquer par la stratégie de l'espèce considérée que par la limitation trophique du milieu, ce qui mériterait d'être testé dans de futurs travaux.

A partir de cette étude, on peut définir la notion de « stimulation de croissance » et proposer la notion « d'optimisation de défoliation » en complément de la compensation de croissance et de l'optimisation de pâturage.

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# References

- Agrawal, A.A. 2000. Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. *Trends in Plant Science* 5: 309–313.
- Alward, R.D., and A. Joern. 1993. Plasticity and overcompensation in grass responses to herbivory. *Oecologia* 95: 358–364.
- Bach, C.E. 2000. Effects of clonal integration on response to sand burial and defoliation by the dune plant *Ipomoea pescaprae* (Convolvulaceae). *Australian Journal of Botany* 48: 159–166.
- Belsky, A.J. 1986. Does herbivory benefit plants? A review of the evidence *The American Naturalist* 127: 870–892.
- Belsky, A.J. 1987. The effects of grazing: confounding of ecosystem, community, and organism scales. *The American Naturalist* 129: 777–783.
- Callaway, R.M., J. Kim, and B.E. Mahall. 2006. Defoliation of *Centaurea solstitialis* stimulates compensatory growth and intensifies negative effects on neighbors. *Biological Invasions* 8: 1386–1397.

- Callaway, R.M., B. Newingham, C.A. Zabinsky, and B.E. Mahall. 2001. Compensatory growth and competitive ability of an invasive weed are enhanced by soil fungi and native neighbours. *Ecology Letters* 4: 429–433.
- de Mazancourt, C., M. Loreau, and L. Abbadie. 1998. Grazing optimization and nutrient cycling: when do herbivores enhance plant production? *Ecology* 79: 2242–2252.
- Esmaeili, M.M., A. Bonis, J.-B. Bouzillé, C. Mony, and M.-L. Benot. 2009. Consequence of ramet defoliation on plant clonal propagation and biomass allocation: example of five rhizomatous grassland species. *Flora* 204: 25–33.
- Forey, E., B. Chapelet, Y. Vitasse, M. Tilquin, B. Touzard, and R. Michalet. 2008. The relative importance of disturbance and environmental stress at local and regional scales in French coastal sand dunes. *Journal of Vegetation Science* 19: 493–502.
- García-Mora, M.R., J.B. Gallego-Fernández, and F. García-Novo. 1999. Plant functional types in coastal foredunes in relation to environmental stress and disturbance. *Journal of Vegetation Science* 10: 27–34.
- Gilbert, M.E., and B.S. Ripley. 2008. Biomass reallocation and the mobilization of leaf resources support dune plant growth after sand burial. *Physiologia Plantarum* 134: 464–472.
- Gonzalez-Teuber, M., and E. Gianoli. 2007. Tolerance to simulated herbivory in two populations of *Concolvulus chilensis* (Convolvulaceae). *Acta Oecologica* 32: 119–123.
- Harris, D., and A.J. Davy. 1986. Strandline colonization by *Elymus farctus* in relation to sand mobility and rabbit grazing. *Journal of Ecology* 74: 1045–1056.
- Hik, D.S., H.A. Sadul, and R.L. Jefferies. 1991. Effects of the timing of multiple grazings by geese on net above-ground primary production of swards of *Puccinellia phryganodes*. *Journal of Ecology* 79: 715–730.
- Hilbert, D.W., D.M. Swift, J.K. Detling, and M.I. Dyer. 1981. Relative Growth Rates and the grazing optimization hypothesis. *Oecologia* 51: 14–18.
- Huhta, A.P., K. Hellström, P. Rautio, and J. Tuomi. 2000. A test of the compensatory continuum: fertilization increases and below-ground competition decreases the grazing tolerance of tall wormseed mustard (*Erysimum strictum*). *Evolutionary Ecology* 14: 353–372.
- Klimes, L., and J. Klimesova. 2002. The effects of mowing and fertilization on carbohydrate reserves and regrowth of grasses: do they promote plant coexistence in species-rich meadows? *Evolutionary Ecology* 15: 363–382.
- Lennartsson, T., P. Nilsson, and J. Tuomi. 1998. Induction of overcompensation in the field gentian, *Gentianella* campestris. Ecology 79: 1061–1072.
- Liu, H.D., F.H. Yu, W.M. He, H. Chu, and M. Dong. 2009. Clonal integration improves compensatory growth in heavily grazed ramet populations of two inland-dune grasses. *Flora* 204: 298–305.
- Macedo, J.A., P. Alves, Â. Lomba, J. Vicente, R. Henriques, H. Granja, and J. Honrado. 2010. On the interest of plant functional classifications to study community-level effects of increased disturbance on coastal dune vegetation. *Acta Botanica Gallica* 157: 305–315.

- Maizeret, C., and D. Tran Manh Sung. 1984. Etude du régime alimentaire et recherche du déterminisme fonctionnel de la sélectivité chez le chevreuil (*Capreolus capreolus*) des Landes de Gascogne. *Gibier Faune Sauvage* 3: 5–19.
- Maschinski, J., and T.G. Whitham. 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *The American Naturalist* 134: 1–19.
- McIntire, E.J.B., and D.S. Hik. 2002. Grazing history versus current grazing: leaf demography and compensatory growth of three alpine plants in response to a native herbivore (*Ochotona collaris*). Journal of Ecology 90: 348–359.
- McNaughton, S.J. 1983. Compensatory plant growth as a response to herbivory. *Oikos* 40: 329–336.
- Moser, B., and M. Schütz. 2006. Tolerance of understory plants subject to herbivory by roe deer. *Oikos* 114: 311–321.
- Paige, K.N. 1999. Regrowth following ungulate herbivory in *Ipomopsis aggregata*: geographic evidence for overcompensation. *Oecologia* 118: 316–323.
- Paige, K.N., and T.G. Whittam. 1987. Overcompensation in response to mammalian herbivory: the advantage of being eaten. *The American Naturalist* 129: 407–416.
- Parra-Tabla, V., V. Rico-Gray, and M. Carbajal. 2004. Effect of defoliation on leaf growth, sexual expression and reproductive success of *Cnidoscolus aconitifolius* (Euphorbiaceae). *Plant Ecology* 173: 153–160.
- Rautio, P., A.P. Huhta, S. Piippo, J. Tuomi, T. Juenger, M. Saari, and J. Aspi. 2005. Overcompensation and adaptative plasticity of apical dominance on *Erysimum strictum* (Brassicaceae) in response to simulated browsing and resource availability. *Oikos* 111: 179–191.
- Strauss, S.Y., J.A. Rudgers, and R.E. Irwin. 2002. Direct and ecological costs of resistance to herbivory. *Trends in Ecology and Evolution* 17: 278–285.
- Tiffin, P. 2000. Mechanisms of tolerance damage: what we know? *Evolutionary Ecology* 14: 523–536.
- Trumble, J.T., D.M. Kolodny-Hirsch, and I.P. Ting. 1993. Plant compensation for arthropod herbivory. *Annual Review of Entomology* 38: 93–119.
- Wallage-Drees, J.M., and B. Deinum. 1986. Quality of the diet selected by wild rabbits (*Oryctolagus cuniculus* (L.)) in autumn and winter. *Netherlands Journal of Zoology* 36: 438–448.
- Wallage-Drees, J.M., and N.C. Michielsen. 1989. The influence of food supply on the population dynamics of rabbits, *Oryctolagus cuniculus* (L.), in a Dutch dune area. *Zeitschrift für Säugetierkunde* 54: 304–323.
- Wang, Z., L. Li, X. Han, and M. Dong. 2004. Do rhizome severing and shoot defoliation affect clonal growth of *Leymus chinensis* at ramet population level? *Acta Oecologica* 26: 255–260.
- Zeevalking, H.J., and L.F.M. Fresco. 1977. Rabbit grazing and species diversity in dune area. *Vegetatio* 35: 193–196.
- Zhao, W., S.P. Chen, and G.H. Lin. 2008. Compensatory growth responses to clipping defoliation in *Leymus chinensis* (Poaceae) under nutrient addition and water deficiency conditions. *Plant Ecology* 196: 85–99.

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# Long-term changes in calcareous grassland vegetation in North-western Germany – No decline in species richness, but a shift in species composition

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#### ABSTRACT

We aimed to answer the question of whether the species richness and composition of calcareous grasslands in North-western Germany had changed over the last 70 years as a result of atmospheric nitrogen (N) deposition. In total, 1186 plots of *Festuco-Brometea* (alliance *Bromion erecti*) grasslands from the suboceanic regions of the country were compiled (1061 plots from literature sources spanning a time period from 1936 to 1996, 125 new plots from 2008). Environmental descriptors recorded for each plot included geographic coordinates, altitude, heat index (combining slope and aspect), mean Ellenberg indicator values for light, soil moisture, soil pH and soil N, and cumulative N deposition (the latter being highly positively correlated with the year of sampling).

In a Detrended Correspondence Analysis, the sample plot scores along axis one were highly correlated with the mean Ellenberg N-values, those along axis two were significantly affected by the year of sampling. In a general linear model, species richness of vascular plants showed a markedly hump-shaped relationship with mean Ellenberg N-value, whereas it was weakly affected by year (cumulative N load). Species with a significant negative trend over time were more often (than expected by chance) habitat specialists of dry grasslands, small, light-demanding and winter-green or evergreen with smaller seeds and scleromorphic leaves. In contrast to what has been found for acidic grasslands, N deposition in calcareous grasslands did not result in a decline in species richness, most likely because calcareous grasslands are water- and phosphorus-limited, and are well-buffered in terms of soil pH. To prevent a further change in species composition towards more mesophytic communities, grassland management by the site managers needs to be intensified.

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# 1. Introduction

Atmospheric nitrogen (N) deposition and the subsequent eutrophication of terrestrial and aquatic ecosystems have been recognized as global processes where the planetary thresholds have already been overstepped (Rockström et al., 2009). In Central Europe the deposition of N exceeds 10–15 kg ha<sup>-1</sup> year<sup>-1</sup> in semi-natural vegetation and 20 kg ha<sup>-1</sup> year<sup>-1</sup> in coniferous forest, and the values are higher or even much higher in areas of intensive agriculture (Sutton et al., 2011). In Germany, the emission rates for nitrogen oxides have considerably decreased since 1990, whereas those for ammonium have been largely stable over the past years (http://www.umweltbundesamt.de/). In general, the emissions of N in Europe and other parts of the world are projected to increase during the next decades (e.g., Gruber and Galloway, 2008). The effects of N deposition on plant-species composition and richness have been documented for several vegetation types, based on three lines of evidence. First, experiments conducted in various habitat







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types have shown that N addition is often associated with an increase in biomass and a simultaneous decrease in species richness (Bobbink et al., 2010). In a recent meta-analysis, however, De Schrijver et al. (2011) showed that these effects on plant biomass and richness are vegetation type-dependent and that species of different life forms are differently affected. A second source of evidence relies on descriptive comparisons of areas situated along a geographic gradient in N deposition, for example in North European forests (Diekmann et al., 1999) and in British grasslands and heathlands (Stevens et al., 2004, 2006; Maskell et al., 2010). These spatial studies have largely supported the experimental results, showing a general decline in species richness, a decrease in species indicative of nutrient-poor sites, and a shift in functional types from forbs to grasses. The third way to examine effects of atmospheric pollution is a temporal approach, comparing plots sampled at a time with low N deposition with more recent plots having been exposed to higher deposition levels. Such time-series analyses have mainly been conducted in forests based on the monitoring of permanent plots (e.g., Diekmann et al., 1999), but have also been applied using a non-permanent design (Diekmann and Duprè, 1997; Gaudnik et al., 2011). Provided that the number of plots is large and that the data set is not spatially or temporally biased, this approach has some advantages compared to a permanent plot design in that it can be more representative through covering larger regions and more plots.

Eutrophication-driven changes in European vegetation are particularly well studied in acidic grasslands, which have suffered from a steep decline in species richness mainly caused by an increase in a few competitive grasses at the expense of forbs and dwarf shrubs (Stevens et al., 2006, 2011a; Duprè et al., 2010; Maskell et al., 2010). These temporal trends coincide closely with the results of N-addition experiments in grasslands (see De Schrijver et al., 2011). Surprisingly, the effects of N deposition in calcareous grasslands are less well documented, although these grasslands are among the most species-rich habitat types in Europe and of focal interest to nature conservationists across the continent. In one of the few long-term studies from Britain. Bennie et al. (2006) re-surveved 92 plots from English chalk grasslands that were first surveyed in 1952-53. They showed a decline in species richness, a decrease in species associated with inherently infertile conditions and an increase in species typical of more mesotrophic grasslands, thereby indicating an effect of nutrient enrichment. In contrast, an increase in  $\alpha$  diversity over 70 years was observed in a re-survey of 88 calcareous grassland sites in the English county of Dorset (Newton et al., 2012). In another permanent plot study from southern Germany, revealing similar results, the vegetation changes were attributed mainly to a change in management, namely a decrease in grazing intensity (Hagen, 1996). Again, these changes and their interpretation coincide with results from various N-addition experiments (Bobbink, 1991; Willems et al., 1993; Jacquemyn et al., 2003). Several of the experimental studies suggest that the increasing dominance of Brachypodium pinnatum is one of the main proximal reasons for the observed species loss (e.g., Bobbink and Willems, 1987). The evidence for a decrease in species richness and the role of N enrichment in this process, however, are not unequivocal. In an N-addition experiment with chalk grassland species, Wilson et al. (1995) did not observe an increasing cover of grasses (such as Brachypodium) and a subsequent loss of species diversity. A recent spatial study from Britain also did not give evidence for a negative impact of N deposition on plant species richness in calcareous grasslands (Maskell et al., 2010).

Another limiting nutrient in many ecosystems is phosphorus (P). The input of P into terrestrial ecosystems from weathering and from the atmosphere is usually low, and if N deposition is high, P may become the limiting nutrient and species with efficient P economies may be favoured (Bobbink et al., 2010; Vitousek et al.,

2010). Both N and P are likely to play an important role for the species richness and variation in species composition, as evidenced for grasslands by, for example, Kleijn et al. (2008) and Ceulemans et al. (2011, 2013). Compared to N, however, P has received only little attention in the study of vegetation changes in semi-natural terrestrial vegetation.

Apart from a recent analysis of the trends in the frequencies of calcareous grassland species in Germany (Jandt et al., 2011), our study is to our knowledge the first large-scale study of temporal changes in the vegetation of calcareous grasslands. It is a follow-up of a similar analysis conducted in acidic grasslands in North-western Europe (Duprè et al., 2010) where the impact of N deposition on species assemblages and diversity were found to be dramatic. Based on an analysis of historical and recent vegetation data from North-western Germany, we aimed to: (1) examine changes in the species composition and richness in calcareous grasslands over the last 70 years in response to N deposition. (2) compare the temporal trends of species with different life-history traits and ecological strategies, (3) assess the role of P for the species composition and richness in calcareous grasslands, and (4) contrast the results with those obtained in acidic grasslands and discuss the underlying causes for the differences between the two grassland types.

## 2. Material and methods

#### 2.1. Vegetation data

Plot-scale data from calcareous grasslands were compiled from the sub-oceanic areas of North-western Germany (Fig. 1). More southern and continental regions with a much larger pool of calcareous grassland species were avoided to obtain a vegetation data



Fig. 1. Map of the study areas in North-western Germany from where plots of calcareous grasslands were compiled.

set with relatively uniform species composition. We searched exclusively for plots that had been assigned by their authors to the alliance Bromion erecti, including grasslands on usually relatively shallow, infertile and high-pH soils, often occurring on south-facing slopes. The decreasing intensity or abandonment of grazing and mowing in semi-natural landscapes has resulted in a strong reduction in the area of traditional calcareous grasslands (Ellenberg and Leuschner, 2010). To minimize confounding management effects, plots were considered for analysis only if active grazing or mowing at the time of sampling was indicated, and if there was no evidence of fertilization. Managed B. erecti communities are characterized by a sward of graminoids (the most common species being the grasses B. pinnatum, Festuca ovina agg., Koeleria pyramidata, Briza media, Helictotrichon pratense and Bromus erectus, as well as Carex flacca and C. caryophyllea) and a large number of forbs such as Lotus corniculatus. Pimpinella saxifraga. Sanguisorba minor. Thymus pulegioides and Cirsium acaule (Ellenberg and Leuschner, 2010). Calcareous grasslands in the area have a relatively high proportion of threatened and protected plant species, including many orchids like Gymnadenia conopsea and Ophrys insectifera. Plots were only retained if information on plot size and geographic location were available. The historical data (1061 plots) were obtained mainly from published sources, and to a lesser extent also from unpublished reports. These were supplemented by 125 newly sampled vegetation plots from 2008. The final data sets included 1186 plots, the data sources are listed in Table A1. The oldest plots of calcareous grasslands from the study area available to us were from 1936, the highest number of plots was sampled in the 1980s.

The vegetation data including all vascular plants were entered into a table and standardized. As the precision of species records differed between the studies and also the taxonomic concepts for some species had changed over time, several taxa had to be aggregated. The taxonomic standardization was based on the nomenclature of Wisskirchen and Haeupler (1998). A further standardization concerned the species-abundance measures that were re-coded to conform to the simplified 6-degree scale of Braun-Blanquet (Dierschke, 1994).

#### 2.2. Environmental data

For a causal analysis of the vegetation changes, we compiled a number of environmental descriptors for each plot. These included geographic location (latitude, longitude, altitude) and information about the topography of the site. As slope and aspect are known to have a strong and coupled impact on the plant-species composition of calcareous grasslands (Bennie et al., 2006), we calculated an integrative heat index value =  $\cos(\operatorname{aspect} - 225) \cdot \tan(\operatorname{slope})$ according to Parker (1988). As surrogates for measured environmental variables, we calculated mean Ellenberg indicator values (Ellenberg et al., 1992) for light (meanL), soil moisture (meanF), soil pH (meanR), and soil nutrient status (meanN, originally described as reflecting nitrogen availability) for all plots. These values have successfully been applied in other studies examining temporal changes of the vegetation in response to atmospheric pollution (Diekmann and Duprè, 1997; Duprè et al., 2010). As recommended by most researchers (see Diekmann, 2003), the calculation of mean values was based on species presence-absence instead of quantitative data, especially to avoid a bias owing to inter-observer differences in cover estimation. We also did analyses with coverweighted data, but the results were very similar.

To examine the long-term effects of atmospheric pollution on species composition and richness, we estimated cumulative values of total (wet and dry) N deposition (cumNdep), based on deposition values for the year 2000 obtained from the model FACEM (Pieterse et al., 2007) and the change of deposition values over time. Details of the calculation are described in Duprè et al. (2010). Cumulative values of sulfur (S) deposition were calculated in a similar manner, but not used in the final analysis for two reasons: first, we assumed that the acidifying effect of S would be well buffered in the calcareous soils, and second, there was a high multi-collinearity between N and S deposition.

No environmental measurements were available for the plots compiled from the literature. For the plots surveyed in 2008, soil samples for the determination of pH, total carbon (C), total N and plant available phosphorus (P) were collected. Soil samples consisted of two mixed sub-samples taken at a depth of 0–10 cm below the litter layer with a core from two opposing corners of the 1 m × 1 m quadrat. In the laboratory, the samples were air dried and passed through a 2 mm sieve prior to analysis. Soil pH was determined in a solution of 10 g of soil and 25 ml of 0.01 M CaCl<sub>2</sub> using a standard glass electrode. Using soil ground to fine powder, C and N were determined with an elemental analyzer. After extraction with ammonium lactate, the content of P was measured by flow-injection analysis. Soil depth was determined as the mean value of five measurements carried out with a marked metal pin.

#### 2.3. Life-history traits

One aim of our study was to examine how far temporal changes in species composition and richness are caused by changing frequencies of species with different life-history traits and ecological strategies. For this, we collected data from the literature on species attributes that we considered to be relevant in the context of the study. We compiled information on the following traits: habitat preference (distinguishing specialists of Festuco-Brometea and Koelerio-Corynephoretea communities typical of dry and sunny, nutrient-poor habitats, and all other species), general life form, Raunkiær life form, life span, plant height, ability of vegetative reproduction, leaf anatomy, leaf longevity, seed mass, and Grime strategy (for data sources, trait categories and species values/ assignments, see Table A2). The ecological optima of species relative to important soil variables and light were assessed by using the Ellenberg indicator values for soil moisture, soil pH, soil nutrients and light (Ellenberg et al., 1992). Except for seed mass, plant height (interval) and Ellenberg values (ordinal), all variables were nominal.

#### 2.4. Statistical analysis

Differences between plots and changes in species composition over time were analyzed by means of ordination. The data set with the species-plot matrix was examined by Detrended Correspondence Analysis (DCA), using the 'decorana' function of the VEGAN package implemented in R version 2.12.1 (Oksanen et al., 2011). Apart from a down-weighting of rare species (to counteract the influence of outliers), we applied the default options of the program. A second (environmental variable-plot) matrix served to interpret the results. We used the function 'envfit' in VEGAN to fit the environmental variables (year, latitude, longitude, altitude, heat index, meanL, meanF, meanR, meanN, and cumNdep) post hoc onto the DCA ordination. The direction of an environmental vector in the resulting diagram shows the direction of the respective gradient, while the length of an arrow reflects the importance of the variable for the variation in species composition. The P values for the variables were based on 999 random permutations, meaning that only those variables are displayed in the ordination diagram that had a significant effect on the variation in species composition at *P* < 0.001. The variables 'year' and 'cumNdep' were highly positively correlated (Pearson correlation: r = 0.948, P < 0.001), therefore only the vector for 'year' is shown.

The relationship between species richness and environmental variables was analyzed by general linear models and generalized linear models using R version 2.12.1. Plot sizes varied considerably between 1 and 225 m<sup>2</sup>, and the relationship between species richness and area was best described by a regression of log species richness against log plot size ( $R^2 = 0.279$ ). To correct for varying plot sizes we then used the residuals of this regression in the GLM instead of the original species-richness values. A large variation in plot size, however, has been shown to in some cases distort species-richness analyses in vegetation data sets (Dengler and Boch, 2008). Therefore, we also created two sub-sets, one excluding all plots larger than 20 m<sup>2</sup> (sub-set 1: 822 plots), the second including only plots up to 10 m<sup>2</sup> (sub-set 2: 389 plots).

To avoid multi-collinearity, separate models were run for 'year' and 'cumNdep'. MeanF and meanN were highly positively correlated. As we assumed that soil moisture is partly captured in the variable 'heat index', we used meanN in the model runs (The results were, however, largely identical to those obtained when using meanF). As species richness often shows unimodal relationships with environmental factors, quadratic terms for all variables were also included in the initial models (after centering of the variables). Each model run started with the full model including all variables, and variables were removed in a stepwise manner until the AIC value was minimized. These models only included variables with a significant or marginally non-significant impact on species richness. After having fitted models for the total number of vascular plant species, we additionally tested the effects of the explanatory variables on the number of typical dry grassland species (habitat specialists according to Table A2) and the percentage of typical dry grassland species. The residuals of total species richness and the number of typical dry grassland species followed a normal distribution, and binomial distribution was applied for the proportion of typical dry grassland species. The main aim of the study was to monitor the change in species richness over time and to examine the roles of cumulative N deposition as well as N and P availability. We therefore do not show all appropriate models based on AIC criteria (Burnham and Anderson, 2002), but only report on one model for each of the dependent variables.

For the statistical analyses for single species, those with fewer than 40 occurrences in the whole data set were omitted in order to reduce the influence of random differences in the abundance of the rarest species between older and more recent plots. To examine whether species had increased or decreased in abundance, the abundance scores were related to the variable 'year' across all sites with linear regression, resulting in three groups of species showing (1) a significant increase over time, (2) a significant decrease over time, or (3) no temporal change in abundance. Using presence-absence values instead of abundance values gave similar results, but fewer species showed significant changes over time. We then compared the life history traits and ecological strategies between increasing and decreasing species using  $\chi^2$ -tests (all categorical variables) and Mann-Whitney U-test (Ellenberg values, plant height, and seed mass). For the typical dry grassland species with fewer than 40 occurrences in the whole data set, we additionally examined the temporal change in geographic range size, using an analysis by Welk (2002) for whole Germany and a distribution atlas of vascular plants by Garve (2007) for the North-west German region of Niedersachsen from where more than a third of the plots were obtained.

#### 3. Results

The number of vascular plants per plot ranged from 7 to 68. The averages of mean Ellenberg values for soil moisture, pH, nitrogen and light (3.9, 7.1, 2.9 and 7.2, respectively) indicate the dry, base-rich and nutrient-poor soil conditions of the calcareous grass-lands and their high light availability. There were pronounced

differences in annual N deposition between sites, with the most polluted grasslands receiving >3 times more N than the least polluted grasslands (840–2882 eq  $ha^{-1}$  year<sup>-1</sup>; average: 1441).

The DCA analysis based on the species-plot matrix resulted in relatively low eigenvalues and gradient lengths for the first two axes, confirming that the data set included plots with a relatively uniform species composition that can be assigned to the alliance B. erecti (i.e., did not comprise plots with a greatly different composition belonging to other alliances of calcareous grasslands, which are widespread under warmer and more continental conditions) (Fig. 2). The site scores in the ordination diagram were well dispersed. Most environmental variables passively fitted to the ordination were significantly correlated to the species composition. The strongest correlation along axis 1 was observed for meanN, its vector pointing to the left. MeanR showed a strong and negative relationship with axis 2. Also year was significantly related to the site scores along axis 2 with its vector pointing down. The perpendicular position of the vectors for meanN and year are in accordance with the weak correlation of the two variables across sites (Pearson correlation: r = 0.058). The vector for cumNdep was almost exactly congruent with the one for year (and was, for the sake of legibility, omitted from the graph).

The most common and typical dry grassland species are found in the center of the ordination diagram. Species situated at the left-hand side of the graph associated with high meanN values include *Bromus inermis*, *Chaerophyllum temulum*, *Galium aparine*, *Stellaria media*, and *Torilis japonica*, indicative of half-open vegetation on relatively nutrient-rich soils. Species at the right-hand side are, for example, elements of sun-exposed fringes (*Laserpitium latifolium*, *Seseli libanotis*, *Thesium bavarum*) and calcareous rocks (*Gymnocarpium robertianum*, *Gypsophila repens*).

In the GLM, vascular plant species richness was influenced by several environmental variables, often in a unimodal way (Table 1). When year was included in the models, the total  $R_{adj}^2$  value amounted to 0.377; with cumNdep instead of year the value (and the whole model) was almost exactly the same. Among the



**Fig. 2.** DCA ordination diagram based on a matrix of 1186 plots and 516 species of calcareous grassland from North-western Germany. The arrows represent an overlay of vectors of the environmental variables fitted *post hoc* to the ordination. Only variables with a significant effect on species composition at P < 0.001 are included; the vector for cumulative nitrogen deposition (cumNdep), being almost fully congruent with the one for year, was omitted for better legibility. Mean-F = mean Ellenberg value for soil moisture, meanR = mean Ellenberg value for soil reaction (pH), meanN = mean Ellenberg value for soil nitrogen. Eigenvalues and gradient lengths are: axis 1 – 0.165 and 4.245 sd, axis 2 – 0.090 and 2.872 sd.

#### Table 1

Effects of geographic variables (latitude, longitude, altitude), local environmental descriptors and year on species richness (corrected for plot size, see Section 2) in calcareous grasslands (n = 1186) in Germany. The number of vascular plants and the number of typical dry grassland species were analyzed with general linear models, and the proportions of typical dry grassland species were analyzed with general linear models (applying binomial distribution). Each column refers to one model. *T* values and significance probabilities ( ${}^{**P} < 0.001$ ,  ${}^{*P} < 0.05$ ,  ${}^{*}0.05 < P < 0.10$ ) as well as total  $R_{adj}^2$  values are given. The last line shows the  $R_{adj}^2$  values of models where year is replaced by cumulative N deposition (cumNdep). Variables that did not remain in the reduced final models are indicated with '-'. MeanL = mean Ellenberg value for light, meanR = mean Ellenberg value for soil nitrogen, sub-set 1 includes only plots with an area ranging from 1 to 20 m<sup>2</sup> (n = 822), sub-set 2 plots ranging from 1 to  $10 m^2$  (n = 389).

	No. of vascular plants		No. of typical dry grassland species			Proportion of typical dry grassland species			
	All plots	Sub-set 1	Sub-set 2	All plots	Sub-set 1	Sub-set 2	All plots	Sub-set 1	Sub-set 2
Latitude	-	-	-	-9.81***	-	-3.79***	$-1.74^{+}$	$-1.76^{+}$	-0.75
Longitude	$-8.16^{***}$	-	-	-	$-6.50^{***}$	-	-		-
Altitude	-	5.40***	-	3.62***	8.68***	0.74	-		-
Altitude <sup>2</sup>	-	-	-	$-2.96^{**}$	$-5.15^{***}$	$-1.78^{*}$	-	-	-
Heat index	5.31***	$-1.84^{+}$	-0.30	-	5.06***	0.75	0.53	0.30	0.31
Heat index <sup>2</sup>	$-7.67^{***}$	$-6.02^{***}$	$-5.34^{***}$	-	$-5.76^{***}$	$-5.28^{***}$	-	-	-
MeanL	1.30	6.83***	4.50***	6.89***	6.72***	5.64***	$1.97^{*}$	1.42	1.28
MeanL <sup>2</sup>	$-7.99^{***}$	$-6.74^{***}$	$-4.73^{***}$	$-13.24^{***}$	$-8.54^{***}$	$-5.37^{***}$	-	-	-
MeanR	$-6.51^{***}$	4.31***	3.22**	1.95+	1.30	5.75***	$2.33^{*}$	1.70+	1.10
MeanR <sup>2</sup>	$-6.15^{***}$	$-4.09^{***}$	$-3.39^{***}$	$-10.90^{***}$	$-10.65^{***}$	$-5.41^{***}$	-	-	-
MeanN	11.48***	7.24***	6.18***	$-3.19^{**}$	$-3.19^{***}$	4.35***	$-2.80^{**}$	$-2.12^{\circ}$	-1.18
MeanN <sup>2</sup>	$-9.05^{***}$	$-6.69^{***}$	$-6.10^{***}$	$-7.43^{***}$	$-7.06^{***}$	$-4.94^{***}$	-	-	-
Year	4.15***	$2.45^{*}$	1.83+	-	-	3.44***	-0.97	-0.61	-0.52
Total $R_{adj}^2$	0.377	0.324	0.374	0.524	0.542	0.527	(0.699)	(0.715)	(0.636)
Total $R_{adj}^2$ with cumNdep	0.376	0.321	0.387	0.452	0.504	0.531	(0.715)	(0.734)	(0.655)

soil variables, the strongest effect on species richness was found for meanN showing a hump-shaped curve (Fig. 3a). Species richness peaked at intermediate heat index values, with a strong decrease towards the steepest and most sun-exposed slopes. Heat index and meanN were negatively correlated (Pearson correlation: r = -0.124, P < 0.001). Year and cumNdep had significantly positive effects on the total number of species, which was confirmed by the simple linear model, albeit with a  $R^2$  of only 0.047 (Fig. 3b). The same relationship as described above was obtained for meanN when analyzing sub-sets 1 and 2 (Table 1), while the effect of year on species richness was only marginally or no longer significant.

With the number of typical dry grassland species as the response variable, the models based on all plots gave similar results to those found for total species richness with respect to mean Ellenberg values, but had higher total  $R_{adj}^2$  values (Table 1). There was, however, a strong negative (instead of a hump-shaped) relationship between the number of dry grassland specialists and meanN, while year had no significant effect. The geographic variables had generally stronger effects on the number of specialist species than on total species richness. Latitude had a pronounced negative effect (also in sub-set 2), indicating the decline in the number of typical dry grassland species towards the north. The species richness of this group peaked at intermediate altitudes. The heat index did not remain in the final model for all plots, whereas it was found to be significant in the models for the two sub-sets. For sub-set 2, year was included in the final model having a positive effect on the number of typical dry grassland species. This effect was, however, no longer visible in a simple linear model (results not shown).

For the proportion of dry grassland species, the final models did not contain any quadratic terms (Table 1). Here, meanN had a negative effect (Fig. 3c) and meanR as well as meanL a positive impact, while year tended to show a negative relationship with the proportion of dry grassland species (Fig. 3d). The results were similar for the models for the two sub-sets, except for much lower significance probabilities. The number and proportion of species typical of mesotrophic conditions increased with time in all data sets (results not shown).

The analysis of temporal trends of species gave several significant differences between different life-history traits. Whereas generalist species showed an overall increase over time, the number of calcareous grassland specialists has declined ( $\chi^2 = 6.206$ , P = 0.012, df = 1; Fig. 4a). Species with a mesomorphic leaf anatomy tended to increase at the expense of those with a scleromorphic or intermediate anatomy ( $\chi^2 = 4.721$ , P = 0.094, df = 2; Fig. 4b). For leaf longevity, summer-green and vernal species have increased over time, while winter-green and evergreen species showed the opposite trend ( $\chi^2$  = 4.773, *P* = 0.029, *df* = 1; Fig. 4c). No significant differences in temporal trends were observed between species differing in Raunkiær life form, life span, or general life form (all P > 0.5); eight annual and biennial species have decreased over time, five have increased. There were about as many increasing (8) as decreasing (7) grasses (Poaceae), and two out of seven woody species showed a decline. For the Grime strategies we found only weak trends in that competitive species tended to increase at the expense of species with other (intermediate) strategies  $(\chi^2 = 4.441, P = 0.218, df = 3;$  Fig. 4d).

Taller plants were over-represented among the increasing species (Mann–Whitney *U*-test: W = 502.5, P = 0.033; Fig. 4e), and species with a positive temporal trend tended to have a higher seed mass compared to decreasing species (W = 468.5, P = 0.063; Fig. 4f). Except in one case (light: W = 1027, P = 0.020) there were no differences between increasing and decreasing species in their Ellenberg indicator values (P > 0.15). *B. pinnatum* did not increase in frequency over the years or in response to cumulative N deposition, and there was no effect of the abundance of the species on total species richness (all P > 0.05).

Among the 22 rarest species typical of dry grasslands in the study area for which data were available, 18 have shown a decline or strong decline in Germany over recent decades (Welk, 2002). In the North-west German region of Niedersachsen, 16 out of 27 rare dry grassland species have decreased in their area of occupancy (number of occurrences in grid squares) by 50% or more compared to the period up to 1981 (Garve, 2007). The other species have declined less strongly or remained constant, meaning that none of the species is currently more common than three decades ago.

Across the 125 calcareous grassland plots sampled in Germany in 2008, vascular plant species richness was only weakly, albeit significantly, correlated to the soil P content (linear regression:  $R^2 = 0.047$ , P = 0.016; Fig. 5a). In contrast, P had a strong effect on



**Fig. 3.** Relationships between the total number of vascular plants (corrected for plot size) and (a) meanN (mean Ellenberg value for soil nitrogen), and (b) year, and relationships between the proportion of typical dry grassland species and (c) meanN, and (d) year (for all regression analyses *n* = 1186).

the species composition as reflected by the DCA ordination ( $R^2 = 0.540$ , P < 0.001; Fig. 5b). None of the N variables (total N, C/N ratio, N deposition) explained much of the variation in species composition (all  $R^2 < 0.09$ ).

#### 4. Discussion

#### 4.1. Species richness

The analysis of the historical data sets of calcareous grasslands from Germany revealed that there has been no or only a slight positive change in total species richness over the last 70 years since the first vegetation plots from the region were available. The ordination diagram indicated that changes in species composition were more pronounced than those found for species richness, which is also reflected in the partly different temporal trends of species with different life history traits and ecological strategies. These results are in sharp contrast to those observed in acidic grasslands that have undergone a dramatic change both in species richness and composition over the last decades.

During recent decades, calcareous grasslands have suffered from a marked decline in area. While some sites have been fertilized and their agricultural use intensified, most areas, especially on the steeper and drier slopes, have faced an abandonment of management (mowing and/or grazing), followed by succession to scrub and ultimately forest (Ellenberg and Leuschner, 2010). Since the implementation of the EU Habitats Directive, which recognizes Festuco-Brometea communities as a focal habitat type, the remaining grassland sites have largely been protected and preserved by continued management. The results of our study suggest that this management has been successful so far, since plant-species richness has largely remained stable. There was also no evidence of an increase over time of *B. pinnatum*. This species is known to respond positively to experimental N addition (e.g., Bobbink and Willems, 1987) and to a reduction and abandonment of management (Ellenberg and Leuschner, 2010). The lack of a decline in total species richness is unexpected for two reasons. First, the majority of calcareous grasslands in the study area are small in size and often isolated. It has been shown that the fragmentation of these grasslands often has detrimental effects on the fitness of populations of rare species, for example Gentianella germanica (Fischer and Matthies, 1998). In addition, the probability of small populations of short-lived plants of going extinct appears to increase with decreasing population size (Matthies et al., 2004). Second, over recent decades, calcareous grasslands have received considerable amounts of airborne N. These are comparable to those in acid grasslands in the same region in which N deposition has had a strong negative impact on species richness (Duprè et al., 2010).



**Fig. 4.** Temporal trends of species with different life history traits and ecological strategies in German calcareous grasslands. Considered were only those species that showed a significant increase (*n* = 43, gray bars) or decrease (*n* = 39, black bars) over time (see Section 2). For height, the values of seven tree and shrub species were omitted from the analysis; for seed mass, 11 values out of 82 were lacking.

One possible reason for the weak effect of atmospheric N deposition on the number of species in calcareous grasslands emerges from Fig. 3a: total species richness does not generally decline with increasing nutrient availability (as indicated by mean Ellenberg N-values), but shows a hump-shaped relationship with fertility. With respect to total species richness, grasslands with a low natural N supply (those situated in the left-hand side of the Fig. 3a) may thus even benefit from N addition. Furthermore, on the steepest and most S-exposed sites with shallow dry soils (those with a high heat index), N deposition most likely does not result in an increased N availability for the plants. The reason is that, during rainless periods in the growing season, the soils dry out and water



**Fig. 5.** Effect of soil phosphorus (P) content (log-transformed) on the (a) species richness of vascular plants ( $R^2 = 0.047$ , P = 0.016), and on (b) the main gradient in species composition as reflected by DCA axis 1 ( $R^2 = 0.540$ , P < 0.001) across 125 calcareous grassland plots in Germany sampled in 2008.

instead of N becomes the main limiting factor for most species. Dryness also results in a low biological activity of soil organisms and a reduced N mineralization (Ellenberg and Leuschner, 2010). The scarce literature on long-term changes in unimproved calcareous grasslands supports the observation that the species richness of these grasslands is not strongly affected by N deposition. This was, for example, noted by Partzsch (2000) in a study of porphyry outcrop vegetation in Eastern Germany. This interpretation also coincides with observations made by Bennie et al. (2006) in British chalk grasslands where the total number of species had generally declined over recent decades, most likely as an effect of nutrient enrichment, but these changes were less pronounced on the steepest and most sunny slopes. Another resurvey of calcareous grasslands in the UK also showed a significant, but weak decline in species richness (from on average 41 species in 1990-93 to on average 39 species in 2006-09 in 144 m<sup>2</sup> plots) (van den Berg et al., 2011). These results coincide with the lack of significant changes in species richness observed in long-term field experiments in the UK (Phoenix et al., 2012). Similar to the results of our study, an increase in local species richness over the last decades was observed in calcareous grassland sites in the county of Dorset in England, owing to a pronounced increase in species typical of mesotrophic conditions (Newton et al., 2012).

Another limiting factor in calcareous grasslands that is not directly associated with nor affected by N deposition is phosphorus. The availability of P to plants is generally low on high-pH soils (Carroll et al., 2003; Phoenix et al., 2003; Olsson and Tyler, 2004; Ellenberg and Leuschner, 2010), and the atmospheric input of P is generally low. P may thus be more limiting for species richness than N. The large importance of P for the differentiation of calcareous grassland vegetation becomes evident in the spatial comparison of plots from 2008 in Germany (Fig. 5). This is in clear contrast to acidic grasslands for which there is much evidence (Roem and Berendse, 2000) that P probably plays a subordinate role for the historical changes in species richness and composition compared to N (but see Ceulemans et al., 2011 for a different view).

Finally, another reason for the relatively weak effect of atmospheric N input on the species richness of calcareous grasslands is related to soil pH. N deposition in acidic grasslands results not only in direct N addition, but also indirectly in acidification and increased ammonium contents or higher ammonium/nitrate ratios, and these changes together cause the deleterious effects on species richness on soils with already low pH (Stevens et al., 2011a,b). The rendzina soils of calcareous grasslands, in contrast, have a large buffering capacity against acidification, and N is predominantly mineralized as nitrate. In general, the effects of N deposition on soil chemistry and mineralization have been shown to depend on soil and habitat type in extensively managed habitats (Rowe et al., 2012).

#### 4.2. Species composition

Species composition showed a directional change, for which there are three lines of evidence: (1) the variable 'year' had a significant effect on the differentiation of the grassland vegetation as reflected by the 2nd DCA axis (Fig. 2), (2) the proportion of dry grassland species responded differently to the explanatory variables than did the total species richness, with 'year' having a negative effect on the frequency of specialist species in the German grasslands (Fig. 3d), and (3) species with different attributes showed different temporal trends (Fig. 4). Over-represented among the taxa with a significant negative trend over time were species that were smaller and more light-demanding, had smaller seeds and winter-green or evergreen, more scleromorphic leaves, i.e., features typical for dry grassland specialists. Examples include Acinos arvensis, Arenaria serpyllifolia, Gentianella ciliata and Trifolium campestre. This coincides well with the results found by Jandt et al. (2011). The slow, but significant change towards a community with a higher share of taller species typical of more mesotrophic grasslands or thermophilic fringes is unlikely to be caused by N deposition and increasing N availability to the plants. First, the mean Ellenberg N scores of plots are unrelated to 'year' and 'cumNdep'. Second, there were no differences in N indicator values between increasing and decreasing species. A re-survey of UK grasslands also revealed no effect of N deposition on the change in mean Ellenberg N values (van den Berg et al., 2011).

We believe that the main reason for the shift in species composition is related to land-use history and management that has a strong long-term effect on species richness and composition (Alard et al., 2005; Köhler et al., 2005; Ellenberg and Leuschner, 2010). Several species with a positive temporal trend are typical elements of thermophilic fringe communities that are known to increase in calcareous grasslands after abandonment (at the expense of typical dry grassland species), such as *Clinopodium vulgare*, *Helictotrichon pubescens*, *Ranunculus polyanthemos* and *Viola hirta* (see also Jandt et al., 2011). This does not mean that the grasslands are no longer managed, but that the management is less intensive than in previous times (see also Hagen, 1996). Apart from management intensity, the form of management may also have changed, with a trend towards a transition from grazing to mowing. The effects of these two processes are difficult to separate: the marked increase in *B. erectus*, a tall-growing grass, may be caused by lower grazing intensity, but also by the abandonment of management. The same applies to the decline of several thorny or unpalatable species that are indirectly favored by grazing and suffer from canopy closure and shrub encroachment, for example *Carlina vulgaris*, *C. acaule, Euphorbia cyparissias* and *G. ciliata* (Ellenberg and Leuschner, 2010).

We are aware that any historical survey of vegetation data not based on truly permanent plots faces the risk of bias, which is introduced if plots from different time periods are sampled with different methods. This may concern differences in plot size (Dengler et al., 2009) or the range of community types, season and phenology (Vymazalová et al., 2012). Estimates of species richness derived from phytosociological data – the majority of plots considered here – also can be associated with a certain bias (Chytrý, 2001; Diekmann et al., 2007). However, there is no indication of any systematic difference between different time periods in how, when and which data were sampled.

In conclusion, unlike acidic grasslands and forests, calcareous grasslands have not suffered from a general decline in vascular plant species richness. However, the different temporal trends of species with different attributes in the German data set reflect a change in species composition. Most notable is the loss of typical dry grassland species, which are often threatened and of focal interest to nature conservation. This loss is alarming as many of these species are long-lived, but have a transient seedbank. Although this trend appears to be slow, it indicates that management not only needs to be maintained in general, but also on an appropriate and sufficiently intensive level. The dry grasslands least susceptible to species loss and change are those on steep and sun-exposed slopes, where water and phosphorus limitation are most pronounced.

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#### **Appendix A. Supplementary material**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocon.2014.02. 038.

#### References

- Alard, D., Chabrerie, O., Dutoit, T., Roche, P., Langlois, E., 2005. Patterns of secondary succession in chalk grasslands: can we distinguish the influence of former land uses from vegetation present data? Basic Appl. Ecol. 6, 161–173.
- Bennie, J., Hill, M.O., Baxter, R., Huntley, B., 2006. Influence of slope and aspect on long-term vegetation change in British chalk grasslands. J. Ecol. 94, 355–368.
- Bobbink, R., 1991. Effects of nutrient enrichment in Dutch chalk grasslands. J. Appl. Ecol. 28, 28–41.
- Bobbink, R., Willems, J.H., 1987. Increasing dominance of *Brachypodium pinnatum* (L.) Beauv. in chalk grasslands: a threat to a species-rich ecosystem. Biol. Conserv. 40, 301–314.

- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J.W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L., De Vries, W., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecol. Appl. 20, 30–59.
- Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: A Practical Information-theoretic Approach, second ed. Springer, New York.
- Carroll, J.A., Caporn, S.J.M., Johnson, D., Morecroft, M.D., Lee, J.A., 2003. The interaction between plant growth, vegetation structure and soil processes in semi-natural acidic and calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen deposition. Environ. Pollut. 121, 363–376.
- Ceulemans, T., Merckx, R., Hens, M., Honnay, O., 2011. A trait-based analysis of the role of phosphorus vs. nitrogen enrichment in plant species loss across Northwest European grasslands. J. Appl. Ecol. 48, 1155–1163.
- Ceulemans, T., Merckx, R., Hens, M., Honnay, O., 2013. Plant species loss from European semi-natural grasslands following nutrient enrichment – is it nitrogen or is it phosphorus? Global Ecol. Biogeogr. 22, 73–82.
- Chytrý, M., 2001. Phytosociological data give biased estimates of species richness. J. Veg. Sci. 12, 439–444.
- De Schrijver, A., De Frenne, P., Ampoorter, E., Van Nevel, L., Demey, A., Wuyts, K., Verheyen, K., 2011. Cumulative nitrogen input drives species loss in terrestrial ecosystems. Global Ecol. Biogeogr. 20, 803–816.
- Dengler, J., Boch, S., 2008. Sampling-design effects on properties of species-area curves – a case study from Estonian dry grassland communities. Folia Geobot. 43, 289–304.
- Dengler, J., Löbel, S., Dolnik, C., 2009. Species constancy depends on plot size a problem for vegetation classification and how it can be solved. J. Veg. Sci. 20, 754–766.
- Diekmann, M., 2003. Species indicator values as an important tool in applied plant ecology a review. Basic Appl. Ecol. 4, 493–506.
- Diekmann, M., Duprè, C., 1997. Acidification and eutrophication of deciduous forests in northwestern Germany demonstrated by indicator species analysis. J. Veg. Sci. 8, 855–864.
- Diekmann, M., Kühne, A., Isermann, M., 2007. Random vs non-random sampling, Effects on patterns of species abundance, species richness and vegetation– environment relationships. Folia Geobot. 42, 179–190.
- Diekmann, M., Brunet, J., Rühling, Å., Falkengren-Grerup, U., 1999. Effects of nitrogen deposition: results of a temporal-spatial analysis of deciduous forests in South Sweden. Plant Biol. 1, 471–481.
- Dierschke, H., 1994. Pflanzensoziologie. Eugen Ulmer, Stuttgart, DE.
- Duprè, C., Stevens, C.J., Ranke, T., Bleeker, A., Peppler-Lisbach, C., Gowing, D.J.G., Dise, N.B., Dorland, E., Bobbink, R., Diekmann, M., 2010. Changes in species richness and composition in European acidic grasslands over the past 70 years – the contribution of cumulative atmospheric nitrogen deposition. Glob. Change Biol. 16, 344–357.
- Ellenberg, H., Leuschner, C., 2010. Vegetation Mitteleuropas mit den Alpen, sixth ed. Eugen Ulmer, Stuttgart, DE.
- Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W., Paulissen, D., 1992. Zeigerwerte von Pflanzen in Mitteleuropa, second ed. Scripta Geobot. 18, 1–248.
- Fischer, M., Matthies, D., 1998. The effect of population size on performance in the rare plant *Gentianella germanica*. J. Ecol. 86, 195–204.
- Garve, E., 2007. Verbreitungsatlas der Farn- und Blütenpflanzen in Niedersachsen und Bremen. Natursch. Landschaftspfl. Nieders. 43, 1–507.
- Gaudnik, C., Corcket, E., Clement, B., Delmas, C.E.L., Gombert-Courvoisier, S., Muller, S., Stevens, C.J., Alard, D., 2011. Detecting the footprint of changing atmospheric nitrogen deposition loads on acid grasslands in the context of climate change. Glob. Change Biol. 17, 3351–3365.
- Gruber, N., Galloway, J.N., 2008. An earth-system perspective of the global nitrogen cycle. Nature 451, 293–296.
- Hagen, T., 1996. Vegetationsveränderungen in Kalk-Magerrasen des Fränkischen Jura. Laufener Forschungsber. 4, 1–218.
- Jacquemyn, H., Brys, R., Hermy, M., 2003. Short-term effects of different management regimes on the response of calcareous grassland vegetation to increased nitrogen. Biol. Conserv. 111, 137–147.
- Jandt, U., von Wehrden, H., Bruelheide, H., 2011. Exploring large vegetation databases to detect temporal trends in species occurrences. J. Veg. Sci. 22, 957–972.
- Kleijn, D., Bekker, R.M., Bobbink, R., De Graaf, M.C.C., Roelofs, J.G.M., 2008. In search for key biogeochemical factors affecting plant species persistence in heathland and acidic grasslands: a comparison of common and rare species. J. Appl. Ecol. 45, 680–687.
- Köhler, B., Gigon, A., Edwards, P.J., Krüsi, B., Langenauer, R., Lüscher, A., Ryser, P., 2005. Changes in the species composition and conservation value of limestone grasslands in Northern Switzerland after 22 years of contrasting managements. Persp. Plant Ecol. Evol. Syst. 7, 51–67.
- Maskell, L.C., Smart, S.M., Bullock, J.M., Thompson, K., Stevens, C.J., 2010. Nitrogen deposition causes widespread loss of species richness in British habitats. Glob. Change Biol. 16, 671–679.
- Matthies, D., Bräuer, I., Maibom, W., Tscharntke, T., 2004. Population size and the risk of local extinction, empirical evidence from rare plants. Oikos 105, 481–488.
- Newton, A.C., Walls, R.M., Golicher, D., Keith, S.A., Diaz, A., Bullock, J.M., 2012. Structure, composition and dynamics of a calcareous grassland metacommunity over a 70-year interval. J. Ecol. 100, 196–209.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2011. Vegan: Community Ecology Package. R Package Version 2.0-1. <a href="http://CRAN.r-project.org/package=vegan">http://CRAN.r-project.org/package=vegan</a>.

- Olsson, P.A., Tyler, G., 2004. Occurrence of non-mycorrhizal plant species in south Swedish rocky habitats is related to exchangeable soil phosphate. J. Ecol. 92, 808–815.
- Parker, K.C., 1988. Environmental relationships and vegetation associates of columnar cacti in the northern Sonoran Desert. Vegetatio 78, 125–140.
- Partzsch, M., 2000. Die Porphyrkuppenlandschaft des unteren Saaletals Strukturwandel ihrer Vegetation in den letzten vier Jahrzehnten. Tuexenia 20, 153–187.
- Phoenix, G.K., Booth, R.E., Leake, J.R., Read, D.J., Grime, J.P., Lee, J.A., 2003. Effects of enhanced nitrogen deposition and phoshorus limitation on nitrogen budgets of semi-natural grasslands. Glob. Change Biol. 9, 1309–1321.
- Phoenix, G.K., Emmett, B.A., Britton, A.J., Caporn, S.J.M., Dise, N.B., Helliwell, R., Jones, L., Leake, J.R., Leith, I.D., Sheppard, L.J., Sowerby, A., Pilkington, M.G., Rowe, E.C., Ashmore, M.R., Power, S.A., 2012. Impacts of atmospheric nitrogen deposition, responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. Glob. Change Biol. 18, 1197–1215.
- Pieterse, G., Bleeker, A., Vermeulen, A.T., Wu, Y., Erisman, J.W., 2007. High resolution modelling of atmosphere-canopy exchange of acidifying and eutrophying components and carbon dioxide for European forests. Tellus 59B, 412–424.
- Rockström, J. et al., 2009. A safe operating space for humanity. Nature 461, 472–475. Roem, W.J., Berendse, F., 2000. Soil acidity and nutrient supply ratio as possible
- factors determining changes in plant species diversity in grassland and heathland communities. Biol. Conserv. 92, 151–161.
- Rowe, E.C., Emmett, B.A., Frogbrook, Z.L., Robinson, D.A., Hughes, S., 2012. Nitrogen deposition and climate effects on soil nitrogen availability: influences of habitat type and soil characteristics. Sci. Total Environ. 434, 62–70.
- Stevens, C.J., Dise, N.B., Mountford, J.O., Gowing, D.J.G., 2004. Impacts of nitrogen deposition on the species richness of grasslands. Science 303, 1876–1879.
- Stevens, C.J., Dise, N.B., Gowing, D.J.G., Mountford, J.O., 2006. Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls. Glob. Change Biol. 12, 1823–1833.

- Stevens, C.J., Duprè, C., Dorland, E., Gaudnik, C., Gowing, D.J.G., Bleeker, A., Diekmann, M., Alard, D., Bobbink, R., Fowler, D., Corcket, E., Mountford, J.O., Vandvik, V., Aarrestad, P.A., Muller, S., Dise, N.B., 2011a. The impact of nitrogen deposition on acid grasslands in the Atlantic region of Europe. Environ. Pollut. 159, 2243–2250.
- Stevens, C.J., Manning, P., van den Berg, L., Lamers, L., de Graaf, M.C.C., Wamelink, W., Boxman, A., Bleeker, A., Vergeer, P., Arroniz-Crespo, M., Limpens, J., Bobbink, R., Dorland, E., 2011b. Ecosystem responses to differing ratios of reduced and oxidised nitrogen inputs. Environ. Pollut. 159, 665–676.
- Sutton, M., Howard, C.M., Erisman, J.W., Billen, G., Bleeker, A., Grennfelt, P., van Grinsven, H., Grizzetti, B. (Eds.), 2011. The European Nitrogen Assessment. Cambridge University Press, Cambridge, UK.
- Van den Berg, L.J.L., Vergeer, P., Rich, T.C.G., Smart, S.M., Guest, D., Ashmore, M.R., 2011. Direct and indirect effects of nitrogen deposition on species composition change in calcareous grasslands. Glob. Change Biol. 17, 1871–1883.
- Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A., 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecol. Appl. 20, 5–15.
- Vymazalová, M., Axmanová, I., Tichý, L., 2012. Effect of intra-seasonal variability on vegetation data. J. Veg. Sci. 23, 978–984.
- Welk, E., 2002. Arealkundliche Analyse und Bewertung der Schutzrelevanz seltener und gefährdeter Gefäßpflanzen Deutschlands. Schriftenr. Vegetationsk. 37, 1– 337.
- Willems, J.H., Peet, R.K., Bik, L., 1993. Changes in chalk grassland structure and species richness resulting from selective nutrient additions. J. Veg. Sci. 4, 203– 212.
- Wilson, E.J., Wells, T.C.E., Sparks, T.H., 1995. Are calcareous grasslands in the UK under threat from nitrogen deposition? – an experimental determination of a critical load, J. Ecol. 83, 823–832.
- Wisskirchen, R., Haeupler, H., 1998. Standardliste der Farn- und Blütenpflanzen Deutschlands. Eugen Ulmer, Stuttgart, DE.

# Differential Effects of Oxidised and Reduced Nitrogen on Vegetation and Soil Chemistry of Species-Rich Acidic Grasslands

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Abstract Emissions and deposition of ammonia and nitrogen oxides have strongly increased since the 1950s. This has led to significant changes in the nitrogen (N) cycle, vegetation composition and plant diversity in many ecosystems of high conservation value in Europe. As a consequence of different regional pollution levels

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K. Wotherspoon · D. J. G. Gowing Department of Environment, Earth and Ecosystems, The Open University, Walton Hall, Milton Keynes MK7 6AA, UK and of the increased importance of reduced N in the near future, determining the effect of different forms of N is an important task for understanding the consequences of atmospheric N inputs. We have initiated three replicated N addition experiments in species-rich, acidic grasslands spanning a climatic gradient in the Atlantic

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biogeographic region of Europe in Norway, Wales and France at sites with low levels of pollution. N was added in two doses (0 and 70 kg N ha<sup>-1</sup> year<sup>-1</sup> above background) and in three forms (oxidised N, reduced N and a 50-50 combination). After 2.5 years of N additions, the effects of these treatments on plant biomass, plant nutritional status, soil pH and soil nutrient availability were determined. Impacts of the N additions were observed within the 2.5-year research period. In some cases, the first signs of differential effects of N form could also be demonstrated. In the French site, for example, grass biomass was significantly increased by the oxidised N treatments but decreased by the reduced N treatments. In the Norwegian site, the reduced N treatments significantly reduced soil pH, whereas oxidised N did not. Effects on nutrient availability were also observed. These experiments will be continued to elucidate the longer term impacts of N deposition on these grasslands.

Keywords European acidic grasslands · Nitrogen deposition · Nitrogen supply rate · Oxidised nitrogen · Reduced nitrogen · Species richness

# **1** Introduction

Emissions of ammonia  $(NH_3)$  and nitrogen oxides  $(NO_x)$ have strongly increased since the 1950s (Galloway et al. 2008). Ammonia is volatilised from intensive agricultural systems, such as dairy farming and intensive animal husbandry, whereas nitrogen oxides originate mainly from burning of fossil fuel by traffic, industry and households. Because of short- and long-range transport of these nitrogenous pollutants, atmospheric nitrogen (N) deposition has clearly increased in many natural and semi-natural ecosystems across the world (Bobbink et al. 2010). Areas with currently high atmospheric N deposition (20-100 kg N ha<sup>-1</sup> year<sup>-1</sup>) are central and western Europe, eastern USA and, since the 1990s, eastern Asia and India. Estimated background inputs (pre-1900s) range between 1 and 3 kg N ha<sup>-1</sup> year<sup>-1</sup> (e.g. Asman et al. 1998; Dentener et al. 2006; Galloway and Cowling 2002).

Nutrient availability is one of the major factors that determine the plant species composition in ecosystems. N is the primary limiting nutrient for plant growth in many natural and semi-natural ecosystems, especially under oligotrophic and mesotrophic conditions in temperate and boreal regions (Bobbink et al. 2010; Sala et al. 2000). Many plant species in such ecosystems are adapted to nutrient-poor conditions and can only survive or compete successfully on soils with low N availability (Aerts and Chapin 2000; Tamm 1991). The series of events which occur when N inputs increase in an area with originally low background deposition rates is complex. Many ecological processes interact and operate at different temporal and spatial scales. As a consequence, large variations in sensitivity to atmospheric N deposition have been observed between different natural and semi-natural ecosystems (e.g. Maskell et al. 2010). Despite this diverse sequence of events, the impacts of increased N deposition are (a) direct foliar toxicity; (b) changes in structure and function by eutrophication; (c) soil-mediated effects of acidification; (d) negative impacts of reduced N  $(NH_{\nu})$ such as stunted root growth, acidification of plant cells and toxicity effects; and (e) increased sensitivity for stress and disturbances. Because of these processes, significant changes in the N cycle, in vegetation composition and in plant diversity have been observed in many ecosystems of high nature conservation value in Europe (Bobbink et al. 2010, 1998).

Species-rich grasslands are an important component of European biodiversity, supporting a wide range of plant, invertebrate and bird species. Many of these semi-natural grasslands are listed in the Conservation of Natural Habitats and of Wild Fauna and Flora Directive (92/43/EEC) (the "Habitats Directive") and are thus a major part of the European nature conservation network. Species-rich acidic grasslands are present throughout Europe on soil of intermediate pH (4.5-6.0) in both lowland and mountainous regions. They were originally widespread but have been heavily affected by land-use changes (e.g. abandonment) or intensification of agricultural use (Ellenberg 1996). Species-rich Nardus grasslands (Violion caninae alliance; Schwickerath 1944) are a characteristic form of these acidic grasslands in the Atlantic biogeographic region of Europe. These species-rich, acidic grasslands are today highly threatened in parts of Europe and are protected for nature conservation under the Habitats Directive (habitat 6230; Directive 92/43/EEC).

As in many other species-rich grasslands, increased N deposition can have profound impacts on the structure and function of these acidic and oligotrophic systems. A decline of plant species richness in Britain and in the Atlantic zone of continental Europe was significantly correlated with increasing ambient atmospheric N deposition in the range 2–44 kg N ha<sup>-1</sup> year<sup>-1</sup> (Stevens et al. 2004, 2010). Forb richness in particular was negatively

correlated to N deposition, while the more competitive graminoids increased (Stevens et al. 2011b). The same negative correlations between N deposition and species richness were also detected by a temporal comparison of vegetation data spanning a period of almost 70 years (Duprè et al. 2010). Furthermore, correlative field studies in the Netherlands showed the importance of high soil NH<sub>4</sub><sup>+</sup> concentrations and/or high soil NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> ratios for the absence of many Red List plant species of these Violion grasslands (De Graaf et al. 2009; Kleijn et al. 2007). Experiments in water culture, containers and mesocosms have also revealed the sensitivity of characteristic endangered species of these grasslands for high  $NH_4^+$  or high soil  $NH_4^+$  to  $NO_3^-$  ratios (De Graaf et al. 1998; van den Berg et al. 2005, 2008). However, few N addition field studies have been done in these species-rich acidic grasslands to unravel the mechanisms of the possible decline of diversity (Bobbink and Hettelingh 2011). Berlin (2000) found an increase in graminoids within a 3year N addition experiment on a Festuca ovina grassland but not on an Agrostis capillaris grassland. A longer term experiment at Wardlow Hay Cop (UK) has shown impacts on vegetation species composition (Carroll et al. 2003), vegetation chemistry (Arroniz-Crespo et al. 2008) and soil chemistry (Horswill et al. 2008; Phoenix et al. 2003), but this experiment is on a more acidic soil (pH 4.4) with high ambient N deposition. In addition, both experiments have focussed on the impact of N dose, and not on N form. As a result of different regional pollution climates and potential shifts in emission ratios toward reduced N in the near future, determining the effect of the different N forms is now considered as main task in understanding the consequences of atmospheric N inputs (Achermann and Bobbink 2003; Bobbink and Hettelingh 2011).

The main aim of this research is to determine the processes behind the susceptibility of biodiversity to N deposition by carrying out replicated N manipulation experiments in species-rich acidic grasslands in regions with low levels of pollution. More specifically, we pose the following research questions:

- (a) What is the effect of N addition on the biomass and species richness of the vegetation and on soil chemistry?
- (b) What is the difference between adding N as oxidised N (NO<sub>3</sub><sup>-</sup>) or as reduced N (NH<sub>4</sub><sup>+</sup>)?
- (c) Are there any signs of negative effects of NH<sub>4</sub><sup>+</sup> on certain plant groups?

To address these questions, a set of three replicated N addition experiments were initiated spanning a climatic gradient in the Atlantic biogeographic region of Europe, in Norway, Wales and France. In this paper, we report the findings after 2.5 years of these field experiments contrasting the impacts of  $NH_4^+$  and  $NO_3^-$  in species-rich acidic (pH 5–5.5) grasslands. Biomass, species richness and soil chemistry are described.

# 2 Materials and Methods

# 2.1 Site Descriptions

Nitrogen addition experiments were carried out in acidic grasslands (EUNIS code 1.7) in three countries along the Atlantic climate gradient: Norway, Wales (UK) and France (Fig. 1). All sites belonged to the *V. caninae* alliance (Schwickerath 1944) and had, for Europe, low background N deposition (6–10 kg N ha<sup>-1</sup> year<sup>-1</sup>). Site meteorological and soil characteristics are summarised in Table 1.

The experimental site in Norway is located at Revne, in the Fusa municipality (Hordaland). Bedrock consists of green schist/mica schists overlain by a shallow soil. The site was traditionally managed for haymaking with annual mowing and grazing by sheep in spring and autumn (grazing was discontinued in 2005). Low levels of fertiliser were applied up to 2000, although neither the type nor amounts of fertiliser is known. Part of the area has been ploughed and was probably used for arable farming prior to ca. 1940 (potato, grains). The vegetation is dominated by grasses such as Anthoxanthum odoratum, Festuca rubra and Agrostis capillaris. Rhytidiadelphus squarrosus is the dominant bryophyte. Several sedges (Carex nigra, Carex panicea) and forbs (Viola palustris, Leontodon autumnalis, Plantago lanceolata, Prunella vulgaris) are also present. Initial species richness was 14.9 species per  $2 \times 2$  m plot for vascular plants and 2.7 for bryophytes.

The experimental site in the UK is situated at Trefor, North Wales. The site sits on a slight slope at the top of sea cliffs. Although formerly heathland, the site has been managed for extensive grazing of sheep for many years and consists of a short grassland sward with scattered shrubs. The grass sward is dominated by *Agrostis capillaris*, *F. rubra* and *Potentilla erecta*. Initial species richness was 16.6 species per 2×2 m plot for vascular plants and 3.0 for bryophytes. **Fig. 1** Locations of the experimental sites. *1*, Revne, Norway; *2*, Trefor, Wales (UK); *3*, Léognan, France



The experimental site in France is located at Léognan, near Bordeaux. The soil is an organic podzol overlaying a quaternary deposition of sand on top of limestone. Situated on the edge of an airfield, the site has been maintained as grassland with four to five mowings (with clippings removed) between April and September. The vegetation is dominated by grasses (*Agrostis capillaris, Agrostis curtisii, Danthonia decumbens* and *Pseudarrhenatherum longifolium*) and includes dwarf shrubs such as *Calluna vulgaris, Erica* spp. and *Ulex minor*; sedges (*Carex pilulifera, Carex binervis*) and forbs (*Potentilla erecta* and *Polygala* spp.). Initial species richness was 21.7 species per 2×2 m plot for vascular plants and 2.3 for bryophytes.

# 2.2 Experimental Design

At each of the three sites, 40 experimental plots of  $2 \times 2$  m in a randomised block design with five blocks

were set up in early spring 2007. Eight treatments were applied; four of these were used for the analysis presented in this paper (20 plots).

Nitrogen was applied in solution at a rate of 70 kg N ha<sup>-1</sup> year<sup>-1</sup> in three different forms: reduced N (as NH<sub>4</sub>Cl, referred to as N70-red), oxidised N (as NaNO<sub>3</sub>, N70-ox) or in combination (as NH<sub>4</sub>NO<sub>3</sub>, N70). These treatments provided the opportunity to analyse the effects of reduced and oxidised N separately and in combination. Control plots (N0) received corresponding amounts of deionised water only. The yearly number of applications depended on the length of the growing season at each site (eight additions in Trefor and Léognan and five in Revne). Intensive sampling of vegetation and soil was carried out in early spring 2007 to establish an experimental baseline and again in 2009 after 2.5 years of N application.

The distance between replicate blocks was at least 2 m. One metre wide buffer zones were laid out between

 Table 1
 Overview of site and meteorological characteristics of the experimental sites. The meteorological data are averages over the period 1971–2000 unless otherwise stated

	Revne (Norway) <sup>a</sup>	Trefor (Wales) <sup>b</sup>	Léognan (France) <sup>c</sup>
Site characteristics			
Coordinates	60° 09 29 N 5° 44 31.5 E	52° 59 56 N 4° 26 04 W	44° 42 11 N 0° 35 54 W
Altitude (m asl)	160	40	53
Slope (°)	13	6	_
Aspect	North-west	West	0
Average background N deposition <sup>d</sup>	6.1	9	8.8
Climate characteristics			
Mean annual temperature (°C)	6–8	9.5-10.5	13.3
Mean maximum daily temperature (°C)	9.6	13.1	18.1
Mean minimum daily temperature (°C)	4.2	7.5	8.5
Mean annual rainfall (mm)	1,773.4	827.9	984
Mean annual sun hours	1,186 <sup>e</sup>	1,621.4	1,992
Mean number of rainfall days (>1 mm)	202 <sup>e</sup>	140.9	128
Soil characteristics			
Soil depth (cm)	13	15-65	>150
Soil pH	5.4	5.1	5.2
Extractable $NH_4^+$ (mg kg <sup>-1</sup> dry soil, NaCl extract)	1.5	0.5	3.0
Extractable $NO_3^-$ (mg kg <sup>-1</sup> dry soil, NaCl extract)	16.6	0.8	7.4
Extractable $PO_4^{2-}$ (mg kg <sup>-1</sup> dry soil, NaCl extract)	2.9	0.1	0.1
Exchangeable $Ca^{2+}/K^+/Mg^{2+}$ (mg kg <sup>-1</sup> dry soil, NaCl extract)	419.4/13.7/53.5	159.7/69.3/177.6	62.1/4.9/14.4

<sup>a</sup> Average values at weather station at Bergen (http://retro.met.no)

<sup>b</sup> Average values for Station de Bordeaux, Merignac (1991–2000 for mean annual sun hours; http://climat.meteofrance.com)

<sup>c</sup> Average values for weather station Valley (http://www.metoffice.gov.uk)

<sup>d</sup> CBED (RoTAP 2012) was used for the UK; EMEP-based IDEM models were used for Norway and France (Pieterse et al. 2007)

<sup>e</sup> Data from http://www.climatedata.eu

plots to avoid edge effects and interactions between treatments. In Trefor and Revne, plots were fenced to exclude grazing by sheep and red deer, respectively. Grazing by large herbivores did not occur in Léognan.

# 2.3 Vegetation Sampling and Analysis

In each  $2\times 2$  m plot, all higher plants and bryophytes were identified to a species level and visual estimates of percentage cover were made in July 2009. Species richness and Shannon diversity index and evenness were calculated per plot. Biomass of each plot was collected in 50×50 cm subplots cut to 3 cm above soil level and separated into five functional groups (grasses, forbs, legumes, shrubs and mosses). Dry weight of biomass was measured after drying at 55 °C for 72 h. N, P and K contents were determined in subsamples of the collected biomass for forbs and grasses. Dried plant material was ground to <1 mm using a ball mill and digested using a modified Kjeldahl method (Bremner and Mulvaney 1982). Nutrient contents were analysed colorimetrically (N and P) and flame photometrically (K) on a Skalar SA-40 continuous flow analyser (Skalar BV, Breda, the Netherlands).

# 2.4 Soil Sampling and Analysis

In July 2009, two topsoil samples (0-10 cm) were collected with an auger (diameter 2.5 cm) from opposing corners of each plot. Samples from each plot were pooled, homogenised by hand removing stones and large roots, stored on ice and transported to the laboratory. Two extractions were performed, both used 15 g

of fresh soil extracted on a rotary shaker (100 rpm) for 1 h. One extraction used 100 ml 0.4 M NaCl and the second used the same amount of demineralised water. The soil suspensions were centrifuged for 5 min at 4,000 rpm and a subsample of 20 ml of the supernatant was filtered through a Whatman GF/C filter. The NaCl extracts were stored at -20 °C until analysis of  $NH_4^+$  and  $NO_3^-$ . Soil pH-H<sub>2</sub>O was determined in the demineralised water extract. Extractable concentrations of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were analysed colorimetrically using a continuous flow analyser (Skalar SA-40, Skalar Analytical BV, Breda, the Netherlands). Soil inorganic P availability was measured by Olsen P extraction and colorimetric analysis (MAFF, 1986). Soil moisture content was measured after drying 15 g of fresh soil at 105 °C for 24 h.

Plant N (both NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub>-N) supply rates were assessed by 1-month incubations of Plant Root Simulator probes (PRS<sup>TM</sup>, Western AG Innovations Inc., Saskatoon, Canada). The ion exchange resins in the probes give a measure of N in the soil that is available to plants (nutrient surplus rather than net mineralisation due to competition from plant roots) integrated over 10 cm<sup>2</sup> for the duration of the burial (Western Ag Innovations, 2008). Probes were incubated in July 2009. After incubation, the probes were cleaned in deionized water and returned to Western Ag Innovations for analysis. Probes were eluted with HCl and analysed for NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N colorimetrically using an autoanalyser.

Potential NH<sub>4</sub><sup>+</sup> oxidising activities (PAA) were determined for each plot. Soil slurries consisting of 15 g fresh, sieved (4 mm) soil in 100 ml buffered medium with 2 mM  $(NH_4)_2SO_4$  were kept in 250 ml glass Erlenmeyer flasks. The buffer solution was composed of 2 mM phosphate buffer (an equimolar mixture of KH<sub>2</sub>PO<sub>4</sub> and K<sub>2</sub>HPO<sub>4</sub>, adapted to the prevailing soil pH of 5). During the PAA measurements, the slurries were permanently shaken on a rotary shaker (RO 20, Gerhardt, Bonn, Germany; 100 rpm) in the dark at a temperature of 25 °C. Subsamples of 3 ml were taken at t=0.5, 2, 4, 6, 24 and 96 h, and centrifuged for 5 min at 13,000 rpm (Biofuge pico, Heraeus instruments, South Plainfield, USA), decanted and stored frozen (-20 °C) until further analysis. At each sampling time, the pH of the incubation medium was checked and set to its original value with 0.1 N NaOH or 0.1 N HCl, if necessary. Concentrations of NO3<sup>-</sup> and NO2<sup>-</sup> in the subsamples were measured on a continuous flow analyser (Skalar 40, Skalar Analytical BV, Breda, the Netherlands). PAA rates were determined from the changes in NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> concentrations in time, based on the slopes of the linear parts of the regression lines. The  $R^2$  values of the linear regressions were all 0.99 (±0.01).

# 2.5 Data Analysis

All soil, nutrient and vegetation variables were analysed for differences between treatments within sites using analysis of variance (SPSS 15, Microsoft Corporation, release 15.0.1., 2006) with N treatment as fixed factor and block as random factor. When necessary, data were logarithmically or square root transformed to obtain normality and homogeneity of variances. Post hoc pair-wise comparisons between all treatments were made using Tukey's method. For calculations of post hoc statistics, interaction terms (treatment x block) were excluded. Differences between sites were tested using analysis of variance. When it was not possible to normalise the data with transformations, data were analysed using a Mann–Whitney U test. The level of significance was set at p=0.05.

# **3 Results**

Outcomes of data analysis for all results are summarised in supporting electronic material Table 1.

# 3.1 Vegetation

The average species numbers of the control treatments in 2009 was 16.4 (s.d. $\pm$ 2.2), 20.4 (standard deviation 0.9) and 23.8 (standard deviation 3.1) for Revne, Trefor and Léognan, respectively, and did not differ significantly from those in 2007. None of the experimental sites showed significant effects of the N treatments on average species numbers per 2×2 m sample after 2.5 years of N addition. The Shannon index and evenness were also not affected by the N treatments (data not shown). However in Revne, there was a trend of declining Shannon index in the N70-ox addition compared to the control treatment (p=0.065).

Total biomass production differed considerably between the experimental sites. In Léognan and Trefor, the mean total biomass in 2009 in control plots was 358 and 431 g m<sup>-2</sup>, respectively. In the Revne control plots, the total biomass of the vegetation was only 239 g m<sup>-2</sup> (Fig. 2). Due to the relatively high variability in biomass, significant effects of N treatments on total biomass of the vegetation were not found, but some trends could be observed. In Trefor, all N treatments resulted in a non-significant increase in total biomass production.

Forb biomass in Revne was decreased by the N treatments compared to the control, with a significant decrease for the N70-red treatment (p=0.037, Fig. 2). An opposite effect, although non-significant, was found in Léognan and Trefor, where forb biomass tended to be higher following N additions (Léognan p=0.244; Trefor p=0.081).

Grass biomass in Léognan was increased by the N70-ox treatment, whereas it was decreased by the N70-red treatment resulting in a significant difference (p=0.015; Fig. 2). However, treatment effects did not differ from control.

In Trefor and Revne, grass biomass showed a nonsignificant increase with N additions, irrespective of the N form applied (Trefor p=0.547 and Revne p=0.201).

Although significant differences were not found in Léognan, the N treatments resulted in a non-significant decrease the grass to forb ratio, with the lowest ratios found in the N70-red treatments. In Trefor, no effects were found, but in Revne the N treatments resulted in increased grass/forb ratios with N70-red being significantly higher than control (p=0.037).

All N treatments led to increased plant N contents of both grasses and forbs in Revne (legumes were not measured due to insufficient biomass), although these differences were only significantly higher compared to the control treatments for reduced N (Grass N70 p=0.170, N70-ox p=0.118, N70-red p=0.029; Forb N70 p=0.118, N70-ox p=0.060, N70-red p=0.015; Table 2). In Trefor, only grasses seemed to have (non-significant; p=0.72) higher N contents compared to the control, whereas no effects were found in forbs or legumes. In Léognan, there was a non-significant trend (p=0.06) of increasing N content of legumes with the N treatments, but no effects of the N treatments were found in the other functional groups. Plant P content was not affected by the treatments (Table 2). Average plant N/P ratios of both grasses and forbs in the control plots were lowest in Revne, intermediate in Trefor and highest in Léognan (Table 2). The N treatments resulted in a non-significant trend of increased plant N/P ratios for grasses in Revne and a significant increase in Trefor (p=0.02), and for forbs in Revne (p=0.009). In Léognan, an opposite, non-significant trend was found, with the N treatments leading to a trend of decreased plant N/P ratios of all functional groups (p=0.07). Average C/N ratios of grasses in the control plots did not differ between sites (between 42.5 and 47.7, Table 2). In Revne, the average C/N ratios of grasses and



Fig. 2 Average ( $\pm$ s.e) total biomass production (*left*), and that of forbs (*middle*) and grasses (*right*) in the three experimental sites (gram per square metre). Significant differences between groups are indicated by *different letters* 

N treatment	Revne <sup>a</sup>	Revne <sup>a</sup>				Léognan		
	Grasses	Forbs	Grasses	Forbs	Legumes	Grasses	Forbs	Legumes
N content								
Control	9.5±1.7	22.6±5.1	9.8±1.4	12.8±1.2	23.9±0.5	10.3±0.4	15.4±1.2	13.2±0.5
N70	13.4±1.4	31.7±6.5	12.2±1.6	13.4±0.7	24.0±1.5	11.3±0.7	15.3±1.1	16.4±1.1
N70-ox	13.8±1.8	32.3±4.9	13.7±2.0	13.8±1.0	23.4±1.2	10.6±0.6	15.4±1.8	15.2±1.0
N70-red	15.3±2.0	38.8±5.5	10.2±1.0	11.7±1.2	22.1±1.1	10.3±0.5	14.7±1.1	16.1±1.0
P content								
Control	2.2±0.3	4.6±0.5	1.2±0.1	1.1±0.1	1.5±0.2	$0.5\pm0.0$	$0.8\pm0.0$	0.5±0.0
N70	2.6±0.1	5.0±0.3	1.2±0.1	1.1±0.1	$1.4\pm0.1$	$0.5\pm0.0$	0.9±0.1	0.7±0.1
N70-ox	2.2±0.2	4.6±0.3	1.3±0.1	1.3±0.2	1.3±0.1	0.5±0.0	0.9±0.1	0.6±0.1
N70-red	2.9±0.3	5.3±0.2	1.1±0.1	1.1±0.1	1.5±0.1	0.6±0.1	0.8±0.1	0.7±0.1
N/P ratio								
Control	4.3±0.3	4.7±0.6	8.9±0.5	10.6±0.0	16.5±1.5	21.1±1.0	19.4±0.7	27.5±1.6
N70	5.2±0.4	6.1±0.9	10.8±0.2	11.1±0.5	17.5±1.2	21.1±0.7	17.6±1.6	24.5±0.4
N70-ox	6.2±0.7	$6.9\pm0.8$	10.3±1.2	10.6±0.3	$18.0\pm0.8$	19.7±1.0	18.2±1.6	24.5±0.4
N70-red	5.2±0.3	$7.2 \pm 0.8$	9.5±0.5	10.5±0.1	15.0±0.6	18.3±2.6	18.6±2.0	25.7±2.9
C/N ratio								
Control	43.3±7.0	21.5±3.7	42.5±2.1	31.6±0.5	17.7±1.1	47.7±3.2	29.3±2.2	33.5±1.2
N70	30.5±2.8	13.8±2.7	38.2±1.1	31.7±3.4	17.4±0.9	42.2±2.6	30.8±2.3	27.2±1.5
N70-ox	30.8±4.4	13.6±2.6	40.7±2.0	29.6±0.9	16.8±0.7	45.9±2.9	30.0±3.4	35.4±0.1

31.3±1.4

17.7±2.5

**Table 2** Average ( $\pm$ s.e.) plant N and P contents (milligram per square metre), N/P and C/N ratios in aboveground tissues for functional groups (grasses (G), forbs (F), and legumes (L)) (n=5 replicates per treatment)

<sup>a</sup> Legumes are absent in Revne

28.0±3.4

forbs were decreased by all N treatments, but differences were only significant for grasses (Table 2). In Trefor and Léognan, the N treatments did not affect the average C/N ratios in grasses and forbs, but in Léognan, legumes did show a significant decrease (p=0.02).

12.5±1.9

37.5±1.8

# 3.2 Soil

N70-red

Average soil pH-H<sub>2</sub>O values of control treatments were not significantly different between sites: Trefor (5.1), Revne (5.4) and Léognan (5.2; Table 3). In Trefor, no effects of N treatments were found on soil pH. In Léognan, all N treatments seemed to increase soil pH slightly (but not significantly), but differential effects were not observed. In Revne, the N70-red treatment significantly decreased soil pH compared to the control and N70-ox treatments (p=0.039). This effect was not observed when NH<sub>4</sub><sup>+</sup> was added in combination with NO<sub>3</sub><sup>-</sup> (N70). Average soil NO<sub>3</sub>-N concentrations in the control treatments were highest in Revne, lowest in Trefor and intermediate in Léognan (Table 3; p<0.05). Results for NH<sub>4</sub>-N showed a similar pattern but showed a greater within site variability and the difference between sites was not significant (p=0.14). Soil extractable NO<sub>3</sub>-N more than doubled in Revne due to the N treatments (p=0.03). No effects of N addition on extractable NH<sub>4</sub>-N concentrations were found at this site. In Trefor and Léognan, no effects of treatments on extractable N concentrations were found.

49.0±1.9

32.0±2.4

29.2±1.4

Soil P concentrations (Olsen P) were highest in Revne (average in control plots of 149 mg kg<sup>-1</sup> dry soil, indicative of its former agricultural use). Soil P concentrations were more than tenfold lower in Trefor and even lower in Léognan (average in control plots of 12.5 and 5.5 mg kg<sup>-1</sup> dry soil, respectively). No significant effects of N treatments on soil P concentrations were found in any of the experimental sites (Table 3).

**Table 3** Average ( $\pm$ s.e.) values for soil parameters. Soil nutrients are all expressed in milligram per kilogram dry soil. Potential nitrification is NO<sub>3</sub><sup>-</sup> concentrations (mg kg<sup>-1</sup> dry soil) after

96 h of incubation. Plant N supply rates are in micrograms 10 per square centimetre per burial length (*n*=5 replicates per treatment)

Site	No.	рН	Extractable NO <sub>3</sub> -N	Extractable NH <sub>4</sub> -N	PO <sub>4</sub> -P (Olsen P)	Plant NO <sub>3</sub> -N supply rates	Plant NH <sub>4</sub> -N supply rates	Potential nitrification
Revne	Control	5.4±0.1 ab	2.3±1.2	2.4±0.5	149.3±10.6	5.4±0.9 a	5.6±0.3 ab	141.7±40.5
	N70	5.2±0.2 bc	6.3±2.0	2.4±0.1	127.7±15.7	275.0±82.7 b	10.0±1.4 ab	158.9±32.4
	N70-ox	5.8±0.4 a	5.6±1.8	3.7±0.9	130±16.2	264.4±76.4 b	5.1±0.6 a	163.8±41.3
	N70-red	4.9±0.2 c	5.1±2.2	2.2±0.1	143.8±11.3	24.7±16.5 a	14.1±3.7 b	156.2±37.0
Trefor	Control	5.1±0.1	0.4±0.1	1.5±0.2	12.5±1.1	1.1±0.3 a	14.1±2.1	а
	N70	5.1±0.1	0.9±0.3	4.0±1.3	12.4±2.3	5.1±1.6 b	14.8±2.5	а
	N70-ox	5.2±0.1	0.4±0.1	1.6±0.2	10.8±0.5	3.1±0.9 ab	13.6±1.7	а
	N70-red	5.2±0.1	0.4±0.1	1.7±0.3	13.4±1.1	0.8±0.3 a	21.5±5.7	а
Léognan	Control	5.2±0.2	0.9±0.4	0.6±0.2	5.5±2.2	74.0±38.5	56.0±18.8	11.2±4.5
	N70	5.4±0.4	1.0±0.5	0.9±0.3	4.2±0.8	74.7±27.4	54.5±16.4	12.9±6.6
	N70-ox	5.4±0.4	0.9±0.2	0.5±0.1	5.3±1.1	123.6±51.9	29.4±6.1	13.4±5.2
	N70-red	5.4±0.6	0.6±0.2	0.6±0.1	5.6±2.1	34.4±17.0	62.9±17.8	8.4±2.8

Significant differences between treatments are indicated by different letters

<sup>a</sup> In Trefor no nitrification could be measured

Large differences in plant N supply rates in control plots were found between sites (Table 3). Both NO<sub>3</sub>-N and NH<sub>4</sub>-N supply rates were ten times higher in Léognan than those in Revne and Trefor (significant differences for total N, NO<sub>3</sub>-N and NH<sub>4</sub>-N; p<0.001). In Trefor, N was predominantly present as NH<sub>4</sub>-N, whereas in Léognan and Revne, NO<sub>3</sub>-N and NH<sub>4</sub>-N supply rates were more or less equal. The effects of N additions on N supply rates differed between countries. In Revne, the N70 and N70-ox treatments resulted in significantly increased supply rates of total N (p < 0.05 for both) and NO<sub>3</sub>-N (p<0.05 for both), but not of NH<sub>4</sub>-N (N70 p=0.47; N70ox p=0.99). The N70-red treatment significantly increased NH<sub>4</sub>-N supply rates compared to the N70-ox treatment (p < 0.05), but not compared to the control or N70 treatment (p=0.06; p=0.54). In Trefor, the N70 treatment resulted in significantly higher NO<sub>3</sub>-N rates than the control (p < 0.05), the N70-red treatment was also significantly lower than the N-70 treatment (p < 0.05), and there were no effects of N treatment on total N or NH<sub>4</sub>-N (p=0.29; p=10). In Léognan, no effects of N treatment were found (total N p=0.60; NO<sub>3</sub>-N p=0.9; NH<sub>4</sub>-N *p*=0.24).

In Trefor, nitrification rates were below detection limit (Table 3). Potential nitrification rates in the control plots of Revne were considerably higher than those in Léognan (p<0.001). None of the N treatments had significant effect on potential nitrification.

# **4** Discussion

#### 4.1 Differences Between Sites

The three N addition experiments were replicated spanning a climatic gradient in the Atlantic biogeographic region of Europe. This resulted in differences between the three experimental sites in terms of climate, soil conditions (Table 1) and, to some degree, species composition. Soil depth also varied considerably between sites, and the deep soils at Léognan may have reduced the impact of the warmer drier climate, resulting in higher water availability than if soils had been shallow. Total biomass was highest in Trefor and lowest in Revne (p<0.001) and did not reflect nutrient availability (Table 3). The differences between biomass at the sites are most likely to be driven by climate: Revne has a much shorter growing season and lower temperatures than the other sites and Léognan experienced a drought in 2009.

There were also some differences in the soil pH between the sites (Table 1). There was no significant difference in pH between sites (p=0.14) but there was

a significant site × treatment interaction for soil pH (p < 0.01). Combined with climate, these differences appear to have an impact on N processing. Revne is the least acidic site and this is also where we observed highest levels of nitrification as we would expect since nitrification is hampered by low soil pH (e.g. Ste-Marie and Parè 1999). We also saw a significant reduction in soil pH in Revne as a consequence of the reduced N treatment, this is probably because the high rates of nitrification release more H<sup>+</sup> and thus have greater potential to acidify the soil than with oxidised N input (Johnston et al. 1986). Remarkably, in Trefor, nitrification was below the detection limit. Although this is the site with the lowest pH in 2009 (5.1 in control plots), we would still have expected to see nitrifying activity because nitrification is known to be hampered strongly below soil pH values of 4.5 (Roelofs 1986). Smits et al. (2010) found similarly low levels of nitrification in an acidic matgrass sward in the Netherlands. They hypothesised that the nitrifying bacteria are strongly suppressed, possibly by root exudates of the dominant Violion plants (Smits et al. 2010). A number of studies have identified the exudation of biological nitrification inhibitors from crop species (e.g. Subbarao et al. 2007; Zakir et al. 2008). As the grasslands in this study are a similar grassland type to that used in the study of Smits et al., it is possible that the same phenomenon is being observed here.

Sites responded differently to the N treatments as a consequence of varying conditions, and over time we would expect differences between sites to become more apparent.

# 4.2 Impacts of N Addition and Form

Several gradient studies have shown clear negative correlations between species richness and N deposition in this habitat (Duprè et al. 2010; Maskell et al. 2010; Stevens et al. 2010) but, consistent with other controlled field experiments (Carroll et al. 2003), we did not observe a strong effect in the first 2.5 years. This is not surprising in areas with low background deposition as impacts on species composition may take many years to become apparent and are likely to be cumulative, especially with respect to the accumulation of N in the system and with soil acidification (Duprè et al. 2010). We did observe a non-significant decreasing trend in the species diversity in Revne which indicates that some early phase changes are starting to occur in species composition of the vegetation.

Plant biomass was quite variable but non-significant trends in biomass, especially for grasses were observed. Several experiments have reported increased plant biomass as a consequence of N addition (e.g. Clark and Tilman 2008; Jones et al. 2004; Mountford et al. 1993) and this is a typical response in vegetation where N is the limiting nutrient (LeBauer and Treseder 2008). Grasses typically respond more favourably to N addition than forbs (Bobbink 1991; Stevens et al. 2006), although somewhat surprisingly, we saw an increasing trend in forb biomass in Trefor and Léognan. These results suggest there may be N limitation on biomass production, especially in Trefor where all forms of N addition increased biomass. Reduced forb biomass in Revne with the N70red treatment but not the N70 or N70-ox treatments may suggest that this group is more sensitive to reduced N inputs. This is in line with the N/P ratios of the plant tissues; at Trefor and Revne, the low N/P ratios indicate N limitation but in Léognan higher values could indicate colimitation or P limitation (Güsewell and Koerselman 2002). Over time we would typically expect a changed species composition as a consequence of increased biomass production as small stature plants (e.g. forbs) cannot compete for light with tall grass species (Hautier et al. 2009) and as soils become more acidic (Stevens et al. 2011b). In Léognan, the reduced N treatment resulted in a non-significant reduction in total and grass biomass. This may have been related to an interaction with the drought in Bordeaux in the summer of 2009. Experiments investigating the interactions between N addition and drought have shown that N can exacerbate the impacts of drought (Friedrich et al. 2012). Tissue N content showed a non-significant trend towards being higher at Revne and in grasses at Trefor, but was unchanged at other sites. Tissue N content has been suggested as an indicator of N deposition (Pitcairn et al. 2001). However, in sites such as Trefor, where background levels of N are low and there has never been fertiliser addition, we may expect additional N resources to be used for growth rather than luxury storage (when plants take up more nutrients than they need storing the excess in plant tissues (Barraclough 1993)). Although a number of N addition experiments have found impacts on tissue N, including in comparable plant communities (Carroll et al. 2003; Morecroft et al. 1994), Stevens et al. (2011a) did not find any relationship between N inputs and tissue N concentration of vascular plants in a European survey of acidic grasslands. At Revne, where soil nutrient status is typically a bit higher due to past fertilisation, we see slight increases in tissue N concentration.

Most of the soil variables remained unchanged by the N addition but, given the low background levels of deposition, and the relatively short period of N addition, we would only expect to see the first signs of impact on soil chemistry. Soil pH showed a significant reduction with the addition of reduced N at Revne and a slight increase with N treatments at Léognan. We would typically expect N addition to reduce soil pH (Skiba et al. 1989) but, depending on the buffering capacity of the soil, changes in soil pH may take many years to become apparent. Extractable soil  $NO_3^{-}$  and NH4<sup>+</sup> showed small, non-significant increases at Revne, but not at other sites. Since both NO<sub>3</sub><sup>-</sup> and, to a lesser extent, NH<sub>4</sub><sup>+</sup> are relatively mobile in soil and both are readily utilised by plants (Matson et al. 2002), it is not surprising that we do not see clear trends. However, plant N supply rates did show significant results. Increases in plant N supply rates were observed with the N70 and N70-ox treatments in Revne and with the N70-red treatment in Trefor. This shows differential impacts of N form and, if this continues, is likely to result in changes in plant biomass and/or tissue nutrient concentrations, both of which frequently respond positively to experimental N additions (Arroniz-Crespo et al. 2008; Hautier et al. 2009; Phoenix et al. 2012). Nitrification did not increase at any of the sites in response to N addition and was not measureable at Trefor (see Section 4.1). Morecroft et al. (1994) found similar results in an acid grassland experiment with low nitrification rates, which did not increase significantly at lower N addition treatments (35 and 70 kg N  $ha^{-1}$  year<sup>-1</sup>), but were increased by additions of  $140 \text{ kg N ha}^{-1} \text{ year}^{-1}$ . We may expect to see larger changes as N additions are applied for a longer time period and a cumulative effect became apparent.

# **5** Conclusion

We find the first effects of N addition in three species-rich acidic grasslands in low pollution regions have become apparent within 2.5 years. There were some changes in the vegetation biomass apparent although many of these were not significant and were not consistent across sites. There were few significant changes in vegetation nutrient contents. Over time, these differences may become more apparent. In some cases, the first signs of differential effects of N form could be demonstrated, forb biomass decreased significantly in Revne with the reduced N treatment indicating a negative effect of ammonium. Other differences in the impact of N form were observed for soil response variables. These experiments will be continued to elucidate the longer term impacts of N deposition in formerly unaffected European grasslands.

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## References

- Achermann, B. & Bobbink, R. eds. (2003) Empirical critical loads for nitrogen. Environmental Documentation No. 164 Air. Swiss Agency for Environment, Forest and Landscape SAEFL, Berne, 327p.
- Aerts, R., & Chapin, F. S. (2000). The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research*, 30, 1–67.
- Arroniz-Crespo, M., Leake, J. R., Horton, P., & Phoenix, G. K. (2008). Bryophyte physiological responses to, and recovery from, long-term nitrogen deposition and phosphorus fertilisation in acidic grassland. *New Phytologist*, 180, 864–874.
- Asman, W. A. H., Sutton, M. A., & Schjorring, J. K. (1998). Ammonia: emission, atmospheric transport and deposition. *New Phytologist*, 139, 27–48.
- Barraclough, P. B. (1993). Nutrient storage pool concentration in plants as diagnostic indicators of nutreint sufficiency. In N.
   J. Barrow (Ed.), *Plant nutrition: from genetic engineering* to field practice (pp. 195–198). Dordecht: Kluwer.
- Berlin, G. A. I., Linusson, A., and Olsson, E. G. A. (2000). Vegetation changes in semi-natural meadows with unchanged management in Southern Sweden, 1965–1990. *Acta Oecologica*, 21, 125–138.
- Bobbink, R. (1991). Effects of nutrient enrichment in Dutch chalk grassland. *Journal of Applied Ecology*, 28, 28–41.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., et al. (2010). Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications*, 20, 30–59.
- Bobbink, R. & Hettelingh, J-P. (Eds.) (2011) Review and revision of empirical critical loads and dose-response. Coordination Centre for Effects, National Institute for Public Health and the Environment (RIVM), The Netherlands.
- Bobbink, R., Hornung, M., & Roelofs, J. G. M. (1998). The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, 86, 717–738.

- Bremner, J. M., & Mulvaney, C. S. (1982). Nitrogen—total. In A. L. Page, R. H. Miller, & D. R. Keeney (Eds.), *Methods of soil analysis*. Wisconsin: American Society of Agronomy.
- Carroll, J. A., Caporn, S. J. M., Johnson, D., Morecroft, M. D., & Lee, J. A. (2003). The interactions between plant growth, vegetation structure and soil processes in semi-natural acidic and calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen deposition. *Environmental Pollution*, 121, 363–376.
- Clark, C. M., & Tilman, D. (2008). Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature*, 451, 712–715.
- De Graaf, M. C. C., Bobbink, R., Smits, N. A. C., Van Diggelen, R., & Roelofs, J. G. M. (2009). Biodiversity, vegetation gradients and key biogeochemical processes in the heathland landscape. *Biological Conservation*, 142, 2191–2201.
- De Graaf, M. C. C., Verbeek, P. J. M., Bobbink, R., & Roelofs, J. G. M. (1998). Restoration of species-rich dry heaths, the importance of appropriate soil conditions. *Acta Botanica Neerlandica*, 47, 98–111.
- Dentener, F., Drevet, J., Lamarque, J. F., Bey, I., Eickhout, B., Fiore, A. M., et al. (2006). Nitrogen and sulfur deposition on regional and global scales: a multimodel evaluation. *Global Biogeochemical Cycles*, 20.
- Duprè, C., Stevens, C. J., Ranke, T., Bleeker, A., Peppler-Lisbach, C., Gowing, D. J. G., et al. (2010). Changes in species richness and composition in European acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. *Global Change Biology*, 16, 344–357.
- Ellenberg, H. (1996). Vegetation Mitteleuropas mit den Alpen in ökologischer, dynamischer und historischer Sicht. Stuttgart: Ulmer.
- Friedrich, U., von Oheimb, G., Kriebitzsch, W. U., Schlesselmann, K., Weber, M. S., & Hardtle, W. (2012). Nitrogen deposition increases susceptibility to drought—experimental evidence with the perennial grass *Molinia caerulea* (L.) Moench. *Plant* and Soil, 353, 59–71.
- Galloway, J., & Cowling, E. B. (2002). Reactive nitrogen and the world: 200 years of change. *Ambio*, *31*, 64–71.
- Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., et al. (2008). Transformation of the nitrogen cycle: recent trends, questions and potential solutions. *Science*, 320, 889–892.
- Güsewell, S., & Koerselman, W. (2002). Variation in nitrogen and phosphorous concentrations of wetland plants. *Perspectives* in Plant Ecology, Evolution and Systematics, 5, 37–61.
- Hautier, Y., Niklaus, P. A., & Hector, A. (2009). Competition for light causes plant biodiversity loss after eutrophication. *Science*, 324, 636–638.
- Horswill, P., O'Sullivan, O., Phoenix, G. K., Lee, J. A., & Leake, J. R. (2008). Base cation depletion, eutrophication and acidification of species-rich grasslands in response to long-term simulated nitrogen deposition. *Environmental Pollution*, 155, 336–349.
- Johnston, A. E., Goulding, K. W. T., & Poulton, P. R. (1986). Soil acidification during more than 100 years under permanent grassland and woodland at Rothamstead. *Soil Use and Management*, 2, 3–10.
- Jones, M. L. M., Wallace, H. L., Norris, D., Brittain, S. A., Haria, S., Jones, R. E., et al. (2004). Changes in vegetation and soil characteristics in coastal sand dunes along a gradient of atmospheric nitrogen deposition. *Plant Biology*, *6*, 598–605.

- Kleijn, D., Bekker, R. M., Bobbink, R., De Graaf, M. C. C., & Roelofs, J. G. M. (2007). In search for key biogeochemical factors affecting plant species persistence in heathland and acidic grasslands: a comparison of common and rare species. *Journal of Applied Ecology*, 45, 680–687.
- LeBauer, D. S., & Treseder, K. K. (2008). Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, 89, 371–379.
- Maskell, L. C., Smart, S. M., Bullock, J. M., Thompson, K., & Stevens, C. J. (2010). Nitrogen deposition causes widespread species loss in British Habitats. *Global Change Biology*, 16, 671–679.
- Matson, P., Lohse, K. A., & Hall, S. J. (2002). The gloabalization of nitrogen deposition: consequences for terrestrial ecosystems. *Ambio*, 31, 113–119.
- Morecroft, M. D., Sellers, E. K., & Lee, J. A. (1994). An experimental investigation into the effects of atmospheric deposition on two semi-naural grasslands. *Journal of Ecology*, 82, 475–483.
- Mountford, J. O., Lakhani, K. H., & Kirkham, F. W. (1993). Experimental assessment of the effects of nitrogen addition under hay-cutting and aftermath grazing on the vegetation of meadows on a Somerset peat moor. *Journal of Applied Ecology*, 30, 321–332.
- Phoenix, G. K., Booth, R. E., Leake, J. R., Read, D. J., Grime, P., & Lee, J. A. (2003). Effects of enhanced nitrogen deposition and phosphorus limitation on nitrogen budgets of seminatural grasslands. *Global Change Biology*, *9*, 1309–1321.
- Phoenix, G. K., Emmett, B. A., Britton, A. J., Caporn, S. J. M., Dise, N. B., Helliwell, R., et al. (2012). Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. *Global Change Biology*, 18, 1197–1215.
- Pieterse, G., Bleeker, A., Vermeulen, A. T., Wu, Y., & Erisman, J. W. (2007). High resolution modelling of atmosphere-canopy exchange of acidifying and eutrophying components and carbon dioxide for European forests. *Tellus*, 59B, 412–424.
- Pitcairn, C. E. R., Leith, I. D., Fowler, D., Hargreaves, K. J., Moghaddam, M., Kennedy, V. H., et al. (2001). Foliar nitrogen as an indicator of nitrogen deposition and critical loads exceedance on a European scale. *Water, Air, and Soil Pollution, 130*, 1037–1042.
- Roelofs, J. G. M. (1986). The effect of airborne sulphur and nitrogen deposition on aquatic and terrestrial heathland vegetation. *Experientia*, 42, 372–377.
- RoTAP (2012) Review of transboundary air pollution: acidification, eutrophication, ground level ozone and heavy metals in the UK. Ediburgh, Centre for Ecology and Hydrology.
- Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., et al. (2000). Biodiversity—global biodiversity scenarios for the year 2100. *Science*, 287, 1770–1774.
- Schwickerath, M. (1944). Das Hohe Venn und seine Randgebiete. *Pflanzensoziologie*, 6, 1–278.
- Skiba, U., Cresser, M. S., Derwent, R. G., & Futty, D. W. (1989). Peat acidification in Scotland. *Nature*, 337, 68–70.
- Smits, N. A. C., Bobbink, R., Laanbroek, H. J., Paalman, A. J., & Hefting, M. M. (2010). Repression of potential nitrification activities by matgrass sward species. *Plant and Soil*, 337, 435–445.
- Ste-Marie, C., & Parè, D. (1999). Soil, pH and N availability effects on net nitrification in the forest floors of a range of

boreal forest stands. Soil Biology and Biochemistry, 31, 1579–1589.

- Stevens, C. J., Dise, N. B., Gowing, D. J., & Mountford, J. O. (2006). Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls. *Global Change Biology*, *12*, 1823–1833.
- Stevens, C. J., Dise, N. B., Mountford, J. O., & Gowing, D. J. (2004). Impact of nitrogen deposition on the species richness of grasslands. *Science*, 303, 1876–1879.
- Stevens, C. J., Dupre, C., Dorland, E., Gaudnik, C., Gowing, D. J. G., Bleeker, A., et al. (2011a). The impact of nitrogen deposition on acid grasslands in the Atlantic region of Europe. *Environmental Pollution*, 159, 2243–2250.
- Stevens, C. J., Duprè, C., Dorland, E., Gaudnik, C., Gowing, D. J. G., Bleeker, A., et al. (2010). Nitrogen deposition threatens species richness of grasslands across Europe. *Environmental Pollution*, 158, 2940–2945.
- Stevens, C. J., Dupre, C., Gaudnik, C., Dorland, E., Dise, N. B., Gowing, D. J., et al. (2011b). Changes in species composition of European acid grasslands observed along a gradient of nitrogen deposition. *Journal of Vegetation Science*, 22, 207–215.

- Subbarao, G. V., Rondon, M., Ito, O., Ishikawa, T., Rao, I. M., Nakahara, K., et al. (2007). Biological nitrification inhibition (BNI)—is it a widespread phenomenon? *Plant and Soil*, 294, 5–18.
- Tamm, C. O. (1991). Nitrogen in terrestrial ecosystems: questions of productivity, vegetational changes, and ecosystem stability. Berlin: Springer.
- van den Berg, L. J. L., Dorland, E., Verger, P., Hart, M. A. C., Bobbink, R., & Roelofs, J. G. M. (2005). Decline of acid sensitive plant species in heathland can be attributed to ammonium toxicity in combination with low pH. *New Phytologist*, 166, 551–564.
- van den Berg, L. J. L., Peters, C. J. H., Ashmore, M. R., & Roelofs, J. G. M. (2008). Reduced nitrogen has a greater effect than oxidised nitrogen on dry heathland vegetation. *Environmental Pollution*, 154, 359–369.
- Zakir, H. A. K. M., Subbarao, G. V., Pearse, S. J., Gopalakrishnan, S., Ito, O., Ishikawa, T., et al. (2008). Detection, isolation and characterization of a root-exuded compound, methyl 3-(4-hydroxyphenyl) propionate, responsible for biological nitrification inhibition by sorghum (*Sorghum bicolor*). New Phytologist, 180, 442–451.



# Tracking human impact on current tree species distribution using plant communities

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#### Keywords

*Fagus sylvatica*; Human impact; Niche model; Plant community composition; Potential distribution; Range margin; Silviculture

Nomenclature for taxa Flora Europaea (Tutin et al. 1964–1980)

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# Abstract

**Questions:** Can we adequately model beech distribution based on accompanying vegetation, and how does this compare with a climate model? To what extent does accompanying vegetation predict larger extension of beech at its SW margin compared with current distribution? Do human factors explain the discrepancy between current and potential distribution?

Location: Lowland forests at the SW European range margin of beech.

**Methods:** We built a logistic regression model of beech presence based on accompanying understorey vegetation using 66 976 plots in the French National Forest Inventory (NFI) network. Explanatory variables were obtained by multivariate reduction of 252 species in NFI plots. The model was calibrated in regions where human impact on beech has been comparatively low. The probability of beech presence was estimated at its SW margin and compared with current distribution. We tested whether forest management factors could explain beech absence in locations where the flora predicted its presence. Performance of this model was compared with a classical climatic-envelope model.

**Results:** Modelled potential distribution of beech along its SW margin was larger than observed distribution, suggesting area shrinkage under man's influence. Sites where beech was predicted as present but was currently absent were significantly less common in high forest stands, and more common in coppices, plantations and private forests. Characteristics of species replacing beech (earlysuccessional, plantation or exotic species) confirmed the role of forest management and disturbance in beech disappearance. The floristic model provided a finer resolution distribution map than the climatic-envelope model.

**Conclusions:** Plant communities, together with precise tree species maps, allowed us to estimate manipulation of beech by man, and main silvicultural causes of its disappearance. The improvement in quality of current distribution models has important implications for modelling of niches under future climate scenarios.

#### Introduction

As climate is considered to be the main factor influencing the distribution of tree species (Woodward 1987; Davis & Shaw 2001; Normand et al. 2009), improving our ability to model tree species climatic niche is necessary to better predict future suitability and possible migration in the context of climate change (Pearson & Dawson 2003), especially at their range margins (McKenney et al. 2007). Most models have predicted a reduction at the trailing edge of distribution ranges in the coming decades in response to climate change (Benito-Garzon et al. 2008; Badeau et al. 2010).

Niche modelling used in such approaches establishes a statistical relationship between species presence and environmental descriptors; but dispersal limitations can increase discordances between observed distribution and fundamental niche. Svenning & Skov (2004) found that less than 50% of the climatically suitable range of numerous tree species was currently occupied when modelled from data available in grid atlases. The authors argued that in distal zones of tree species ranges, recent postglacial recolonization could remain incomplete because of limited dispersal ability. However, Welk & Bruelheide (2006) emphasized the crucial role of data resolution. Niche models most often rely on coarse-scale spatial climate data (Araujo et al. 2005), which are usually obtained by the interpolation of time series of parameters measured at a limited number of meteorological stations. Thus, such models cannot pick up fine-scale effects of topography or aspect, which alter the microclimate and therefore species presence. Local site conditions play an especially important role at the range margins of species distribution (Lennon et al. 2002) through the mechanism of compensating factors. Isolated populations occur in locally favourable spots, thus overriding the effects of a globally unsuitable climate. A species distribution model that combines climate and site variables may be poorly reliable, because spatial resolutions of these variables differ; such a model would depict many more climatic components than site conditions as climate is the major determining factor of tree species distribution, and site conditions usually suffer from a lack of quality data. Moreover, the observed distribution on which such models are based can be impacted by human activities, and as a consequence, the assumption of a concordance between the current distribution of a species and its niche is not entirely realistic. This may result in decreased reliability of the models.

Beech (Fagus sylvatica) is a major tree species in European forests, and is the third most important tree species in both France and Europe in terms of percentage forest area. The species reaches the warm, meridional edge of its lowland European distribution in the southwest of France (Appendix S1). Indeed, in parallel to increasing water deficit in summer (Lebourgeois et al. 2005; Badeau et al. 2010) and increasing winter temperatures (Seynave et al. 2008), the probability of presence (Badeau et al. 2010) as well as radial (Lebourgeois et al. 2005) and height (Seynave et al. 2008) growth performance decreases. As a consequence, Badeau et al. (2010) and Kramer et al. (2010) predicted a significant decline of beech in the coming decades in this SW border zone. Further south of this margin, in the Iberian Peninsula, the species is present only at higher elevations such as in the Montseny Mountains (Peñuelas & Boada 2003) and along the northern Atlantic coast (Supporting Information, Appendix S2), where the climate is less constraining. Jump et al. (2007) showed that in the Montseny Mountains the limiting effects of drought and high temperatures on tree establishment and growth observed at lower altitudes disappeared at higher elevation sites. However, the exact limit of the potential distribution of beech along its margins is not known. The impacts of silvicultural practices on its current

distribution may be important in these zones, where conditions for the species are somewhat less suitable. Because beech grows in competition with oak, which has a higher timber value, and with other species of higher productivity (e.g. chestnut and pines), forest management has long disadvantaged beech in favour of other species (Bolte et al. 2007; Leuschner et al. 2009). Indeed, several studies have shown that beech reinvaded oak stands when they were no longer managed, e.g. in forest reserves in Western and Central Europe (Vera 2000). At the local scale, compensating factors have been suggested as an explanation for beech presence in the warm margin of its distribution range. In the peri-Mediterranean region (Thiebaut 1982) and in SW France (Comps 1972), fog or topography could compensate for drought.

The use of data on understorey vegetation could improve niche models by solving some of the previous problems. The composition of plant communities depends on both global and local factors and includes not only climatic but also edaphic constraints (Ellenberg et al. 1992; Wamelink et al. 2005). If one dominant tree species is artificially replaced by another, effects on the ground flora will be chiefly on non-forest and shade-intolerant species (Barbier 2007; Wulf & Naaf 2009). Consequently, the floristic composition will still indicate the ecological conditions that allowed the presence of the former species. In addition, an artificial replacement of the dominant tree species would not necessarily lead to radical changes in soil and microclimate conditions. Thus, understorey vegetation could help to identify the potential distribution of a tree species, independently of its current presence or absence. Few attempts have been made to model the distribution of tree species from understorey vegetation. Rasmussen & Kollmann (2004) tried to define the niche of Sorbus torminalis as a function of plant communities. Based on phyto-sociological surveys, the characteristic flora of beech woodlands has been clearly identified in many parts of Europe, including the southwest border zone of this species (Comps et al. 1986). It should therefore be possible to use forest floor vegetation to model beech presence. Moreover, large databases have recently been made available through national forest inventories, which provide vegetation descriptions at a fine scale.

In this study, we focused on the lowland distribution of beech and investigated the following issues:

• Can we adequately model beech distribution based on accompanying vegetation, and how does such a model compare with a climate model? We expected to find that a model based on plant community composition would capture the patchiness of tree distribution in its border area, whereas a climate model would not.

• To what extent does accompanying vegetation predict a larger extension of beech at its southwest margin in com-

parison with its current distribution? We expected to find that, due to human effects, current beech distribution had shrunk in comparison with its presence predicted by the understorey flora.

• How can human factors explain the discrepancy between current and potential distribution? We hypothesized that (i) disappearance of beech may be related to management practices, such as silvicultural regime or type of ownership, and that (ii) the effects of human impact increased at its southwest margin.

# Methods

# Approach

In the first stage, we calibrated a model of beech presence based on accompanying flora. All data were recorded in the National Forest Inventory (NFI). Because we hypothesized that beech had been heavily manipulated by man in its southwestern range, this region was discarded from the calibration data set. We calibrated the model with data from the northern and northeastern lowlands of France (the core of the species range) and from the southeastern lowlands (Fig. 1), where the absence of beech is due to high climatic constraints. We called this calibration zone the north-and-east zone. Beech limitation in this zone is natural, as supported by Seynave et al. (2008), who showed that in SE France, the current limit of beech distribution closely corresponds to a sharp decrease in height growth. Because we focused on beech behaviour in European lowlands, all forested areas above 350 m were discarded from our analyses. Above this threshold elevation, we observed a general and sharp increase in beech frequency of occurrence throughout France. From the same data set, we also calibrated a statistical, climate-envelope model to predict potential beech presence based on climate variables.



**Fig. 1.** Observed presence and absence of beech in National Forest Inventory plots. A total of 42 417 plots in the north-and-east lowland zone; 24 559 plots in the southwest lowland zone; 60 187 plots above 350 m elevation; 127 163 plots in France.

In the second stage, the previous models were used to predict beech presence in NFI plots of the southwest zone (Fig. 1).

In the third stage, we compared the map of predicted beech presence that we had obtained and the current observed distribution to identify plots where beech could have been either limited or favoured by man. We looked for human and climate factors that could explain the absence of beech on plots where it was predicted by the vegetation model in the southwestern zone.

#### Study area and data sets

French forests are sampled by the NFI (Drapier & Cluzeau 2001) in a stratified random manner, which aims to assess the state of metropolitan forests to guide French forestry policy. We used data collected during the last three inventory cycles that took place between 1981 and 2004. All vascular plant species and common soil bryophytes found in a circular 700-m<sup>2</sup> area were recorded in 127 163 sample plots (Fig. 1). Soil and stand characteristics and local topography were also recorded at the plot level (Table 1). Due to the stratified sampling scheme, each NFI plot represents a certain surface area of the French forest, 103 ha on average.

Climatic data (monthly precipitation, temperatures and number of frost days) were provided by the AURELHY Meteo France model at 1 km  $\times$  1 km resolution (Benichou & Le Breton 1987). We used monthly radiation for the period 1996–2002, provided by Satmos with a precision of 3 km, to estimate monthly Turc potential evapotranspiration (Turc 1951). Monthly and seasonal precipitation deficits were calculated as the difference between potential evapotranspiration and precipitation.

#### Data analysis

# Model development

Logistic regression models for the probability of beech presence were built using either floristic or climate data. Because predictor variables used in both the floristic (plant species presence) and climate (monthly climate variables) models were numerous and correlated, we first applied a dimension reduction obtained through correspondence analysis for floristic data (Saporta & Niang 2006) and principal components analysis for climate data (Aguilera et al. 2006). The first *n* multivariate axes (n = 27 and n = 19 for the floristic and the climate models, respectively) were used as potential independent variables in the logistic regressions.

Among the total set of floristic inventories (1285 species), 746 species occurred in both the north-and-east and the southwest border zones. Only species displaying at

Table 1. Edaphic, topographic and anthropogenic variables recorded by the French NFI and used in the present study.

Factor	Variable	Number of classes	Description
Soil	Available water capacity	10	Gradient: low to high capacity
	Waterlogged soil characteristics	3	Absent – intermediate – high
	Depth	10	Gradient: superficial to deep
	Acidity	3	Gradient: basic to acid
	Humus activity	8	Gradient: mor to eumull
	Waterlogged humus	2	Presence – absence
	Rock outcropping	10	Percentage cover of rock outcrops
	Gravel content	10	Percentage of soil volume
	Texture	9	Gradient: sandy to clayey
Topography	Slope	2	Slope $\leq$ 5° – slope > 5°
	Local radiation	Continuous	Gradient: low to high local radiation
	Aspect	Continuous	Gradient: from south to north
	Valley confinement	2	Yes-no
	Effects on water balance	4	Gradient: worsening to improving
Human	Ownership status	2	State – private
	High forest management	2	Yes-no
	Coppice management	2	Yes-no
	Coppice with standards management	2	Yes-no
	Afforestation or species substitution by plantation	2	Yes-no
	Predominance of an exotic species	2	Yes – no

least 50 occurrences in the north-and-east zone and ten in the border zone were considered for correspondence analysis (427 species). Overstorey composition may influence understorey vegetation (Augusto et al. 2003; Barbier et al. 2008), but mainly affects non-forest and shade-intolerant species (Barbier 2007; Wulf & Naaf 2009); as a precaution, we discarded these from the analysis. We referred to Rameau et al. (1989, 2008) and Ellenberg et al. (1992) to identify shade-intolerant species and to Julve (1998) to establish the list of non-forest species. Introduced species, mostly ligneous, were also discarded because they often display a distribution that is not in a state of equilibrium with the climate. Beech was also removed. Finally, 252 species were retained for correspondence analysis. Only plots with at least five of these species present were kept (59 670 in the calibration zone).

For the climatic model, we applied a principal components analysis to the matrix of the same 59 670 relevés and 133 climatic variables.

We used logistic regression to determine the relationship between beech presence and the floristic or climatic axes of either correspondence analysis or principal components analysis. The regressions produced probabilities *P* of beech being present in each NFI plot (0 < P < 1). We used a logit link and binomial error distribution (McCullagh & Nelder 1989), where logit transformation of *P* was modelled as a linear function of the *n* possible explanatory axes ( $x_k$ , k = 1, n):

$$logit(P) = log\left(\frac{P}{1-P}\right) = \alpha + \sum_{k=1}^{n} \beta_k x_k + \varepsilon.$$

Only the first axes of previous multivariate analyses, which retained the largest part of total variance, were used in model construction. Models were fitted using a maximum likelihood method (McCullagh & Nelder 1989) and forward stepwise selection among these axes. We kept the number of axes in each model that offered the best compromise between the number of variables and model performances according to Nagelkerke  $R^2$  (Nagelkerke 1991), AIC values and  $\chi^2$  score.

The models were adjusted on a randomly selected twothirds of all plots from the north-and-east zone (n = 24 291) and validation was performed on the remaining one-third (n = 12 174). Each model was then used to calculate the probability of beech occurrence in the southwest zone.

#### Model evaluation

First, we evaluated the reliability and discriminatory capacity of the two models (Pearce & Ferrier 2000). The first test was for concordance between estimated probabilities and observed frequencies of beech, while the second assessed the ability of the model to correctly discriminate between occupied and unoccupied sites.

Reliability was evaluated with the Hosmer-Lemeshow goodness-of-fit test (Hosmer & Lemeshow 2000).

Discrimination capacity was determined by visual inspection of the relative distribution of predicted values among plots for presence or absence of beech. We also calculated the area under the receiver-operating curve (ROC), denoted AUC. According to Swets (1988), we
considered an AUC between 0.90 and 1.00 as excellent, between 0.80 and 0.90 good, between 0.70 and 0.80 fair, between 0.60 and 0.70 poor, and between 0.50 and 0.60 as fail. We also used several criteria derived from the two-bytwo cross-classification table which confronts expected and observed presences and absences (Supporting Information, Appendix S3a and S3b). We chose sensitivity (Ss) and specificity (Sp), which respectively represent the proportion of correct predictions for presence or absence, and precision of presence (*Pp*) and absence (*Pa*), providing the proportion of correct predictions when a presence or an absence is expected. In order to build such a table, a probability threshold *P*<sub>fair</sub> must be chosen, above or below which the species is considered to be present or absent. As recommended by Liu et al. (2005), we used the Ss and Sp equality method to determine the  $P_{\text{fair}}$  threshold value.

Frequency of beech occurrence (prevalence) in the fitting data set can impact the results of distribution modelling (Manel et al. 2001). Allouche et al. (2006) developed a model evaluation criterion independent of prevalence, the true skill statistic (*TSS*). From the cross-classification tables, we calculated *TSS* in the north-and-east and the southwest border zones in order to evaluate the change in global performance of our models between these two areas.

# Indicator species for beech presence

For the purpose of illustration, we listed the plant species significantly associated with the presence of beech, either positively or negatively. We used a two-sided Fisher's exact test at the P < 0.001 level of significance to compare the frequencies of occurrence of all of the 1285 species present in the French lowland plots where beech was present, with those where it was absent.

# Determining factors related to the absence of beech where forecast by the floristic model

We estimated the influence of anthropogenic variables on beech presence in the southwest border zone by calculating ratios of the frequencies of each management condition in sites where beech was absent ( $f_0$ ) to those where it was present ( $f_1$ ). In this way, we tested the role of management regime, type of regeneration, type of ownership and exotic species introduction. The probability of occurrence given by the climate model represented a measurement of the global climatic constraints on beech, and the ratio of its mean in sites where beech was absent ( $m_0$ ) to that where beech was present ( $m_1$ ) was calculated. We also identified the dominant tree species related to the presence of beech in the southwest border zone by calculating the ratio of their observed frequencies in sites where beech was absent ( $f_0$ ) to those where it was present ( $f_1$ ). We also identified which management conditions and dominant tree species were related to the disappearance of beech (beech predicted by the flora but not observed) in the southwest border zone. For this purpose, we calculated the ratios  $(f_b/f_d)/(f_a/f_c)$ , where  $f_a$ ,  $f_b$ ,  $f_c$  and  $f_d$  were the observed frequencies of each management condition or dominant tree species in cells *a*, *b*, *c* and *d*, respectively, of the cross-classification table (Supporting Information, Appendix S3a). We also calculated the ratios  $(m_b/m_d)/(m_a/m_c)$ , where  $m_a$ ,  $m_b$ ,  $m_c$  and  $m_d$  were the observed means of the modelled climatic probability of presence in the cross-classification table. Values of the ratios higher than one indicated a silvicultural condition related to the disappearance of beech, or a tree species replacing beech in sites where it had been predicted to occur, but was recorded absent.

All computations and statistical analyses were carried out using SAS/STAT<sup>®</sup> 9.2 software version (SAS/STAT<sup>®</sup> Institute Inc., Cary, NC, US).

# Results

# Indicator species of beech presence

In the French lowlands, 103 plant species were significantly associated with the presence of beech, at the P < 0.001 level. The 30 most significant are listed in Table 2. We found 154 species significantly more associated with beech absence. Species preferring beech were more shade tolerant than those avoiding it: mean Ellenberg light indicator values were 4.6 and 5.8, respectively (P < 0.0001). See Appendices S4 and S5 for the full lists of species.

# Modelling beech potential distribution

Both floristic and climatic regression models included 13 multivariate axes (Table 3). We tested for over-dispersion: Pearson  $\chi^2$  and deviance were not significant (P > 0.05), suggesting that the models fitted well. The threshold probability value, sensitivity and specificity were  $P_{\text{fair}} = 0.45$ , Ss = 0.74 and Sp = 0.74 for the floristic model, and  $P_{\text{fair}} = 0.44$ , Ss = 0.72 and Sp = 0.73 for the climate model.

#### Model evaluation

Floristic and climate models showed good discriminatory power following the classification proposed by Swets (1988) based on AUC values (Table 4; Appendix S6), both in the calibration and validation data sets. Low probabilities were well represented by the models and corresponded to a first frequency peak for unoccupied sites only (Fig. 2). The second peak was located at probability values above  $P_{\text{fair}}$  and mainly concerned occupied sites. This was indicative of the good discrimination capacity of the models.

**Table 2.** Species most significantly associated with beech presence in theFrench lowlands ( $\leq$ 350 m elevation). Species are classified by decreasingratio of presence to absence. \* Tree species.

Species	Frequency of occurrence (%) i plots where beech is:				
Species Vaccinium myrtillus Galium odoratum Dxalis acetosella Abies alba* .uzula sylvatica Melica uniflora Sorbus aria* Milium effusum .uzula pilosa Dryopteris dilatata Rubus idaeus .amiastrum galeobdolon Athyrium filix-femina Anemone nemorosa Cornus mas* lex aquifolium* Polytrichum formosum Atrichum undulatum Acer pseudoplatanus* Carex sylvatica Quercus petraea*	absent	present			
Vaccinium myrtillus	1.1	8.8			
Galium odoratum	1.5	10.2			
Oxalis acetosella	1.2	6.6			
Abies alba*	1.4	7.5			
Luzula sylvatica	1.1	4.9			
Melica uniflora	1.8	7.2			
Sorbus aria*	2.0	7.3			
Milium effusum	4.2	14.0			
Luzula pilosa	2.8	8.4			
Dryopteris dilatata	2.1	6.1			
Rubus idaeus	1.9	5.4			
Lamiastrum galeobdolon	4.4	12.0			
Athyrium filix-femina	3.4	9.1			
Anemone nemorosa	4.7	12.1			
Cornus mas*	2.7	6.9			
llex aquifolium*	13.4	31.7			
Polytrichum formosum	15.6	36.9			
Atrichum undulatum	5.9	13.7			
Acer pseudoplatanus*	7.2	16.7			
Carex sylvatica	12.6	28.1			
Quercus petraea*	26.1	58.0			
Dryopteris carthusiana	4.0	8.7			
Crataegus laevigata	6.4	13.2			
Carpinus betulus*	30.5	59.2			
Dryopteris filix-mas	7.1	13.0			
Deschampsia flexuosa	11.0	17.6			
Betula pendula*	24.2	38.2			
Acer campestre*	14.9	23.1			
Corylus avellana	33.1	45.0			
Hedera helix	55.1	64.5			

Performance criteria were calculated for the calibration and validation subsets (Table 4), based on the two-by-two cross-classification tables (Supporting Information, Appendix S7). The floristic model performed slightly better than the climate model for all criteria, although overall, both models were adequate, with both *Ss* and *Sp* above 0.7.

## Prediction of beech presence in the southwest border zone

The behaviour of the floristic and climate models in the southwest border zone was not the same. Both predicted a low value of beech presence in comparison with the north-and-east zone. However, the floristic model globally predicted more presences than were observed (18% vs 11%), while the climate model forecast less presences than were observed (3% vs 11%). The global performance of the floristic model remained stable and was better than the climate model (*TSS* = 0.49 and 0.09, respectively).

**Table 3.** Wald  $\chi^2$  and estimated values of model parameters for the logistic regressions of beech presence fitted on correspondence analysis axes for the floristic model and principal components axes for the climate model. All  $\chi^2$  values were significant at the P < 0.001 level.

Variables entered	Wald $\chi^2$	Standardized estimates
Floristic model		
Intercept	875.7	-
Axis 6	700.4	0.3177
Axis 1	396.5	-1.7861
Axis 4	371.1	-0.2949
Axis 10	292.7	0.4047
Axis 12	142.7	-0.1579
Axis 3	108.4	-0.1778
Axis 2	106.5	0.1804
Axis 18	103.2	0.1427
Axis 19	90.7	0.1009
Axis 14	79.1	0.1954
Axis 24	58.7	0.0714
Axis 17	30.8	-0.0621
Axis 11	22.4	0.0850
Climate model		
Intercept	1193.1	-
Axis 1	1399.6	-1.2211
Axis 15	333.8	-0.1929
Axis 2	324.1	-0.2092
Axis 18	292.1	-0.1763
Axis 14	202.6	0.1690
Axis 3	150.4	0.1453
Axis 11	80.6	0.0980
Axis 10	69.5	0.0920
Axis 4	62.9	-0.0849
Axis 13	33.5	0.0718
Axis 16	30.7	-0.0507
Axis 12	16.7	-0.0493
Axis 17	8.1	0.0308

Predicted probabilities according to the floristic (Fig. 3a) and climate (Fig. 3b) models were mapped for the 59 670 plots in the French lowlands and were compared with the current distribution (Fig. 1). The scattered pattern of current beech presence in the western lowlands of France was preserved in the floristic model, but not in the climate model, which predicted a homogeneous although low probability of presence throughout the area. Combined with the global underestimation by the climate model of beech prevalence mentioned above, this led to a lower sensitivity of the climate model in the southwest border zone (0.11) compared to the floristic model (0.61). In other words, most of the current beech presences in SW France occur ouside the climatic range according to our climate model. Floristically-determined potential sites covered a wider geographical area (Fig. 3a) compared to the climate envelope of the species (Fig. 3b).

The proportion of sites where beech was absent among those where it had been predicted to be present by the floristic model (1 - Pp) was 35% for the north-and-east zone



Fig. 2. Distribution of predicted probability values where beech was present or absent in the validation subset. (a) Floristic model and (b) climate model. The vertical dashed line indicates the threshold probability level P<sub>fair</sub>.

Statistics	Floristic model			Climate model				
	Calibration data set	Validation data set	Southwest border zone	Calibration data set	Validation data set	Southwest border zone		
Hosmer-Lemeshow goodness of fit test ( $\chi^2$ value)	26.4 ( <i>P</i> < 0.001)	21.1 (P < 0.01)	_	66.8 (P < 0.0001)	39.6 (P < 0.0001)	_		
AUC	0.834	0.827	_	0.805	0.802	_		
R <sup>2</sup> Nagelkerke	0.43	0.42	_	0.37	0.37	_		
Range of probabilities	0-0.99	0–0.99	0–0.99	0–0.93	0–0.93	0–0.66		
Sensitivity (Ss)	0.74	0.74	0.61	0.72	0.72	0.11		
Specificity (Sp)	0.74	0.74	0.88	0.72	0.73	0.98		
Precision of presence (Pp)	0.65	0.65	0.39	0.63	0.64	0.46		
Precision of absence (Pa)	0.82	0.82	0.95	0.80	0.79	0.90		
True skill statistic (TSS)	0.49	0.48	0.49	0.44	0.44	0.09		

and 61% for the southwest border zone. This suggested that some beech had disappeared in this latter zone. For the climate model, this proportion was smaller (54%). An area-weighted sum showed that the potential disappearance of beech covered over 67% of the area where it had been predicted by the floristic model, i.e. more than 300 000 ha.

The modelled climatic probabilities showed that the southwest zone is characterized by a regular and gentle climate gradient, while an abrupt spatial transition occurs in the eastern lowlands, along the Rhone Valley (Fig. 3b), as plot distribution did not decrease regularly from null to high probability values (Fig. 4). The range of climatic probabilities observed in the north-and-east zone, used for calibration, encompassed that of the southwest border zone.

# Determining factors related to the disappearance of beech model

In the 23 205 sites in the southwest border zone, human impact played an important role in determining the current presence or absence of beech in sites predicted as favourable. The ratios of the frequencies  $(f_b/f_d)/(f_a/f_c)$  for five management variables among the six tested and the ratio of the means  $(m_b/m_d)/(m_a/m_c)$  for the climatic probability were higher than one (Table 5). The management conditions related to the absence of beech when forecast by the floristic model were presence of coppice with standards, coppice or absence of high forest management regimes, plantation forestry and private ownership status. A large majority of these conditions had a negative effect on beech presence (ratio  $f_0/f_1 < 1$ ). The presence of beech where it was predicted by flora was less dependent on climate suitability than where it was not predicted  $\left[\frac{m_b}{m_d}\right]$  $(m_a/m_c) > 1$ ]. Human impact on beech presence or absence was exacerbated in the south-west margin compared to the north and east plains (Supporting Information, Appendix S8).

The species replacing beech when it was not present on sites predicted as potential are given in Table 6. These were either species favoured by plantation, most often allochthonous (*Castanea sativa, Pinus nigra* ssp. *laricio, Pseudotsuga menziesii, Quercus rubra* and *Robinia pseudoacacia*), more



**Fig. 3.** Probability of beech presence in the French lowlands ( $\leq$ 350 m elevation). Estimated from (**a**) the species composition of plant communities (floristic modelling approach) and (**b**) climate variables (climate-envelope modelling approach).

light-demanding, pioneer species (*Betula* sp., *Populus tremula* and *Quercus robur*) or species usually accompanying beech (*Carpinus betulus* and *Quercus petraea*).

# Discussion

The main result was that the composition of forest plant communities predicted a potential range for beech that is wider than the current distribution at its southwest margin. This discrepancy may be attributed to three causes: (1) model bias, (2) differential range filling, with understorey species spreading faster than beech during the postglacial colonization due to different dispersal abilities, and (3) human impact on species presence.

The quality of the floristic model in the north-and-east zone was good, and even slightly better than the climate model. This calibration zone encompassed a wider range of



**Fig. 4.** Distribution of predicted values of beech presence in the French lowlands by the climate model in the north-and-east zone and in the southwest border zone. (a) For forest areas (proportion of National Forest Inventory (NFI) plots) and (b) for the whole area (proportion of AURELHY grid cells), for each 0.01 probability class. A distribution gap is observed for low probability values, between 0 and 0.3, in the north-and-east zone.

drought and temperature conditions than the southwest border zone. In addition, it has been shown that the niche of herbaceous species is stable between eastern and western France, at least for edaphic conditions (Coudun & Gégout 2005). There was therefore no evidence of bias that would lead to an overestimation of beech presence in the southwest zone.

It would be difficult to attribute the discrepancy between the current limits of beech distribution and its potential range predicted by flora to a less complete postglacial colonization by beech compared to the understorey flora. Glacial refugia of beech have been identified close to the area we studied (Magri et al. 2006), in the north of the Iberian Peninsula and in the western Alps. The same authors have shown that, at around 1000 BP, beech had

Table 5. \	Variables explaining the presence or absence of beech in the southwest border zone ( $n =$	23 205 plots). (i) Independently of the predicted pres
ence and (i	ii) when predicted by the floristic model. (a) Anthropogenic variables; (b) climate variables.	

(d)						
Variables	(i) Proportion (\$ containing bee	ဖ) for sites ch	Ratio of frequencies in sites where beech is absent to those	(ii) Ratio of frequencies in the cells of the cross-classification table $(f_b/f_d)/(f_o/f_c)$		
	Absent (f <sub>0</sub> )	Present ( $f_1$ )	where it is present $(t_0/t_1)$			
Coppice with standards	26	27	0.99	2.08		
Coppice	18	6	3.19	1.93		
<ul> <li>Other management than high forest</li> </ul>	50	36	1.41	1.72		
<ul> <li>Afforestation or species substitution by plantation</li> </ul>	9	7	1.31	1.69		
<ul> <li>Private ownership status</li> </ul>	86	51	1.69	1.17		
Predominance of an exotic species	37	11	3.50	0.98		
(b)						
Variable	(i) Mean value containing be	for sites ech	Ratio of means in sites where beech is absent to those where	(ii) Ratio of means in cells of the cross-classification table		
	Absent (m <sub>o</sub> )	Present ( $m_1$ )	it is present $(m_0/m_1)$	(m <sub>b</sub> /m <sub>d</sub> )/(m <sub>a</sub> /m <sub>c</sub> )		
Climate suitability (modelled probabilities)	0.16	0.27	0.60	1.24		

**Table 6.** Relationships between dominant tree species and beech presence, and species replacing beech when it was predicted by the floristic model but is currently absent in the southwest border zone (n = 23 205). Species that more often occupy sites where beech is absent than those where it present, or species that replace beech when it disappears have ratios >1.

Species	Frequency of s for sites where	pecies (%) beech is:	Ratio of frequencies of species in sites where beech is	Ratio of frequencies of species in cells of the cross-classification table $[(f_{\rm b}/f_{\rm d})/(f_{\rm o}/f_{\rm c})]$	
	absent ( $f_0$ )	present ( $f_1$ )	absent to those where it is present ( $f_0/f_1$ )		
Castanea sativa	3.2	4.1	0.79	5.20	
Robinia pseudoacacia	1.4	0.3	5.10	5.09	
Populus tremula	0.5	0.2	2.90	4.26	
Carpinus betulus	0.8	1.6	0.55	3.87	
Pinus nigra ssp. laricio	2.1	0.8	2.79	3.79	
Quercus robur	23.9	20.3	1.17	2.66	
Pseudotsuga menziesii	1.2	1.5	0.83	2.36	
Quercus rubra	0.4	0.5	0.88	2.35	
Quercus petraea	10.6	41.2	0.26	1.82	
Betula sp. (incl. pendula and pubescens)	1.1	0.2	5.52	1.14	

already reached its maximum extension, which was very similar to that of today. In our study we observed that, like beech, the main plant species associated with beech in the French lowlands are shade tolerant. Moreover, a large number of these accompanying species are known to be ancient woodland species (Hermy et al. 1999), for example, *Anemone nemorosa, Carex sylvatica, Galium odoratum, Lamiastrum galeobdolon, Luzula pilosa, Melica uniflora, Milium effusum* or *Oxalis acetosella,* which, in addition, are especially poor colonizers (Bossuyt et al. 1999). Willner et al. (2009) showed that understorey species richness of beech forest decreased as the distance from the glacial refuge of beech increased, which supposes that the distribution of these

species is limited by postglacial dispersal. So, these accompanying species probably did not colonize the postglacial leading edge faster than beech. Instead, their presence without *Fagus sylvatica* suggests its disappearance.

The relative area of forest potentially affected by the disappearance of beech in the southwest border zone was almost twice as large as in the north-and-east zone. Forest management was the main factor explaining the absence of beech where it had been predicted by the flora. Almost all frequency ratios calculated for the tested management variables differed from one. Stand management regime has a significant impact on beech presence. The negative effects of coppicing and coppice with standards and the positive effect of high forest management that we observed were consistent with specific characteristics of beech (Ellenberg 1988; Peters 1997). The ability of beech to resprout after cutting is limited and so it is progressively eliminated by coppicing. High forests limit light access to the forest floor, thus favouring beech regeneration, unlike coppice with standards.

Plantation forestry has tended to eliminate beech from some suitable sites because of its lower economic value and establishment success rate in comparison with more valuable or light-demanding species. The role of artificial afforestation and regeneration can be illustrated by the species that have replaced beech. They were often exotic species, favoured by large state plantation programmes during the second half of the 20th century, such as Pseudotsuga menziesii or Quercus rubra. Other replacing species, such as Castanea sativa, are native to France but were widely extended by man in the past. However, in some cases, autochthonous species were found instead of beech. This could result from selection against beech due to its lower resprouting ability after coppicing, unsuccessful natural regeneration of beech stands or recent natural afforestation of ancient agricultural lands where beech had not vet colonized.

Ownership status was also related to beech elimination in the southwest border zone. State forests in France are ancient forests, whereas half of today's private forests have replaced arable land during the last two centuries. Forest management cycles are longer on average in state forests. Historical trends have led to higher volumes of wood per hectare in state forests (19% more than in private forests on average throughout France, from NFI data analysis). These three characteristics favour beech, which is a late-successional and shade-tolerant species. In the SW French lowlands, 80% of the inventoried NFI sites are private forests, a higher figure than in the northand-east lowlands (59%). This high proportion partly explains why beech is so often missing at its southwest margin.

Another interesting result of the floristic model was that the predicted potential distribution of beech in the southwest border zone was wider and patchier than that forecast by a classical climate-envelope model. Two explanations are possible. First, although the sampling density was the same for floristic and climate data (about one point for every 100 ha of forest), they represented different scales of ecological phenomena (Randin et al. 2009; Franklin 2010). Climate data were averaged over 1 km × 1 km cells whereas floristic data were collected on 700-m<sup>2</sup> plots. Thus, the latter could capture variation in microclimate that the former could not. Second, because understorey vegetation responds strongly to variations in soil conditions, the vegetation model integrated part of the relationships between beech presence and edaphic parameters, such as soil nutritional status or soil moisture regime, whereas climate models did not. This may be crucial when considering that waterlogging and flooding in alluvial forests exert important controls on beech presence (Ellenberg 1988).

# Conclusions

Our model of beech distribution based on plant community composition, which presents relics of former beech distribution, predicted a wider range than is currently occupied by beech. Moreover, the absence of beech at its southwest margin in sites where the model predicted presence was clearly related to management factors. These two results strongly support the hypothesis that beech distribution has shrunk due to human activities. Stand management regime, plantation forestry and ownership status have had negative effects on beech presence, ultimately leading to elimination of the species. Man has truncated the distribution range of beech by replacing it with more productive, often allochthonous, species. It would be interesting to use the predicted potential range, as shown through our floristic model, to calibrate climate niche models. In this way, bias due to human impact on current tree distribution would be reduced. The quality of projections for future climate scenarios would also be improved. Because most temperate tree species have been manipulated by man, our approach could be profitably applied to species other than beech.

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*Author contributions*: D.E.S., V.B., M.L., E.C. and J.L.D. conceived the ideas; D.E.S., V.B. and J.L.D. analysed the data; and D.E.S. and J.L.D. led the writing.

# Biosketche

Daniel E. Silva is interested in modelling forest species distribution and their responses to environmental constraints. He uses large inventory databases to identify climatic and edaphic controls on tree species niches.

# References

Aguilera, A.M., Escabias, M. & Valderrama, M.J. 2006. Using principal components for estimating logistic regression with high-dimensional multicollinear data. *Computational Statistics & Data Analysis* 50: 1905–1924.

- Allouche, O., Tsoar, A. & Kadmon, R. 2006. Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). *Journal of Applied Ecology* 43: 1223–1232.
- Araujo, M.B., Thuiller, W., Williams, P.H. & Reginster, I. 2005. Downscaling European species atlas distributions to a finer resolution: implications for conservation planning. *Global Ecology and Biogeography* 14: 17–30.
- Augusto, L., Dupouey, J.L. & Ranger, J. 2003. Effects of tree species on understory vegetation and environmental conditions in temperate forests. *Annals of Forest Science* 60: 823–831.
- Badeau, V., Dupouey, J.L., Cluzeau, C., Drapier, J. & Le Bas, C. 2010. Climate change and the biogeography of French tree species: first results and perspectives. In: Loustau, D. (ed.) *Forest, carbon cycle and climate change*. pp. 231–252. Quae, Paris, FR.
- Barbier, S. 2007. Influence de la diversité, de la composition et de l'abondance des essences forestières sur la diversité floristique des forêts tempérées. PhD Thesis, Orléans University, Orléans, FR.
- Barbier, S., Gosselin, F. & Balandier, P. 2008. Influence of tree species on understory vegetation diversity and mechanisms involved – a critical review for temperate and boreal forests. *Forest Ecology and Management* 254: 1–15.
- Benichou, P. & Le Breton, O. 1987. Prise en compte de la topographie pour la cartographie de champs pluviométriques statistiques: la méthode AURELHY. Agrométéorologie des régions de moyenne montagne. Les Colloques de l'INRA 39: 51–69.
- Benito-Garzon, M., Sanchez De Dios, R. & Sainz Ollero, H. 2008. Effects of climate change on the distribution of Iberian tree species. *Applied Vegetation Science* 11: 169–178.
- Bolte, A., Czajkowski, T. & Kompa, T. 2007. The north-eastern distribution range of European beech – a review. *Forestry* 80: 413–429.
- Bossuyt, B., Hermy, M. & Deckers, J. 1999. Migration of herbaceous plant species across ancient–recent forest ecotones in central Belgium. *Journal of Ecology* 87: 628–638.
- Comps, B. 1972. *Essai sur le déterminisme écologique du Hêtre (Fagus sylvatica L.) en* Aquitaine. PhD Thesis, Bordeaux University, Bordeaux, FR.
- Comps, B., Letouzey, J. & Timbal, J. 1986. Étude synsystématique des hêtraies pyrénéennes et des régions limitrophes (Espagne et Piémont aquitain). *Phytocoenologia* 14: 145–236.
- Coudun, C. & Gégout, J.C. 2005. Ecological behaviour of herbaceous forest species along a pH gradient: a comparison between oceanic and semicontinental regions in northern France. *Global Ecology and Biogeography* 14: 263–270.
- Davis, M.B. & Shaw, R.G. 2001. Range shifts and adaptive responses to quaternary climate change. *Science* 292: 673–679.
- Drapier, J. & Cluzeau, C. 2001. La base de données écologiques de l'IFN. *Revue Forestière Française* 3: 365–371.
- Ellenberg, H. 1988. Vegetation ecology of Central Europe. Cambridge University Press, Cambridge, UK.

- Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W. & Paulissen, D. 1992. Zeigerwerte von Pflanzen in Mitteleuropa. Scripta Geobotanica 18: 1–248.
- Franklin, J. 2010. Mapping species distributions. Cambridge University Press, Cambridge, UK.
- Hermy, M., Honnay, O., Firbank, L., Grashof-Bokdam, C. & Lawesson, J.E. 1999. An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. *Biological Conservation* 91: 9–22.
- Hosmer, D.W. & Lemeshow, S. 2000. *Applied logistic regression*. 2nd ed. Wiley, New York, NY, US.
- Julve, P. 1998. Baseflor. *Index botanique, écologique et chorologique de la flore de France*. Version: 2009. URL: http://perso.wana-doo.fr/philippe.julve/catminat.htm.
- Jump, A.S., Hunt, J.M. & Peñuelas, J. 2007. Climate relationships of growth and establishment across the altitudinal range of *Fagus sylvatica* in the Montseny mountains, northeast Spain. *Ecoscience* 14: 507–518.
- Kramer, K., Degen, B., Buschbom, J., Hickler, T., Thuillier, W., Sykes, M.T. & de Winter, W. 2010. Modelling exploration of the future of European beech (*Fagus sylvatica* L.) under climate change – range, abundance, genetic diversity and adaptive response. *Forest Ecology and Management* 259: 2213–2222.
- Lebourgeois, F., Bréda, N., Ulrich, E. & Granier, A. 2005. Climate–tree growth relationships of European beech (*Fagus sylvatica* L.) in the French Permanent Plot Network (RENECOFOR). *Trees* 19: 385–401.
- Lennon, J.J., Kunin, W.E., Corne, S., Carver, S. & Van Hees, W. W.S. 2002. Are Alaskan trees found in locally more favourable sites in marginal areas? *Global Ecology and Biogeography* 11: 103–114.
- Leuschner, C., Kockemann, B. & Buschmann, H. 2009. Abundance, niche breadth, and niche occupation of Central European tree species in the centre and at the margin of their distribution range. *Forest Ecology and Management* 258: 1248– 1259.
- Liu, C.R., Berry, P.M., Dawson, T.P. & Pearson, R.G. 2005. Selecting thresholds of occurrence in the prediction of species distributions. *Ecography* 28: 385–393.
- Magri, D., Vendramin, G.G., Comps, B., Dupanloup, I., Geburek, T., Gomory, D., Latalowa, M., Litt, T., Paule, L., Roure, J.M., Tantau, I., Van Der Knaap, W.O., Petit, R.J. & De Beaulieu, J.L. 2006. A new scenario for the quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. *New Phytologist* 171: 199–221.
- Manel, S., Williams, H.C. & Ormerod, S.J. 2001. Evaluating presence–absence models in ecology: the need to account for prevalence. *Journal of Applied Ecology* 38: 921–931.
- McCullagh, P. & Nelder, J.A. 1989. Generalized linear models. Monographs on statistics and applied probability series. 2nd ed. Chapman & Hall, London, UK.
- McKenney, D.W., Pedlar, J.H., Lawrence, K., Campbell, K. & Hutchinson, M.F. 2007. Beyond traditional hardiness zones:

using climate envelopes to map plant range limits. *BioScience* 57: 929–937.

- Nagelkerke, N.J.D. 1991. A note on general definition of the coefficient of determination. *Biometrika* 78: 691–692.
- Normand, S., Treier, U.A., Randin, C., Vittoz, P., Guisan, A. & Svenning, J.C. 2009. Importance of abiotic stress as a range-limit determinant for European plants: insights from species responses to climatic gradients. *Global Ecology and Biogeography* 18: 437–449.
- Pearce, J. & Ferrier, S. 2000. Evaluating the predictive performance of habitat models developed using logistic regression. *Ecological Modelling* 133: 225–245.
- Pearson, R.G. & Dawson, T.P. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography* 12: 361–371.
- Peñuelas, J. & Boada, M. 2003. A global change-induced biome shift in the Montseny Nountains (NE Spain). *Global Change Biology* 9: 131–140.
- Peters, R. 1997. *Beech forests. Geobotany 24.* Kluwer Academic, Dordrecht, NL.
- Rameau, J.C., Mansion, D., Dumé, G., Timbal, J., Lecointe, A., Dupont, P. & Keller, R. 1989. Flore forestière française. Guide écologique illustré. Tome 1: plaines et collines. Institut pour le Développement Forestier, Paris, FR.
- Rameau, J.C., Mansion, D., Dumé, G., Gauberville, C., Bardat, J., Bruno, E. & Keller, R. 2008. Flore forestière française. Guide écologique illustré. Tome 3: région méditerranéenne. Institut pour le Développement Forestier, Paris, FR.
- Randin, C.F., Engler, R., Normand, S., Zappa, M., Zimmermann, N.E., Pearman, P.B., Vittoz, P., Thuiller, W. & Guisan, A. 2009. Climate change and plant distribution: local models predict high-elevation persistence. *Global Change Biology* 15: 1557–1569.
- Rasmussen, K.K. & Kollmann, J. 2004. Defining the habitat niche of *Sorbus torminalis* from phytosociological releves along a latitudinal gradient. *Phytocoenologia* 34: 639–662.
- Saporta, G. & Niang, N. 2006. Correspondence analysis and classification. In: Greenacre, M. & Blasius, J. (eds.) *Multiple correspondence analysis and related methods*. pp. 371–392. Chapman & Hall, Boca Raton, FL, US.
- Seynave, I., Gegout, J.C., Herve, J.C. & Dhote, J.F. 2008. Is the spatial distribution of European beech (*Fagus sylvatica* L.) limited by its potential height growth? *Journal of Biogeography* 35: 1851–1862.
- Svenning, J.C. & Skov, F. 2004. Limited filling of the potential range in European tree species. *Ecology Letters* 7: 565–573.
- Swets, J.A. 1988. Measuring the accuracy of diagnostic systems. *Science* 240: 1285–1293.
- Thiebaut, B. 1982. Existe-t-il une hêtraie Méditerranéenne distincte des autres forêts de hêtre en Europe occidentale? *Vegetatio* 50: 23–42.

- Turc, L. 1951. Nouvelle formule pour le calcul du bilan de l'eau en fonction des valeurs annuelles moyennes des précipitations et de la température. *Comptes Rendus de l'Académie des Sciences* 233: 633–635.
- Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M. & Webb, D.A. 1964–1980. *Flora Europaea*, vols. 1–5. Cambridge University Press, Cambridge, UK.
- Vera, F.W.M. 2000. *Grazing ecology and forest history*. CAB International, Oxford, UK.
- Wamelink, G.W.W., Paul, W.G., Han, F.V.D. & Frank, B. 2005. Plant species as predictors of soil pH: replacing expert judgement with measurements. *Journal of Vegetation Science* 16: 461–470.
- Welk, E. & Bruelheide, H. 2006. There may be bias in r/p ratios (realized vs. potential range) calculated for European tree species – an illustrated comment on Svenning & Skov 2004. *Journal of Biogeography* 33: 2013–2018.
- Willner, W., Pietro, R.D. & Bergmeier, E. 2009. Phytogeographical evidence for post-glacial dispersal limitation of European beech forest species. *Ecography* 32: 1011–1018.
- Woodward, F.I. 1987. *Climate and plant distribution*. Cambridge University Press, Cambridge, UK.
- Wulf, M. & Naaf, T. 2009. Herb layer response to broadleaf tree species with different leaf litter quality and canopy structure in temperate forests. *Journal of Vegetation Science* 20: 517–526.

# Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. European distribution map of beech.

**Appendix S2.** Beech distribution in the SW European lowlands.

**Appendix S3.** Cross-classification table and performance criteria.

**Appendix S4.** Species accompanying beech in the French lowlands.

**Appendix S5.** Species indicating the absence of beech in the French lowlands.

Appendix S6. Receiver operating curves.

**Appendix S7.** Results of cross-classification tables.

**Appendix S8.** Role of forest management conditions.

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# Forest Ecology and Management

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# Does natural regeneration determine the limit of European beech distribution under climatic stress?

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# ABSTRACT

Climate is considered to be the main factor that determines forest species distribution. In Europe, climatic series show a global warming trend and an increase in frequency of summer droughts, which should affect growth and regeneration of tree populations. Beech (*Fagus sylvatica* L.) is particularly sensitive to drought and high temperatures. Then, this species reaches the rear edge of its lowland European distribution in the south-west of France. In this area, statistical modelling of future distributions based on climate scenarios show a significant potential reduction for *F. sylvatica*'s range. At the moment, failure of beech establishment is still unknown at the regional scale in the rear edge of its distribution range.

The aim of this study was thus to determine if a reduction in natural regeneration of beechwoods could be related to the decrease in the species presence at its range margin. We identified ecological factors related to beech recruitment by estimating seedling density on the forest floor respectively in 71 and 85 beech plots in the south-west lowlands of France. We also determined if a relation between seedling amount and mast-seeding existed.

We showed the importance of local factors in the natural regeneration stages by performing a multivariate reduction of the data and a multiple regression on densities. The inherent capacity of the stand to produce fruits explained a greater part of cupule density variance than did the climatic factor. However, we observed that meso-, micro- and pedo-climates were the main factors controlling seedling amount. Higher soil moisture, precipitation and temperature during the growing season increased seedling density, while late spring and early autumn frosts decreased it. Soil and stand conditions also played a significant role. Fruit production increased in stands that showed tree crown degradation, while seedling amount decreased in this case. The increase in allocation to reproduction could be a strategy of beech to cope with ecological constraints that tend to limit its establishment.

Thus, seedling establishment is related to the factors controlling *F. sylvatica* presence at its southern distribution margins. This illustrates how natural regeneration is a key stage for beech success where the future of the species is jeopardized in the context of climate change.

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Forest Ecology and Managemer

# 1. Introduction

Improving our knowledge of tree species behavior at their range margins is essential to understanding their future suitability in the context of climate changes. During the last few decades in Europe, climate series of mean temperatures have clearly revealed a global warming (IPCC, 2001; Luterbacher et al., 2004) and an increase in extreme climatic events such as high mean summer temperatures and summer drought (Schar et al., 2004; Stott et al., 2004). European species populations at their southern distribution limit might be more vulnerable to the increasing frequency of extreme climatic conditions because they are already growing in the warmest and driest part of their climatic niche. That is why species' southern ranges are considered to be hotspots of vegetation changes. Several results from statistical modelling of future distributions based on climate scenarios have already shown significant potential reductions for several tree species, at their rear edge in Europe (Attorre et al., 2008; Badeau et al., 2010; Benito Garzon et al., 2008; Berry et al., 2002).

Beech is at the moment a major tree species in European forests, but its populations in the southernmost range margins may shift to higher altitudes (Peñuelas et al., 2007; Peñuelas and Boada, 2003) and their distribution shrink. This species is indeed sensitive to climatic variations such as more frequent extreme drought events or global warming, particularly in terms of growth (Dittmar et al., 2003; Granier et al., 2007; Jump et al., 2006b; Lebourgeois et al.,



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2005; Seynave et al., 2008), productivity (Bontemps et al., 2009; Ciais et al., 2005) and physiological performance (Bréda et al., 2006; Granier et al., 2007; Rennenberg et al., 2006). However, at the species distribution margins, climatic and site constraints can also lead to adaptive responses that may enhance populations' tolerance to drier environmental conditions (Fotelli et al., 2009; Jump et al., 2006a; Peñuelas et al., 2008; Peuke et al., 2002). It was shown that beech presented high heat stress tolerance and metabolic plasticity during the heat-wave in the summer of 2003, compared to other Atlantic tree species in south-western Europe (Garcia-Plazaola et al., 2008). But further investigations of beech behavior at its southern European margins are needed to improve our knowledge of tree species potential responses to climate change.

Because of strong regional climatic constraints, beech reaches the rear edge of its lowland European distribution in the southwest of France. In Spain, the species appears only at higher elevations, as the climatic constraints decrease (Jump et al., 2007). Badeau et al. (2010) modelled the potential reduction of this species over the next few decades in this south-west border zone according to future climate scenarios. This disappearance should imply a decline in present populations or a regeneration failure, and qualifies the border zone as a hotspot for change in beech distribution. We hypothesized that signs of decline or establishment failure already exist in the south-western lowlands of France. Consequently, we focused on this zone to study beech responses to climatic limitations.

We considered natural regeneration (i.e. seedling establishment) as a crucial stage in the regeneration of populations located in mature beech stands in the south-western European plains, although it is necessary to consider several demographic indicators in population persistence studies, as the results of Barbeta et al. (2011) underlined. Regeneration of beech has mostly been studied in Central and Western Europe, at the local scale, and authors have mainly focused on the effects of light on seedling behavior.

Above all, drought affects physiological performance (Galle and Feller, 2007), growth and nitrogen uptake (Fotelli et al., 2002, 2001), competitive ability (Fotelli et al., 2005, 2002; Rust and Savill, 2000) and as a result, density (Madsen and Hahn, 2008) of beech seedlings. Besides, light intensity decreases with the degree of canopy closure and is a crucial factor for seedling development, despite the shade-tolerant nature of beech. The effects of gaps in forest cover have been investigated through experimental or natural gap measurements in Southern and Central Europe. Canopy opening changed growth patterns and beech establishment success (Caquet et al., 2009; Collet et al., 2001; Gansert and Sprick, 1998; Modrý et al., 2004; Topoliantz and Ponge, 2000). However, the effects of light are complex as intense sunlight could play a negative role (Emborg, 1998; Peltier et al., 1997; Robson et al., 2009; Tognetti et al., 1998) and water supply could interact with light effect (Petritan et al., 2007). In their recent work, Caquet et al. (2009) and Madsen and Hahn (2008) argued that existing regeneration was a key factor in the success of beech establishment when light intensity increased after a canopy opening. But spatial pattern of stand regeneration along wide and extreme environmental gradients such as the ones present in the southern margins was poorly -if any- investigated.

Seedling density may be related to fruit production and depend on germination success. Few studies have been conducted to determine the relationship between beechnut density and seedling abundance in beech stands. Topoliantz and Ponge (2000) observed that maximum seedling abundance did not occur concomitantly with the highest beechnut abundance. The authors argued that seed production was positively influenced by environmental conditions which tended to decrease seedling survival. Innes (1994) also showed that beechnut production was correlated with crown transparency and dieback.

The objective of the present study was to determine how abiotic environment may control spatial patterns of European beech natural regeneration in mature stands at its south-western, dry limit. We considered seedling density as an indicator of population regeneration and tested the relationship between seedling and cupule densities along a lowlands bioclimatic gradient.

# 2. Materials and methods

# 2.1. Sites

This study was conducted in Frenchsouth-western forests (elevation  $\leq$  350 m) where lowlands European beech reaches its distribution limit. This area presents widely varying climatic conditions (sub-Mediterranean, Atlantic and sub-continental climates) and soil characteristics. French forests were investigated based on information provided by foresters' network and scientists whose research focuses on beech. Beech stands were selected according to a sampling design structured on gradients of precipitation deficit and annual temperature which are closely related to the frequency of beech according to Badeau et al. (2010). In each selected stand, mast beech trees were present in the overstory and accounted for at least 40% of the total basal area. Within-site conditions were homogeneous. We only selected stands with limited impacts of human activities and low presence of roe deer and wild boar tracks. The exact position of plots was randomly selected among the total area of the stand, if the previous criteria were satisfied. No minimum distance between stands was required if low distances were justified by differences in stand or site characteristics. In our sample, the minimum distance between two stands was about 20 m and the average 296 km. The extreme latitudes were 43°04'N and 48°28'N, and the extreme longitudes 2°53'E and 3°33'W. Fig. 1 shows the location of collected plots among beech distribution in France. The plots covered large ranges of values for soil water capacity (20-225 mm) and soil pH (3.7-7.9) and the main humus forms were represented.

## 2.2. Data collection

We estimated cupule density to represent fructification success, as it could influence the seedling density. However, some fruits contain empty seeds and, as a result, we probably overestimated fructification success in some sites. At each site, ecological conditions, stand characteristics, and densities of seedlings and cupules were described in plots with three different surface areas: one



**Fig. 1.** Map of sampled plots (elevation  $\leq 350$  m; *N* = 85) over beech distribution in France (source: French National Forest Inventory).

 $400\text{-m}^2$  circular plot included in one  $700\text{-m}^2$  circular plot, both with the same center point, and five circular subplots of 2 m<sup>2</sup> (0.8 m radius) included in the  $700\text{-m}^2$  plot, placed at a distance of 11.3 m from the center, and systematically distributed (Fig. 2). Data were collected throughout the growing season, from June to September in 2007 and 2008. Cupule density was estimated only during 2007 and corresponded to an intermediate mast year in 2006. Some cupules remaining from 2005 may have also been counted; however, we assumed that this overestimation was moderate because fruiting was low in 2005 compared to 2006, and because cupules usually degrade completely by the following year except in the most acidic soils (where they may last for up to two years due to a lower degradation rate).

In the 700-m<sup>2</sup> plots, we described dendrometric characteristics of the trees and the stand (diameter at breast high, tree species basal area, number of individuals per tree species, dominant beech age, dominant tree and beech heights). We established a beech decline index based on defoliation rate and branch mortality. The decline index was a score ranging from 0 to 4 given to individual dominant or co-dominant beech trees (0: no symptom observed; 1: slight defoliation and/or fine branches dead on the periphery of the crown; 2: more than 50% of dead fine branches, presence of dead branches in the upper half of the crown; 3: more than 50% of dead branches in the crown: 4: dead or moribund tree). Eight variables were derived from this index: the proportion of beech basal area for each of the five classes to the total beech basal area in the stand, the same proportion for beech trees with a decline index value higher than or equal to 2, higher than or equal to 1, and the mean of the individual values noted for all the beech trees in each stand. In the same plot area, topography was described (slope, aspect, topographic position and water supply based on topographic position).

In the 400-m<sup>2</sup> plots, we described the vegetation composition and estimated the vegetation cover in each height layer (from canopy to herbaceous layers at  $\ge 7.0$ ,  $\ge 2.0$ ,  $\ge 0.3$ , and >0 m). Soil was physically described (depth, texture, gravel content, depth of CaCO<sub>3</sub> detection and soil waterlogging) in an excavated soil pit at the center of the plot. Soil water storage capacity was estimated based on root exploration depth and texture coefficient (Baize and Jabiol, 1995). Chemical analyses were performed on one soil sample per site taken at a depth of between 10 and 20 cm (pH, mineral and organic matter contents). Humus type, cover and thickness of soil organic layers (fibric, mesic and humic organic



Fig. 2. Design of sampled plots and subplots. Details of measurements conducted in the different plots.

horizons) were determined. From the vegetation composition of the herbaceous layer, we calculated the mean Ellenberg and Landolt indicator values for light, continentality, temperature, moisture, soil reaction and nitrogen in each plot (Ellenberg et al., 1992; Landolt, 1977).

We determined cupule density in the five subplots at 71 sites. In order to achieve satisfactory accuracy of estimated density, we used progressive areas depending on the number of cupules found. The area in the subplot was  $0.25 \text{ m}^2$  when the number of fruits was higher than 50. This area was extended to 0.50 m<sup>2</sup> when cupule number was between 10 and 50, and to 1 m<sup>2</sup> for lower values. Beech seedling density was estimated over 2 m<sup>2</sup> for individuals smaller than or equal to 0.30 m in height in 85 sites (the same previous 71 sites plus 14 new sites). We included 1-year-old seedlings. The densities were expressed in number of cupules or seedlings per square meter. We identified beech trees that had reached reproductive maturity based on the relationship between age and stem circumference, using the French National Forest Inventory dataset in the studied zone. Beech reached its reproductive maturity between 40 and 60 years old (Thiébaut and Vernet, 1981). The circumference threshold that was relevant to identify a mast beech tree with high confidence was 60 cm. The distance from the subplot center of the nearest mast beech tree was calculated. The cover percentages for beech and for total vegetation as well as the number of beech individuals whose crown projected on the subplot were visually determined in each of the previously defined height layer per subplot.

In this work, we tested relationships between local regeneration of beech and climate at three spatial scales. Mesoclimate was defined as the regional climate, i.e. at the scale of several km<sup>2</sup>. Regional climate values was provided by meteorological stations distant from several kilometers and having topographical and altitudinal characteristics different from the plots. To improve spatial representativeness and variability of these data, we used climatic data from the AURELHY Meteo France model, averaged over thirty years of measurements (1961–1991) and adjusted to topography. Monthly precipitation, minimum and maximum temperatures. monthly number of frost days and annual number of days below 5 and 10 °C were interpolated by kriging to a  $1 \times 1$  km resolution (Benichou and Le Breton, 1987). Monthly and annual mean temperatures, annual precipitation, precipitation continentality (Angot, 1883) and thermal amplitude were calculated from AURE-LHY climatic data. We used monthly radiation delivered by SAT-MOS between 1996 and 2002 with a  $3 \times 3$  km resolution to calculate Turc potential evapotranspiration (Turc, 1951). Monthly and seasonal precipitation deficits were estimated as the difference between potential evapotranspiration and precipitation. Microclimate was defined at the forest stand scale, changing according to topographic variations. These variations were appreciated from quantitative and qualitative variables: slope, aspect, topographic position and water supply based on topographic position. Pedoclimate concerned the combination of the regional climate variables and soil characteristics. We estimated the monthly soil water content by subtracting the monthly precipitation deficit value from the soil water content of the previous month. Monthly soil water deficits and excesses were respectively defined as the precipitation deficit after the soil water content reached the null value, and as the amount of precipitation after the soil water content reached the soil water storage capacity. Table 1 summarizes the collected or calculated variables used in this study.

The spatial resolution of such data  $(1 \times 1 \text{ km} \text{ and } 3 \times 3 \text{ km},$  respectively) did not correspond to the area where seedling density was estimated (700 m<sup>2</sup>). However, the previous cited points concerning the spatial variability and representativeness, the description of micro- and pedo-climate and the reliability of such climate grid data supported their use. Moreover, we observed high Pearson

# Table 1

Description of climatic, stand and soil variables available in the 85 sampled beechwoods in the south-western French lowlands. Climatic factors include mesoclimate derived from the AURELHY model and SATMOS data, microclimate and pedoclimate.

Factor	Variable	Description, units
Climate (meso-, micro- and	Monthly precipitation	$\ln mm \cdot m^{-2}$
pedo-climate)	Monthly minimum temperature	In °C
1	Monthly maximum temperature	In °c
	Monthly mean temperature	In °c
	Monthly number of frost days	
	Annual precipitation	$\ln mm \cdot m^{-2}$
	Annual mean temperature	In °C
	Annual number of days below 5 °C	
	Annual number of days below 10 °C	_
	Precipitation continentality	Ratio of may to october precipitations to november to april precipitations
	Thermal amplitude	Ratio of mean annual minimum to mean annual maximum temperatures
	Monthly radiation	In i.m <sup>-2</sup>
	Monthly Turc potential evapotranspiration	In mm
	Monthly and seasonal precipitation deficit	Monthly and seasonal Turc potential evapotranspiration – precipitation, in mm
	Monthly and seasonal soil water content	Water content of the month <sub><i>m</i>-1</sub> – precipitation deficit of the month <i><sub>m</sub></i> , in mm
	Monthly and seasonal soil water deficit	Precipitation deficit after the soil water content reached 0, in mm
	Monthly and seasonal soil water excess	Amount of precipitation after the soil water content reached the soil water storage
		capacity, in mm
	Index of growing season length	Sum of standardized mean temperatures of May and September
	Index of precipitation deficit length	Sum of standardized precipitation deficits of May and September
	Index of soil water deficit length	Sum of standardized soil water deficits of June and September
	Index of soil water excess length	Sum of standardized precipitation deficits of December and March
	Slope	In °
	Aspect gradient	Quantitative gradient: from south to north : 0.5 $\times$ cosines ( $\pi \times$ aspect/200) + 0.5
	Effects on water balance	4 classes (from decreasing to increasing water supply)
	Mean Ellenberg indicator values for	Only where specific richness $\ge 5$ in the plot
	continentality	
	Mean Ellenberg indicator values for	Only where specific richness $\ge 5$ in the plot
	temperature	
	Mean Ellenberg indicator values for moisture	Only where specific richness $\ge 5$ in the plot
Soil	Soil water storage capacity	$\ln mm \cdot m^{-2}$
	Soil depth	In cm
	Soil organic matter concentration	In g·kg <sup>-1</sup>
	Humus type	Gradient of humus activity: from mor to eumull (8 classes)
	Soil waterlogging characteristics	$-1 \times$ depth of waterlogging appearance
	Gravel content	Percentage of volume
	lexture	Sand, slit and clay, in g-kg
	pH Fusher mobile action concentrations	Water and KCI pn Dhambarua anlainm mannaainm andium nataasium iran mannaaa aluminum
	Excitatigeable cation concentrations	Phosphorus, calcium, magnesium, sourum, potassium, non, mangamese, arummum, in mol $ka^{-1}$
	Organic carbon concentration	In more $\alpha^{-1}$
	Total nitrogen concentration	In g.kg <sup>-1</sup>
	Carbon nitrogen ratio	Carbon/nitrogen
	Proton concentrations	In mol·kg <sup>-1</sup>
	Cation exchange canacity	In mol·kg <sup>-1</sup>
	Cation saturation	In nercent
	Residual soil water content	In $\sigma k \sigma^{-1}$
	Depth of soil reaction	In cm
	Mean Ellenberg indicator values for soil	Only where specific richness $\geq 5$ in the plot
	reaction	······································
	Mean Ellenberg indicator values for nitrogen	Only where specific richness $\geq 5$ in the plot
Stand	Tree basal area	For trees with a diameter $\ge 7.5$ cm, in m <sup>-2</sup> ha <sup>-1</sup>
	Proportion of beech basal area in the stand	-
	Dominant trees basal area	$\ln m^{-2} \cdot ha^{-1}$
	Proportion of dominant beech basal area in the	-
	stand	
	Stand density	In individuals per ha
	Number of individuals per tree species	-
	Proportion of beech individuals in the stand	-
	Number of dominant trees per tree species	-
	Proportion of dominant beech trees in the stand	-
	Mean diameter by tree species	At 1.30 m in height, in m
	Mean diameter of dominant beeches	At 1.30 m in height, in m
	Mean total height of dominant beeches	In m
	Mean diameter of dominant trees in the stand	For the 6 largest trees on the 700 $m^2$ plot, in m
	Mean total height of dominant trees in the	For the 6 largest trees on the 700 $m^2$ plot, in m
	stand	
	Age of dominant beech trees	For dominant beech trees, in years
	vegetation cover in each height layer	Proportion of area covered by the vertical projection of crowns in the 700 m <sup>2</sup> plot
	Variation cover in submission in the late	died
	vegetation cover in subplots in each height	iviean proportion of area covered by the vertical projection of crowns in total subplot
	layci Baach tree cover	area Droportion of area covered by the vertical projection of beach tree crowns in the
		rioportion of area covered by the vertical projection of beech the crowits in the

Table 1 (continued)

Factor	Variable	Description, units
	Beech cover on subplots in each height layer	700 $\mathrm{m}^2$ plot area Mean proportion of area covered by the vertical projection of beech crowns in the total subplot area
	Number of beech individuals on subplots in each height layer	-
	Distance between the nearest mast beech tree and the subplot center	Mean of the five subplots, at 0.30 m height, in m
	Diameter of the nearest mast beech tree from the subplot	Mean of the five subplots, at 1.30 m height, in cm
	Beech tree decline index	DEPEFEU method (Forest Health Department) Nageleisen (1996). Based on defoliation, dead branches and tree mortality.
	Relative beech fertility index	Deviation of beech height from the mean dominant beech height in the 85 plots at and for a given age and forestry practice
	Stand management	High forest, coppice-with standards, coppice, conversion into high forest
	Disturbance cover	Total projected area of natural or human disturbances in the plot
	Light index	Mean cumulative cover of tree, high shrub and low shrub layers in the subplots, in percent
	Cupule density	In number. M <sup>-2</sup>
	Seedling density	In number. M <sup>-2</sup>
	Mean Ellenberg indicator values for light	Only where specific richness $\ge 5$ in the plot

correlation values ( $\geq 0.8$ ) between spatially associated SATMOS and Météo-France meteorological station data for monthly radiations (Supplementary material Appendix S1).

The climatic gradient along our study area was quantified and was stable over the last decades. We found high correlation values between the 1961–1990 period and the decade preceding density measurements (1997–2006) for monthly precipitation and monthly mean, maximal and minimal temperatures variables (Supplementary material Appendix S2), wherever the data sources that we used: Météo-France meteorological station data (Météo-France, 2011) and Climate Research Unit TS 1.2 data (CRU, 2011; 10' grid). The seedling densities estimated in 2007 and 2008 resulted from the survival response of the natural regeneration and the germination stage, and thus, were influenced by the previous years' climate and in particular climatic stress.

### 2.3. Data analysis

Multiple linear regression analysis was used to identify ecological variables related to seedling density. Because of a high level of collinearity among the variables tested in our study, a reduction in their number was necessary. Multivariate dimension reduction techniques have been widely employed in ecology (Aguilera et al., 2006). We used a principal component regression analysis (PCR) (Kendall, 1957), which applies a principal component analysis (PCA) to the data matrix and uses the factorial axes as explanatory variables in a multiple regression analysis. In a first step, we performed a PCA for each of the three types of ecological factors: meso-, micro- and pedo-climate, soil and stand factors. Only the first factorial axes representing at least 95% of the total variance were retained in the analysis. In a second step, we conducted a multiple regression analysis based on a stepwise selection procedure of these factorial axes, to identify those significantly explaining the density. The Akaike Information Criterion (Akaike, 1974) was used as a fit criterion to select the best model. The normality of residuals was assessed by the Shapiro-Wilk test (Shapiro, 1965). We verified that the significant factorial axes between the three PCA were not highly correlated (Pearson correlation values). In order to interpret the results of the regression analysis of the density, we then determined the main variables contributing to the significant axes. To assess the importance of a given axis or factor, we removed this axis or factor from the regression model and calculated the decrease in *R*-squared.

In order to verify that there was no spatial trend for the residuals of the density model, we modelled these residuals using latitude, longitude and the combination of the two variables in a linear regression model. We also assessed spatial autocorrelation of the density and of the model residuals using the Moran's index (Moran, 1950).

In the multiple linear regression relating beech seedling density to ecological variables, we tested cupule density as a covariate. We applied natural logarithm transformations to the seedling density to normalize the residuals. All computations and statistical analyses were carried out using SAS/STAT<sup>®</sup> software (9.2 version).

# 3. Results

Summary statistics of cupule and seedling densities and of the main sites and stand characteristics are given Table 2. Fig. 3 presents the spatial distribution of cupule and seedling densities in the sample. The results of the three PCA are shown in Table 3. For climate, soil and stand factors, respectively 16, 15 and 17 factorial axes were retained (95% of the total variance).

The significant factorial axes (p < 0.05) identified in the multiple regression analysis of the logarithm of seedling density are presented in Table 4. Seven axes were entered by the stepwise procedure and the *R*-squared value reached 0.33. The residuals normality was verified by the Shapiro-Wilk test. No spatial trend and spatial autocorrelation were observed for the seedling density and the residuals of the model, as no geographical variables were related to the model residuals, and as the Moran's index value indicated a random spatial distribution. Cupule density was not significant in the model. All types of factors were contained in the model. Decrease in *R*-squared after removing the explanatory axes of a given factor from the model equalled 0.19 for the climate factor, 0.10 for the soil factor, and 0.08 for the climate factor. The variables with the highest contribution to the eigenvalue of each significant axis are summarized in Table 5.

The main climatic conditions increasing seedling density were high precipitation, soil moisture, temperature and evapotranspiration, and low water deficit and frost, during the growing season. Both excess of water in the soil and low water supply tended to decrease the amount of seedlings. We observed opposite effects for some of these variables, mainly in climatic axis 6, the least explanatory of all the axes.

The soil variables which influenced seedling density were soil mineral element content (mainly negative effect of iron and positive effects of aluminum), sand soil content and biological activity (positive effects).

#### Table 2

Statistical summary of cupule and seedling densities and of the main sites and stand characteristics in the sampled beechwoods in the south-west of France.

Factor	Measured variable (units)	Mean	Median	Min	Max	Standard deviation
General site	Latitude	45°49′N	-	43°04′N	48°28′N	-
characteristics	Longitude	0°20′W	-	2°53′E	3°33′W	-
	Slope (in °)	8.8	3.8	0	34.0	10.3
Regeneration	Cupule density (n b $m^{-2}$ ), in 71 sites	306.4	235.6	38.2	867.2	232.2
	Seedling density (n b $m^{-2}$ ), in 85 sites	0.90	0.35	0	14.7	1.8
Stand	Dominant tree total height (in m)	26.4	26.1	17.2	41.2	5.6
	Dominant beech total height (m)	27	26	18	44	6
	Dominant tree diameter (m)	0.40	0.39	0.23	0.69	0.12
	Dominant beech diameter (m)	0.46	0.45	0.23	0.80	0.13
	Canopy cover (percent)	82	85	15	95	14
	Average age of dominant beech trees (years)	102	97	41	200	36
	Stand density (individuals per ha)	507	393	114	1714	336
	Tree basal area $(m^{-2} \cdot h a^{-1})$	28.9	27.9	14.9	52.0	7.9
	Beech basal area (m <sup>-2</sup> ·h a <sup>-1</sup> )	19.7	18.1	7.3	44.1	8.8
	Proportion of beech basal area in the stand (percent)	68	66	23	100	22
	Proportion of mast tree basal area (percent)	62	60	13	100	23
	Distance between the nearest mast beech tree and the subplot center (m)	4.37	4.36	0.57	8.25	1.51
	Proportion of defoliated or declining beech basal area (percent)	25	15	0	100	29
	Light index (percent)	114	110	27	200	31
	Species richness (number of species)	20	17	7	43	10
Soil	Soil water storage capacity (m m·m <sup>-2</sup> )	122.5	132.0	20.2	224.9	58.9
	Soil ph	5.0	4.7	3.7	7.9	1.2
	Sand soil content $(g \cdot kg^{-1})$	356	294	26	982	246
	Silt soil content $(g \cdot kg^{-1})$	381	414	4	756	180
	Clay soil content $(g \cdot kg^{-1})$	263	226	14	893	179
	Humus type	Dysmull	Dysmull	Mor	Eumull	Two classes
	Carbon/nitrogen ratio	18.9	18.4	12.6	32.1	3.8
Climate	Annual precipitations (mm)	886.6	870.2	665.1	1459.5	150.9
	Mean annual temperature (°C)	11.6	11.6	10.0	13.1	0.8
	Summer soil water deficit (mm)	182.7	185.5	39.4	243.3	32.9



Fig. 3. Distribution of the seedling density of beech. South-western lowlands of France (elevation  $\leqslant\!350$  m). In number of seedling per m², N = 85.

For stand factors, only one axis (axis 4) significantly explained seedling density variations, though the decrease in *R*-squared after removing it was about 8%. A low proportion of healthy beech in the

Table 3

Variance in the correlation matrix for the three principal components analyses. The factorial axes retained correspond to at least 95% of the total variance.

	Factorial axis	Cumulated variance (%)		
_		Meso-, micro- and pedo-climate	Soil	Stand
	1	33.7	35.9	29.7
	2	57.8	52.5	44.1
	3	71.4	64.6	54.6
	4	79.1	70.5	62.6
	5	82.2	76.0	68.5
	6	84.6	79.7	73.1
	7	86.8	83.0	77.0
	8	88.3	86.1	80.3
	9	89.6	88.2	82.8
	10	90.7	90.1	85.1
	11	91.7	91.7	87.3
	12	92.6	92.9	89.1
	13	93.4	93.9	90.6
	14	94.2	94.9	92.1
	15	94.8	95.8	93.2
	16	95.4	-	94.2
	17	_	-	95.2

stand (basal area), canopy closure and low tree and beech densities decreased the seedling amount.

Cupule density was positively correlated to a slight beech decline (Pearson correlation reached 0.28, p < 0.05).

## 4. Discussion

Our approach, based on a reduction in the set of variables and on a multiple regression analysis, allowed us to identify the main ecological effects related to the spatial pattern of natural regeneration in beech stands, over wide drought and temperature gradients.

#### Table 4

Multiple regression of the logarithm for seedling density based on factorial axes (climatic, soil and stand principal components analyses) and using a stepwise selection procedure. Decrease in R-squared ( $R^2$ ) when removing each factorial axis from the regression model. All explanatory variables have one degree of freedom. N = 85 sites.

Factorial	Stepwise selection procedure			Decrease	Parameter	Standard	<i>t</i> -test	P-value	Rank in		
axis	Step	Partial R <sup>2</sup>	Cumulative R <sup>2</sup>	F-value	P-value	in R <sup>2</sup>	estimates	deviation			the model
Intercept	-	-	-	-	-	-	0.466	0.046	10.2	<.0001	-
Climate 5	1	0.074	0.07	6.64	0.0117	0.06	-0.063	0.024	-2.64	0.0102	2
Soil 15	2	0.062	0.14	5.88	0.0175	0.06	-0.221	0.087	-2.54	0.0130	3
Stand 4	3	0.044	0.18	4.35	0.0401	0.07	-0.070	0.024	-2.97	0.0039	1
Climate 10	4	0.040	0.22	4.15	0.0451	0.05	0.094	0.039	2.40	0.0186	4
Climate 14	5	0.036	0.26	3.78	0.0555	0.04	-0.105	0.047	-2.25	0.0276	5
Soil 4	6	0.033	0.29	3.64	0.0599	0.04	0.077	0.034	2.24	0.0280	6
Climate 6	7	0.039	0.33	4.46	0.0379	0.04	0.055	0.026	2.11	0.0379	7

#### Table 5

Variables of the principal components analyses with the highest contribution to the eigenvalue of each (a) stand, (b) soil and (c) climate factorial axis significant in the seedling density model. The axes are listed according to the decrease in *R*-squared ( $R^2$ ) when removing them from the regression model. Only variables with an absolute eigenvalue higher than half of the highest absolute eigenvalue for each axis are presented.

Factor	Axis	Variable	Eigenvalue
(a)			
Stand	4	Basal area proportion of defoliated or	0.35
		declining beech	
		Canopy cover	0.29
		Light	0.26
		Stand density	-0.21
(b)			
Soil	15	Iron soil content	0.46
		Phosphorus soil content	0.37
		Potassium soil content	0.31
		Aluminum soil content	-0.33
		Biological activity (humus type)	-0.27
	4	Phosphorus	0.47
		Silt soil content	-0.38
		Manganese soil contents	0.35
		Sand soil content	0.28
		Potassium soil content	0.24
(c)			
Climate	5	Late spring and early autumn frosts	0.28
		Precipitation continentality	-0.22
		Minimum temperatures during the	-0.20
		growing season	
		Precipitation deficit during summer	-0.16
	10	Southern aspect	-0.53
		Late spring frosts	0.43
	14	Waterlogging	0.43
		Water supply (according to	-0.28
		topography)	
	6	Moisture (Ellenberg indicator values)	-0.29
		Waterlogging	0.27
		Mean temperature	0.26
		Spring temperatures	-0.24
		Evapotranspiration during the	0.18
		growing season	
		Late spring frosts	0.15

4.1. Natural establishment of beech in mature stands at its southwestern European margin

Although climatic factors showed the most influence on seedling density, we observed significant effects of soil and stand characteristics.

Temperature contrasts between the growing season and the rest of the year increased and water excess decreased seedling amount. Precipitation continentality, elevated annual mean temperature and minimum temperature during the growing season (i.e. thermic continentality) increased seedling density, as well as northern aspect. Late spring and early autumn frosts affected the number of seedlings found. Dittmar et al. (2006) already considered late frosts to be important ecological events that strongly affect vitality and competitiveness of beech. The abiotic characteristics related to an increase in seedling density were consistent with the water and temperature needs of beech, as a continental-climate species sensitive to water excess (Le Tacon, 1981) and late spring or early autumn frosts. Continentality provides optimum conditions for seedling establishment and growth in two ways. Firstly, higher moisture during summer tends to increase seedling growth and decrease mortality (Madsen and Larsen, 1997; Petritan et al., 2007) whereas high moisture in winter, as in the case of Atlantic forests, improves pathogens like Rhizoctonia solani for seeds (Perrin and Muller, 1979) and Phytophtora for seedlings (Savoie et al., 1988). Secondly, winter chilling could improve germination quality by the induction of seed dormancy, the conservation of germinative capacity during winter, and the renewal of bud growth and shoot elongation (Falusi and Calamassi, 1990).

Thus, elevated mean temperature and low drought during the growing season, as well as winter severity in such continental climates, might improve seedling success in seed conservation, germination and seedling growth or survival.

The negative effect of humus types rich in organic matter on seedling density could be explained by the thicker organic layers which create a physical barrier to rooting, as has been suggested by Le Tacon (1981). Efficient rooting could be more crucial at the southern limits, as young seedlings would tend to favor root growth rather than shoot growth in drier conditions.

Consistently with many other studies (Caquet et al., 2009; Collet et al., 2001; Gansert and Sprick, 1998; Modrý et al., 2004; Topoliantz and Ponge, 2000), we showed that light level, estimated by canopy cover and related to southern aspect, increased seedling density. We did not observe any negative effects of high light exposure, as did Emborg (1998), Peltier et al. (1997), and Tognetti et al. (1998), probably because we studied regeneration in mature beech stands only.

Aluminum soil content was related to sand soil content, which both increased in acidic soil. Such soil prevents from water excess, which explained the positive relationship with seedling density.

The relation between fruit production and seedling density was not significant in our study. The estimated cupule density depended on fruit production during 2006 and 2005, while the measured seedling density concerned the seedling establishment and persistence for a longer period of time. This could be the main argument explaining the absence of correlation between the two densities. However, too many potential filters exist at each stage from fruit to established seedling: pollination, on which seed quality depends; climatic and edaphic conditions; pathogens and predators, among others. First of all, the variability of empty seed rates, mainly explained by self-fertilization in beech (Wang, 2003), would reduce the pertinence of the relation between the fruit and seedling densities. Then, different rates of degradation could cause varying amounts of beechnuts and cupules. Furthermore, climatic conditions influence germination and seedling survival and humus or soil types potentially influence rooting. These factors are also related to the appearance of seed or seedling pathogens but affect them in different ways. Moreover, the effect of predators is surely higher for seeds than for seedlings, which for beech are seldom browsed, but does not affect cupule abundance. Deer could prevent the establishment of young seedlings (Kenderes et al., 2008) and significantly reduce dense beech regeneration (Olesen and Madsen, 2008), but Modrý et al. (2004) observed that beech was the least impacted by deer herbivory among nine forest tree species studied, so that beech seedling abundance might increase at the expense of more browsed species. However, even if climate controls both the inter-annual variations in mast-seeding (Lindquist, 1931; Piovesan and Adams, 2001) and in dormancy release (Falusi and Calamassi, 1990) in beech, optimum or limiting climatic values could differ between these two stages. Finally, competitive interactions between beech and other species have already been mentioned as key factors in the success of Fagus sylvatica establishment (Fotelli et al., 2002, 2001).

#### 4.2. Regeneration strategy in a constrained environment

Fruit density was positively correlated to a slight decline in beech crown, while seedling density was negatively correlated (variable with the highest contribution to the eigenvalue of stand axis 4). If we consider tree crown decline to be an indicator of ecological constraints, our results were consistent with those found in other studies, where seed production increased (Innes, 1994; Topoliantz and Ponge, 2000) and seedling survival decreased (Topoliantz and Ponge, 2000) under limiting environmental conditions. Water constraints and temperature are the two climatic components spatially affecting regeneration density and tree crown in beech (E Silva, 2010). Genet (2009) showed that stand decline of the species led to a decrease of carbon allocation to radial growth, to the benefit of storage and reproduction functions. These elements suggest that low germination or seedling establishment might be partially offset by a higher seed production.

# 4.3. The future of beech in South-West France

Our results showed that natural regeneration is a key stage for beech success in its south-western margin, where the future of the species is jeopardised in the context of climate change. Climatic characteristics that explained low regeneration density were partly those explaining low frequency of beech presence in this region. In the context of climate change, conditions for beech regeneration are supposed to worsen in this zone, which emphasizes the risk for the future of beech. We showed that spatial variations of seedling density were mainly related to climate, but stand characteristics, that management practices can modify, also influenced seedling establishment. In the most problematic zones for natural regeneration in beech (i.e. the most oceanic ones), favouring an increase in dominance of mast beech trees in the stand by decreasing the canopy competition for light should improve the regeneration capacity. However, an increase in light level generally leads to higher water losses and temperatures in the stand, which could affect seedling establishment. So, further experiments on the optimal equilibrium between light level and microclimate characteristics should be conducted. Adapting management practices in order to improve beech natural regeneration is therefore possible and may be necessary in the context of climate change.

### 5. Conclusion

The number of sites, their distribution and the varied topo-edaphical conditions allowed us to study the determinism of beech regeneration in mature stands along a wide gradient at its southwestern limit. This directly concerns forestry practices related to the regeneration of beech populations with increasing temperatures and drought severity. According to our results, local factors played a significant explanatory role in seedling establishment or survival; this confirms the relevance of our spatial approach. We found that the spatial variation in regeneration density was mainly controlled by climate, involving mesoclimate, microclimate and pedoclimate, with a decrease in density at the edge of the distribution range for beech, and then, by local factors. As a result, beech decline corresponded to lower levels of regeneration, even if fruit production was slightly higher. This suggests that seedling establishment and survival are crucial to the success of beech at its southern margin, where the species' future is seriously at risk in the context of climate change. It is now of particular interest to focus on the interannual variations in beech regeneration along the same type of climatic gradient in order to determine if natural regeneration responds spatially to extreme climatic events, whose frequency is expected to increase over the upcoming decades.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foreco.2011.11.031.

#### References

- Aguilera, A.M., Escabias, M., Valderrama, M.J., 2006. Using principal components for estimating logistic regression with high-dimensional multicollinear data. Comput. Stat. Data Anal. 50, 1905–1924.
- Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control 19, 716–723.
- Angot, A., 1883. Étude sur les Vendanges en France. In: Mascart, E., Legrand, J.P. (Eds.), Fluctuations météorologiques. vendanges et activité solaire. La Météorologie. Annales du Bureau Central Météorologique de France, Paris, pp. 73–89 [in French].
- Attorre, F., Francesconi, F., Scarnati, L., De Sanctis, M., Alfò, M., Bruno, F., 2008. Predicting the effect of climate change on tree species abundance and distribution at a regional scale. IForest – Biogeosciences and Forestry 1, 132– 139.
- Badeau, V., Dupouey, J.L., Cluzeau, C., Drapier, J., Le Bas, C., 2010. Climate change and the biogeography of French tree species: first results and perspectives. In: Lousteau, D. (Ed.), Forest Carbon Cycle and Climate Change. Quae, Paris, pp. 231–252.
- Baize, D., Jabiol, B., 1995. Guide pour la description des sols. Paris, Inra, pp. 1–375 [in French].
- Barbeta, A., Peñuelas, J., Ogaya, R., Jump, A.S., 2011. Reduced tree health and seedling production in fragmented *Fagus sylvatica* forest patches in the Montseny Mountains (NE Spain). For. Ecol. Manage. 261, 2029–2037.
- Benichou, P., Le Breton, O., 1987. Prise en compte de la topographie pour la cartographie de champs pluviométriques statistiques : la méthode AURELHY. Agrométéorologie des régions de moyenne montagne. Colloq. Inra 39, 51–69 [in French].
- Benito Garzon, M., Sanchez De Dios, R., Sainz Ollero, H., 2008. Effects of climate change on the distribution of Iberian tree species. Appl. Veg. Sci. 11, 169–178.
- Berry, P.M., Dawson, T.P., Harrison, P.A., Pearson, R.G., 2002. Modelling potential impacts of climate change on the bioclimatic envelope of species in Britain and Ireland. Glob. Ecol. Biogeogr. 11, 453–462.
- Bontemps, J.D., Herve, J.C., Dhote, J.F., 2009. Long-term changes in forest productivity: a consistent assessment in even-aged stands. For. Sci. 55, 549– 564.
- Bréda, N., Huc, R., Granier, A., Dreyer, E., 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. Ann. For. Sci. 63, 625–644.

- Caquet, B., Montpied, P., Dreyer, E., Epron, D., Collet, C., 2009. Response to canopy opening does not act as a filter to *Fagus sylvatica* and *Acer* sp. Advance regeneration in a mixed temperate forest. Ann. For. Sci. 67, 105–116.
- Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogee, J., Allard, V., Aubinet, M., Buchmann, N., Bernhofer, C., Carrara, A., Chevallier, F., De Noblet, N., Friend, A.D., Friedlingstein, P., Grunwald, T., Heinesch, B., Keronen, P., Knohl, A., Krinner, G., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourcival, J.M., Papale, D., Pilegaard, K., Rambal, S., Seufert, G., Soussana, J.F., Sanz, M.J., Schulze, E.D., Vesala, T., Valentini, R., 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437, 529–533.
- Collet, C., Lanter, O., Pardos, M., 2001. Effects of canopy opening on height and diameter growth in naturally regenerated beech seedlings. Ann. For. Sci. 58, 127–134.
- CRU, 2011. <http://www.cru.uea.ac.uk/>.
- Dittmar, C., Zech, W., Elling, W., 2003. Growth variations of common beech (*Fagus sylvatica* L.) under different climatic and environmental conditions in European dendroecological study. For. Ecol. Manage 173, 63–78.
- Dittmar, C., Fricke, W., Elling, W., 2006. Impact of late frost events on radial growth of common beech (*Fagus sylvatica* L.) in southern Germany. Eur. J. For. Res. 125, 249–259.
- E Silva, D., 2010. Ecology of beech (*Fagus sylvatica* L.) at the southwestern margin of its distribution, Ph.D. thesis of November 2010. INRA-Nancy University, 196p. [In French with English abstract].
- Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W., Paulissen, D., 1992. Zeigerwerte von Pflanzen in Mitteleuropa. Scripta Geob. 18, 1–248 [in German].
- Emborg, J., 1998. Understorey light conditions and regeneration with respect to the structural dynamics of a near-natural temperate deciduous forest in Denmark. For. Ecol. Manage. 106, 83–95.
- Falusi, M., Calamassi, R., 1990. Bud dormancy in beech (*Fagus sylvatica* L.). Effect of chilling and photoperiod on dormancy release of beech seedlings. Tree Physiol. 6, 429–438.
- Fotelli, M.N., Gessler, A., Peuke, A.D., Rennenberg, H., 2001. Drought affects the competitive interactions between *Fagus sylvatica* seedlings and an early successional species, *Rubus fruticosus*: responses of growth, water status and delta C-13 composition. New Phytol. 151, 427–435.
- Fotelli, M.N., Rennenberg, H., Gessler, A., 2002. Effects of drought on the competitive interference of an early successional species (*Rubus fruticosus*) on *Fagus sylvatica* L. seedlings: N-15 uptake and partitioning, responses of amino acids and other N compounds. Plant Biol. 4, 311–320.
- Fotelli, M.N., Rudolph, P., Rennenberg, H., Gessler, A., 2005. Irradiance and temperature affect the competitive interference of blackberry on the physiology of European beech seedlings. New Phytol. 165, 453–462.
- Fotelli, M.N., Nahm, M., Radoglou, K., Rennenberg, H., Halyvopoulos, G., Matzarakis, A., 2009. Seasonal and interannual ecophysiological responses of beech (*Fagus sylvatica*) at its south-eastern distribution limit in Europe. For. Ecol. Manage. 257, 1157–1164.
- Galle, A., Feller, U., 2007. Changes of photosynthetic traits in beech saplings (*Fagus sylvatica*) under severe drought stress and during recovery. Physiol. Plantarum 131, 412–421.
- Gansert, D., Sprick, W., 1998. Storage and mobilization of nonstructural carbohydrates and biomass development of beech seedlings (*Fagus sylvatica* L) under different light regimes. Trees-Struct. Funct. 12, 247–257.
- Garcia-Plazaola, J.I., Esteban, R., Hormaetxe, K., Fernandez-Marin, B., Becerril, J.M., 2008. Photoprotective responses of Mediterranean and Atlantic trees to the extreme heat-wave of summer 2003 in Southwestern Europe. Trees-Struct. Funct. 22, 385–392.
- Genet, H., 2009. Effect of ageing and soil fertility on carbon allocation in sessile oak and common beech, two contrasted broad-leaved species: experimental and modeling approaches, Ph.D. thesis of March 2009. INRA-Nancy University, 237p.
- Granier, A., Reichstein, M., Bréda, N., Janssens, I.A., Falge, E., Ciais, P., Grünwald, T., Aubinet, M., Berbigier, P., Bernhofer, C., Buchmann, N., Facini, O., Grassi, G., Heinesch, B., Ilvesniemi, H., Keronen, P., Knohl, A., Köstner, B., Lagergren, F., Lindroth, A., Longdoz, B., Loustau, D., Mateus, J., Montagnani, L., Nys, C., Moors, E., Papale, D., Peiffer, M., Pilegaard, K., Pita, G., Pumpanen, J., Rambal, S., Rebmann, C., Rodrigues, A., Seufert, G., Tenhunen, J., Vesala, T., Wang, Q., 2007. Evidence for soil water control on carbon and water dynamics in European forests during the extremely dry year: 2003. Agr. For. Meteorol. 143, 123–145.
- Innes, J.L., 1994. The occurrence of flowering and fruiting on individual trees over 3 years and their effects on subsequent crown condition. Trees-Struct. Funct. 8, 139–150.
- IPCC, 2001. Climate Change 2001: impacts, adaptation and vulnerability. In: McCarthy, J.J., Canziani, O.F., Leary, N.A., Dokken, D.J., White, K.S. (Eds.), Contribution of Working Group II to the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press, Cambridge.
- Jump, A.S., Hunt, J.M., Martinez-Izquierdo, J.A., Peñuelas, J., 2006a. Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. Mol. Ecol. 15, 3469–3480.
- Jump, A.S., Hunt, J.M., Peñuelas, J., 2006b. Rapid climate change-related growth decline at the southern range edge of *Fagus sylvatica*. Glob. Chang. Biol. 12, 2163–2174.
- Jump, A.S., Hunt, J.M., Peñuelas, J., 2007. Climate relationships of growth and establishment across the altitudinal range of *Fagus sylvatica* in the Montseny Mountains, northeast Spain. Ecoscience 14, 507–518.
- Kendall, M.G., 1957. A Course in Multivariate Analysis. London, Griffin, pp. 1-183.

- Kenderes, K., Mihok, B., Standovar, T., 2008. Thirty years of gap dynamics in a central European beech forest reserve. Forestry 81, 111–123.
- Landolt, E., 1977. OÈ kologische Zeigerwerte zur Schweizer Flora. Veröffentlichungen des Geobotanischen Institutes der ETH, vol 64. Stiftung Rübel, Zürich [in German].
- Le Tacon, F., 1981. Caractérisation édaphique de la hêtraie. In: Teissier Du Cros, E. (Ed.), Le Hêtre. Les Editions de l'INRA, Paris, pp. 77–84 [in French].
- Lebourgeois, F., Bréda, N., Ulrich, E., Granier, A., 2005. Climate-tree-growth relationships of European beech (*Fagus sylvatica* L.) in the French permanent plot network (renecofor). Trees-Struct. Func. 19, 385–401.
- Lindquist, B., 1931. Den Skandinaviska bogskogens biologi. Sven. Skogsvardsforen. Tidskr. 29, 486–520 (In Danish).
- Luterbacher, J., Dietrich, D., Xoplaki, E., Grosjean, M., Wanner, H., 2004. European seasonal and annual temperature variability, trends, and extremes since 1500. Science 303, 1499–1503.
- Madsen, P., Hahn, K., 2008. Natural regeneration in a beech-dominated forest managed by close-to-nature principles - a gap cutting based experiment. Can. J. For. Res. 38, 1716–1729.
- Madsen, P., Larsen, J.B., 1997. Natural regeneration of beech (*Fagus sylvatica* L.) with respect to canopy density, soil moisture and soil carbon content. For. Ecol. Manage 97, 95–105.
- Météo-France, 2011. < http://climatheque.meteo.fr/okapi/accueil/okapiWebClim/ index.jsp> [in French].
- Modrý, M., Hubený, D., Rejsek, K., 2004. Differential response of naturally regenerated European shade tolerant tree species to soil type and light availability. For. Ecol. Manage. 188, 185–195.
- Moran, P.A.P., 1950. Notes on continuous stochastic phenomena. Biometrika 37, 17– 33.
- Nageleisen, L.M., 1996. Méthode d'évaluation de l'aspect du houppier (protocole depefeu). Département de la Santé des Forêts, Échelon Technique Nord-Est, Nancy [in French].
- Olesen, C.R., Madsen, P., 2008. The impact of roe deer (*Capreolus capreolus*), seedbed, light and seed fall on natural beech (*Fagus sylvatica*) regeneration. For. Ecol. Manage. 255, 3962–3972.
- Peltier, A., Touzet, M.C., Armengaud, C., Ponge, J.F., 1997. Establishment of Fagus sylvatica and Fraxinus excelsior in an old-growth beech forest. J. Veg. Sci. 8, 13– 20.
- Peñuelas, J., Boada, M., 2003. A global change-induced biome shift in the Montseny mountains (NE Spain). Glob. Chang. Biol. 9, 131–140.
- Peñuelas, J., Ogaya, R., Boada, M., Jump, A.S., 2007. Migration, invasion and decline: changes in recruitment and forest structure in a warming-linked shift of European beech forest in Catalonia (NE Spain). Ecography 30, 829– 837.
- Peñuelas, J., Hunt, J.M., Ogaya, R., Jump, A.S., 2008. Twentieth century changes of tree-ring delta C-13 at the southern range-edge of *Fagus sylvatica*: increasing water-use efficiency does not avoid the growth decline induced by warming at low altitudes. Glob. Chang. Biol. 14, 1076–1088.
- Perrin, R., Muller, C., 1979. La pourriture des faines causée par Rhizoctonia solani Kühn: incidence de cette maladie après les fainées de 1974 et 1976. Traitement curatif des faines en vue de la conservation. Eur. J. For. Pathol. 9, 89–103 [in French with English abstract].
- Petritan, A.M., Von Luepke, B., Petritan, I.C., 2007. Effects of shade on growth and mortality of maple (Acer pseudoplatanus), ash (Fraxinus excelsior) and beech (Fagus sylvatica) saplings. Forestry 80, 397–412.
- Peuke, A.D., Schraml, C., Hartung, W., Rennenberg, H., 2002. Identification of drought-sensitive beech ecotypes by physiological parameters. New Phytol. 154, 373–387.
- Piovesan, G., Adams, J.M., 2001. Masting behaviour in beech: linking reproduction and climatic variation. Can. J. Bot. 79, 1039–1047.
- Rennenberg, H., Loreto, F., Polle, A., Brilli, F., Fares, S., Beniwal, R.S., Gessler, A., 2006. Physiological responses of forest trees to heat and drought. Plant Biol. 8, 556– 571.
- Robson, T.M., Rodríguez-Calcerrada, J., Sánchez-Gómez, D.G., Aranda, I., 2009. Summer drought impedes beech seedling performance more in a sub-Mediterranean forest understory than in small gaps. Tree Physiol. 29, 249–259.
- Rust, S., Savill, P.S., 2000. The root systems of *Fraxinus excelsior* and *Fagus sylvatica* and their competitive relationships. Forestry 73, 499–508.
- Savoie, J.M., Comps, B., Letouzey, J., 1988. Bilan hydrique de hêtraies mixtes en relation avec le comportement et la régénération du hêtre (*Fagus sylvatica* L.). Acta Ecol. Ecol. Plant 9, 285–300 [in French].
- Schar, C., Vidale, P.L., Luthi, D., Frei, C., Haberli, C., Liniger, M.A., Appenzeller, C., 2004. The role of increasing temperature variability in European summer heatwaves. Nature 427, 332–336.
- Seynave, I., Gegout, J.C., Herve, J.C., Dhote, J.F., 2008. Is the spatial distribution of European beech (*Fagus sylvatica* L.) limited by its potential height growth? J. Biogeogr. 35, 1851–1862.
- Shapiro, S.S., 1965. An analysis of variance test for normality (complete samples). Biometrica 52, 591–611.
- Stott, P.A., Stone, D.A., Allen, M.R., 2004. Human contribution to the European heatwave of 2003. Nature 432, 610–614.
- Thiébaut, B., Vernet, P., 1981. Biologie de la reproduction sexuée. In: Teissier Du Cros, E. (Ed.), Le Hêtre. Les Editions de l'INRA, Paris, pp. 198–204 [in French].
- Tognetti, R., Minotta, G., Pinzauti, S., Michelozzi, M., Borghetti, M., 1998. Acclimation to changing light conditions of long-term shade-grown beech (*Fagus sylvatica* L.) seedlings of different geographic origins. Trees-Struct. Funct. 12, 326–333.

- Topoliantz, S., Ponge, J.F., 2000. Influence of site conditions on the survival of *Fagus sylvatica* seedlings in an old-growth beech forest. J. Veg. Sci. 11, 369–374. Turc, L., 1951. Nouvelle formule pour le calcul du bilan de l'eau en fonction des
- valeurs annuelles moyennes des précipitations et de la température. CR Acad. Sci. III-Vie 233, 633–635 [in French].
- Wang, K.S., 2003. Relationship between empty seed and genetic factors in European beech (*Fagus sylvatica* L.). Silva Fenn 37, 419–428.

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# Detecting the footprint of changing atmospheric nitrogen deposition loads on acid grasslands in the context of climate change

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# Abstract

Although atmospheric nitrogen (N) deposition and climate changes are both recognized as major components of global change, their interaction at ecosystem level is less well understood. A stratified resampling approach was used to investigate the potential impact of changing levels of atmospheric nitrogen deposition and climate change on species composition of nutrient-poor acid grasslands within the French Atlantic Domain (FAD). The study was based on a comparison, over a period of 25 years, of 162 past and present vegetation records assigned to the species-rich Nardus grasslands and distributed in regional community types (CTs). Similarly, the characterization of N deposition and climate was stratified according to (i) past (1980–1990) and present (1995–2005) periods, and (ii) FAD and CT scales. Despite the relatively short time span between sampling periods, significant N deposition and climate changes were detected as well as vegetation changes. Correspondence analysis showed that the relative importance of N deposition and climate in explaining vegetation changes depended on the spatial scale of investigation (FAD vs. local CTs) and the CT. At the FAD scale, the increase of annual mean temperature and decrease of water availability were clearly related to the changes in floristic composition. At the local scale, the most stable CT experienced no significant climate change and a stable load of N deposition, whereas the CTs characterized by the largest floristic changes were associated with dramatic climate changes and moderate loads in both oxidized and reduced N deposition. Despite the narrow gradient of deposition investigated, N deposition was related to significant grassland community changes, depending on the region, i.e. climate context, and on whether N deposition was in the oxidized or reduced form. Our results suggest that N deposition drives grassland composition at the local scale, in interaction with climate, whereas climate changes remain the predominant driver at the FAD scale.

*Keywords:* climate change, French Atlantic Domain, oxidized and reduced nitrogen deposition, resampling, species composition, species-rich *Nardus* grasslands

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# Introduction

Climate change and atmospheric nitrogen (N) deposition represent two components of contemporary global change which are both considered as important, but unequal, challenges to biodiversity issues (Sala *et al.*,

Correspondence: Didier Alard, UMR1202 BIOGECO, Université de Bordeaux, Bât B8 RdC, Avenue des Facultés, F-33405 Talence, France, tel. + 33 540008774, fax + 33 540003657, e-mail: d.alard@ecologie.u-bordeaux1.fr 2000; GBO-3, 2010). While scientists have mostly focused on climate change as the prime threat to biodiversity conservation (Hannah *et al.*, 2002), air pollution, an acknowledged widespread problem, is increasingly considered in nature planning or management (Lovett *et al.*, 2009) and nowadays recognized for its importance in conservation issues and ecosystem health (Sala *et al.*, 2000; Phoenix *et al.*, 2006; Bobbink *et al.*, 2010). Historic climatic warming is now well documented (Moberg *et al.*, 2005; Osborn & Briffa, 2006) and it is projected that the increase of the global mean surface

temperature will rise from 1.8 at present to 4.0°C over the next 100 years, mainly due to the CO<sub>2</sub> enrichment of the atmosphere by fossil fuel use (IPCC, 2007). Specific climate events, such as regional heatwaves, may also become more frequent and affect ecosystem properties and species physiology, distribution and phenology (Ciais et al., 2005; Peñuelas et al., 2007). However, the effects of climate change on ecosystems remain uncertain due to its spatial variability and the specificity of habitats responses. Similarly, the global N cycle is largely modified by human activities, from intensive agriculture and industrial activities, and has reached the point where more N is fixed annually by humandriven than by natural processes (Vitousek et al., 1997; Galloway, 1998). The atmospheric deposition of reactive N has increased in Europe from an estimated background rate of 1-5 kg N ha<sup>-1</sup> yr<sup>-1</sup> in the early 1900s to  $30-60 \text{ kg N} \text{ ha}^{-1} \text{ yr}^{-1}$  in the 1980s and the early 1990s in the worst-affected regions (NEGTAP, 2001). Although, in most north-western European countries, emissions of oxidized N declined after the 1990s as a result of more stringent legislation (Fagerli & Aas, 2008), current deposition rates are still an order of magnitude higher than in preindustrial time (Galloway et al., 2008). The continental anthropogenic N fixation rate is predicted to increase by about 60% by the year 2020 and expose more terrestrial ecosystems to greater rate of N deposition (Dentener et al., 2006). Although N deposition rates are in decline in some regions, the recovery of ecosystems from eutrophication can lag behind by decades because N has the potential to accumulate in ecosystems (Cunha et al., 2002). As a consequence, the effects of increased atmospheric N deposition will remain a significant problem for many seminatural habitats in the future and recovery may often not be possible without active management and restoration measures (Wamelink et al., 2009).

Enhanced N levels represent a particular threat to temperate seminatural or unimproved vegetation types as most temperate terrestrial systems are N limited (Vitousek & Howarth, 1991). Oligo- and mesotrophic ecosystems are especially at risk because many characteristic species are adapted to nutrient-poor conditions and are outcompeted by species with higher N demands (Wedin & Tilman, 1993; Hautier et al., 2009). In Europe, impacts of N deposition have been mostly studied in countries experiencing high levels of N deposition. Research on N deposition impacts provides a range of indirect effects on plants by changing soil chemistry through base cation depletion from soils and greater rates of nitrification. The resulting acidification, eutrophication, nitrate leaching and increased solubility of phytotoxic elements (Al and Fe) have been identified as the major effects of excessive N deposition on soil, especially those with low acid-buffering capacity and on the ecosystems they support (Carroll *et al.*, 2003; Pilkington *et al.*, 2005; Horswill *et al.*, 2008). Overall, indirect effects of total N deposition on plants have been identified as increasing plant and litter productivity (Tomassen *et al.*, 2003), reducing species richness (Stevens *et al.*, 2004, 2010) and increasing susceptibility of plants to stress and disturbance including pathogens, herbivory (Throop *et al.*, 2004), drought, and frost (Caporn *et al.*, 2000; Sheppard & Leith, 2002). Direct toxicity of ammonia was also observed on plant foliage trough physiological perturbation following assimilation by leaves (Pearson & Stewart, 1993).

In Europe, there are various seminatural ecosystem types that are susceptible to N deposition impacts for which empirical critical loads have been derived (Bobbink & Hettelingh, 2011) Among these ecosystems, Agrostis sp. dominated grasslands are found throughout France and parts of Europe where they occupy various nutrient-poor sites. These grasslands are characterized by acid soils with low capacities to buffer against N enrichment making them particularly vulnerable to the induced acidification. In France, they cover a wide geographic and climatic range from the west coast, characterized by an oceanic humid climate with mild winters and warm summers, to the east border characterized by a relatively dry continental climate with hot summers and cold dry winters. In Europe, these grasslands of high conservation value have declined in recent years (Haines-Young et al., 2003) and they are now considered as habitats of priority interest for the Natura 2000 network under the Habitats Directive (Flora directive 92/43/EEC). These grasslands are representative of the 'species-rich Nardus grasslands (NARD)' (Natura 2000 code 6230) (Bensettiti et al., 2005). In addition to land-use change, habitat loss has resulted from abandonment of traditional management practices followed by successional changes to other vegetation like scrub and woodland. Abandonment of management permits biomass accumulation, which may be increased by enhanced N deposition (Britton et al., 2001). The remaining speciesrich NARD need to be protected especially in countries where low air pollution currently limits the potential damage to these ecosystems.

However, regional variation in plant community composition means that grasslands may respond differently to atmospheric N deposition according to their abiotic environment, especially climatic conditions. Indeed, the extent to which increased N inputs will drive changes in plant productivity and species composition over the next century will depend on how N deposition interacts with other influential global change factors, such as climate warming, to influence the N retention of ecosystems (Magnani *et al.*, 2007; Turner & Henry, 2009; de Vries *et al.*, 2009; Churkina *et al.*, 2010). The impact of air pollution on terrestrial ecosystems and its relationship to climate change are important issues, both on scientific and policy level, which may be strongly dependent on the spatial variation of both climate and atmospheric deposition at local to regional scale.

In this article, we provide a comprehensive analysis of recent climatic, N deposition and species composition changes to acid grasslands within the French Atlantic Domain (FAD) for the last 25 years. Three questions are addressed:

- 1. Have there been detectable changes in N deposition and climate over the last 25 years and if so what is the spatial distribution and magnitude of these changes?
- 2. Can changes in vegetation composition in acid grasslands over the 25 years be detected and are these changes dependent on initial community composition?
- 3. Can observed vegetation trends be linked to N deposition, climate or both, and at which scale?

# Materials and methods

#### Studied grasslands

The studied sites were nutrient-poor and calcifuge grasslands located within the Atlantic biogeographical area of France. They belong to the phytosociological *Nardetea strictae* class (Rivas-Goday & Rivas-Martinez, 1963) and the *Violion caninae* alliance (Schwickerath, 1944) and its vicarious eu-Atlantic *Agrostion curtisii* alliance (De Foucault, 1986). At this biogeographical scale, atmospheric gradients are relatively strong for both N deposition and climate variables (Table 1). Management variation between the sites was minimized as these grasslands are extensively grazed or mown, and are not artificially fertilized. To ensure homogeneity of studied grasslands, the selection of vegetation records was based on floristic similarity as established at the European scale by Stevens *et al.* (2010). Grasslands had to contain at least five characteristic species among grasses [*Agrostis capillaris L., Danthonia decumbens* (L.) DC., *Deschampsia flexuosa* (L.) Trin., *Festuca rubra L./ovina L., Luzula campestris* (L.) DC., *Nardus stricta L.*], sedges (*Carex pilulifera L.*), forbs [*Calluna vulgaris* (L.) Hull, *Campanula rotundifolia L., Galium saxatile L., Polygala* spp. L., *Potentilla erecta* (L.) Räuschel] and mosses [*Rhytidiadelphus squarrosus* (Hedw.) Warnst].

# Past and present vegetation records

A review of the phytosociological literature of acid grasslands (*V. caninae*, Schwickerath, 1944 and *A. curtisii*, De Foucault, 1986) was performed and allowed selection of appropriate areas where grasslands could be fitted to floristic criteria. A dataset containing 137 past vegetation records from 1978 to 1990 was compiled from Brittany (Clément, 1978; Stieperaere, 1990), Limousin (Botineau *et al.*, 1986), North Aquitaine (De Foucault, 1986, 1993), South Aquitaine (De Foucault, 1986) regions. Geographical coordinates (latitude and longitude) were determined from information available in past vegetation records (Table 1). This initial dataset was used to explore changes in community composition over time through a stratified resampling of this vegetation type (Dullinger *et al.*, 2003; Haveman & Janssen, 2008).

Between May and September of 2007, 25 sites distributed in the preselected areas from past data were visited according to protocol established by Stevens *et al.* (2010) and located by their latitude, longitude and elevation coordinates (Table 1). Areas that were affected by animals, tracks or path or were in the rain shadows of trees or hedges were excluded from the

**Table 1** Description of all sites grouped according to their geographical location (Survey region). Number of vegetation records (*n*) in each region is indicated for present (Pres) and past records. Mean geographical coordinates of all vegetation records are given for longitude and latitude. Mean elevation was calculated for the present vegetation records only. Nitrogen deposition and climate variables of all sites are expressed as annual means for the period 1980–2005 (±standard error). Total N deposition values were obtained from the mean grid cell values of EMEP model. Mean temperature, annual precipitation and potential evapotranspiration (PET) were obtained from a spatial interpolation of observed data means on grid reference of the French Meteorological Office (METEO-FRANCE)

Survey region	n (Pres/ Past)	Latitude/longitude (decimal degrees)	Elevation (m)	N deposition (kg N ha <sup>-1</sup> yr <sup>-1</sup> )	Temperature (°C)	Precipitation (mm)	PET (mm)
Brittany	5/12	47.42/-2.41	99	18.3 (±0.98)	11.0 (±0.11)	1038 (±64)	680 (±10)
Limousin	4/46	45.79/1.83	599	14.2 (±0.93)	9.8 (±0.11)	1371 (±15)	704 (±6)
North Aquitaine	5/26	44.75/-1.04	60	9.3 (±0.34)	13.5 (±0.09)	1089 (±38)	817 (±8)
South Aquitaine	6/33	43.36/-1.43	198	11.1 (±1.79)	14.2 (±0.05)	1514 (±12)	881 (±7)
Vosges	5/20	49.04/7.52	257	14.7 (±0.61)	9.8 (±0.06)	918 (±8)	686 (±3)

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survey. Areas with large amounts of *Juncus* sp. and woody species were also excluded to avoid, respectively, wetlands and unmanaged grasslands. In each grassland, five  $2 \times 2$  m quadrats were randomly located. All vascular plants were identified to a plant species level and their cover was estimated from 1 to 10 using the domin scale (see Rich *et al.*, 2005).

Plant species occurrence, i.e. the number of sites where a species can be found compared to the total number of sites, was calculated in both past and present vegetation records. Occurrence reflects a frequency of species presence in an area, irrespective of their cover. Species abundance was also calculated in both past and present vegetation records as the mean cover of one species for all the sites where the species was recorded (Hanski *et al.*, 1993).

# Soil analyses

In each site visited in 2007, the upper 10 cm of the soil was sampled using a 5 cm diameter Dutch auger. Topsoil samples were collected from two opposing corners of each quadrat and pooled for analysis. In all sites, three of the five collected samples were randomly selected and analysed to obtain a mean value.

The pH of fresh soil was measured after shaking 5 g soil with 25 mL deionized water and waiting for stable pH (pH/ Ion 510 pH Meter; Eutech Instruments, Nijkerk, the Netherlands). After drying at room temperature, soil texture was measured with fractions referred to clay, silt and sand (INRA laboratory of Arras, standard AFNOR NF X 31-107). These acidic soils required no decarbonatation process. After destruction of the organic matter and ultrasonic dispersion, sands were separated using wet sieving. Silts and clays were separated according their time of sedimentation using the Robinson pipette method (Day, 1965). Soil composition was expressed as the percentage of each fraction. Soil was ground to a fine powder and analysed for total C and N content (CNS Analyser, Elementar Model; Stockport, UK). Plant-available phosphorus (P) content was measured using an Olsen extrac-

tion and a colorometric determination. For measurements of soil exchangeable ions ( $NH_4^+$ ,  $NO_3^-$ ,  $SO_4^{2-}$ ), samples were shaken with 0.4 M NaCl and analysed using an auto-analyser by colorometric determination. For all samples, metal concentrations ( $Al^{3+}$ ,  $Ca^{2+}$ ,  $Fe^{3+}$ ,  $K^+$ ,  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Si^{2+}$ ,  $Zn^{2+}$ ) were determined using an ICP-MS. Concentrations of all elements were expressed in mg kg<sup>-1</sup> dry soil.

# Plant analyses

For the 25 sites in the 2007 surveys, samples of the *A. capillaris* grass were collected within plant communities where the quadrats were located. Approximately 10 g of above-ground material was collected in each site and washed briefly in deionized water. After oven drying for 72 h at 55 °C, samples were ground to <2 mm before analyses. The total C and N contents were measured on an autoanalyser (CNS Analyser, Elementar Model). To determine plant tissue P, a dry ashing method (Chapman & Pratt, 1985; Ryan *et al.*, 2001) was used followed by a Barton colour complex (MAFF, 1986). Contents of all elements were expressed as a percentage of dry weight.

# Nitrogen deposition and climate data

Total, reduced and oxidized N deposition data were obtained from the EMEP (European Mapping and Emissions Programme) model (Simpson *et al.*, 2003; Fagerli *et al.*, 2004), which is the most accurate N deposition model available in France. Total N deposition was based on wet and dry deposition of reduced (NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup>) and oxidized (NO<sub>2</sub>, NO<sub>3</sub><sup>-</sup> and HNO<sub>3</sub>) N inputs. Mean annual N deposition was calculated from 13 cells of EMEP 50 × 50 km grid at the location of each past and present vegetation record. In each site, averages for a 10-year period for annual reduced, oxidized and total N deposition were calculated from 1980–1990 decade (past data) or 1995–2005 decade (present data). For the 1980–1990, data were only available for three of the years: 1980, 1985 and 1990.

Mean annual temperature, annual precipitation (P) and mean annual potential evapotranspiration (PET), were

**Table 2** Plant community types (CTs) as defined by a cluster analysis after the first correspondence analysis on the 162 vegetation records sampled. Corresponding phytosociological associations and characteristic species were given; *n*, number of present (Pres) and past vegetation records in each survey region

СТ	Phytosociological association	Characteristic species	n (Pres/Past)
AGRO	Agrostietum capillaris–curtisii	Agrostis capillaris, Dactylis glomerata, Galium saxatile, Polygala serpyllifolia, Danthonia decumbens	5/10 Brittany 3/0 North Aquitaine 1/1 South Aquitaine
AVEN	Viscario–Avenetum pratensis/ Aveno pratensis–Genistum sagitallis	Avenula pubescens, Euphorbia cyparissias, Festuca filiformis, Potentilla neumanniana, Thymus pulegiodes	5/20 Vosges
FEST	Galio saxatilis–Festucetum rubrae	Briza media, Centaurea jacea var. nigra, Conopodium majus, Leucanthemum vulgare, Silene vulgaris	4/46 Limousin 2/0 North Aquitaine 0/2 Brittany
PSEC	Carici piluliferae– Pseudarrhenatheretum longifolii	Agrostis curtisii, Brachypodium pinnatum, Erica vagans, Pteridium aquilinum, Cirsium filipendulum	5/32 South Aquitaine 0/5 North Aquitaine
PSES	Simethi planifoliae– Pseudarrhenatheretum longifolii	Agrostis curtisii, Erica scoparia, Pseudarrhenatherum longifolium, Simethis planifolia, Ulex minor	0/21 North Aquitaine



**Fig. 1** Variation in nitrogen deposition for all sites sampled in the French Atlantic Domain (NARD) and community type level between past and present vegetation records; (a) total, (b) oxidized and (c) reduced N deposition. Means  $\pm$  standard error were calculated for the 1980–1990 (grey bars) and 1995–2005 (white bars) decades. Results of one-way anovas were summarized above the bars. (\*)P < 0.1; \*P < 0.05; \*\*P < 0.01; \*\*P < 0.001; ns, not significant. For AVEN and PSEC, no statistical analysis has been performed due to the number of EMEP grids used (n = 2).



**Fig. 2** Climate variation for all sites sampled in the French Atlantic Domain (NARD) and community type level between past and present vegetation records; (a) mean annual temperature, (b) annual precipitation (P), (c) annual potential evapotranspiration (PET) and (d) difference between P and PET (P-PET). Means ± standard error were calculated for the 1980–1990 (grey bars) and 1995–2005 (white bar) decades. Results of one-way anovas were summarized above the bars: (\*)P < 0.1; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; ns, not significant.

obtained from the French Meteorological Office (METEO-FRANCE) from 1980 to 2005. For each location of past and present vegetation records, climatic data were based on spatial interpolation on  $0.125^{\circ} \times 0.125^{\circ}$  grid reference of observed data. Water availability was characterized in term of the difference between annual precipitation and potential evapotranspiration (P-PET). Similarly to N deposition data, mean climatic data were calculated either from past (1980–1990; 11 years) or present (1995–2005; 11 years) decades according to the type of vegetation records.

#### Statistical analysis

To homogenize the vegetation dataset, the cover-abundance coefficients scale for phytosociological relevés used in past dataset (Braun-Blanquet, 1932; Braun-Blanquet, 1964) was converted to fit with the domin scale used in the present dataset (see Rich *et al.*, 2005). For present vegetation records, a mean abundance coefficient was calculated from the five quadrats. Species occurring in only one record were not considered as they cannot be interpreted reliably.

A first correspondence analysis (CA1) was performed on the complete dataset of past and present vegetation records (n = 162). A hierarchical clustering analysis was performed to test the occurrence of different community types (CTs) within the species-rich NARD. A CT (i.e. a cluster) can therefore include both past and present vegetation data to draw a comparison of floristic composition between the two decades within a homogeneous species pool. Indicator species were identified for each CT with IndVal method (Dufrene & Legendre, 1997) which uses both the specialization and the occurrence of the species in the dataset.

To test vegetation differences over time, a cluster including only past vegetation records was excluded, meaning that this vegetation type formerly described in the literature was not sampled in our present vegetation records. Then, a second correspondence analysis (CA2) on the remaining vegetation records was carried out allowing interdate comparisons at the FAD and CT scale. To avoid sample size effects in the comparison between numerous past vegetation records and present ones, a random resampling method without replacement  $(n = 10\ 000)$  was used for past vegetation records coordinates along the Axes 1 and 2 of the CA2. The number of vegetation records used to calculate the mean coordinate of past vegetation was equal to the number of vegetation records occurring in the present data. Overlapping between confidence intervals at 95%, 99% and 99.9%, obtained by random resampling for past vegetation records, and mean coordinates for present vegetation records was examined to test significant differences in plant composition between the two dates. Finally, a spearman's rank coefficient correlation between vegetation records coordinates along Axis 1 and 2 and environmental variables was calculated to assess ecological gradient in CA2 (Becker et al., 1988). Only significant correlations with r > 0.4 were selected.

To determine main species turnover over time, differences in species occurrence and abundance were examined for FAD and CT scale in both past and present vegetation records. Within species which occurred in at least 20% of the past vege-



**Fig. 3** Correspondence analysis (CA2) of the dataset (141 sites  $\times$  210 species) including past and present vegetation records. (a) Distribution of past (black plots) and present (grey plots) records. (b) Community type (CT) was represented by the barycentre of the vegetation records distribution at the two periods: 1980s (grey ellipse) and present data (white ellipse). Arrows are indicated when the shift between the barycentre of the two periods was significant within the same CT. First two axes represent 14.2% of the total variation in species composition.

tation records, species showing a difference in abundance between present and past vegetation records exceeding 2% were selected.

Temporal changes in N deposition and climatic variables were tested between the two decades of interest (1980–1990 and 1995–2005) using one-way analysis of variance (ANOVA). Data and residuals distribution were checked for normality with the Shapiro–Wilk test and for homogeneity of variance with the Bartlett's test. All analyses were conducted using R (R.2.10.1; R Foundation for Statistical Computing, Vienne, Austria).

# Results

# Classification and description of plant assemblages

The hierarchical cluster analysis on all vegetation records (NARD; n = 162) discriminated five CTs on the basis of their floristic composition. CTs were described by their plant assemblage, their phytosociological association and coded following their main characteristic grass species (Table 2). Each CT can be related to one main geographic origin. However, for three of the five CTs, records belong to several geographic origins. AGRO was thermo-Atlantic to eu-Atlantic CT, dominated by A. capillaris and A. curtisii (Clément, 1978; Stieperaere, 1990). AVEN grasslands were characterized by Avenula pratensis (Muller, 1986) and FEST were Festuca rubra grasslands (Botineau et al., 1986). Within thermo-Atlantic Pseudarrhenatherum longifolium grasslands, two CT arose: one characterized by Carex pilulifera (PSEC) and another characterized by Simethis planifolia (PSES) (De Foucault, 1986, 1993). PSES was represented in our dataset only by past vegetation records, suggesting that this CT was not resampled in south-west of France in 2007. Therefore, PSES was not considered for the temporal analyses in this study, reducing the dataset to a total of 141 vegetation records.

# Changes in nitrogen deposition and climate over time

Significant variations in N deposition were found at the different CT sites between the two decades (1980–1990 and 1995–2005) (Fig. 1). Total N deposition was elevated above background levels but with all values observed below 20 kg N ha<sup>-1</sup> yr<sup>-1</sup> and showed a general decreasing trend occurred over the past 30 years (Fig. 1a). Oxidized N deposition showed reductions for all sites considered together (NARD), but also for FEST and AVEN sites separately (Fig. 1b). On the contrary, for the same decades, reduced N deposition indicated a significant decrease only for FEST sites (Fig. 1c). Reduction in N deposition for FEST sites resulted both from oxidized and reduced N deposition, whereas N deposition

tion for AVEN sites was only induced by oxidized N deposition.

Overall, annual mean temperature changed significantly between 1980s and recent years (Fig. 2a). In our dataset, temperature increased globally by 1.1 °C, with strong variation according to the CT sites from 0.9 °C for AVEN to 1.8 °C for AGRO and 1.9 °C for FEST while there was no significant change for PSEC sites. Analysis of variance showed a significant reduction in mean annual precipitation by 183 mm occurring over the past 30 years for all sites (NARD) (Fig. 2b). At the

**Table 3** Correlations between the coordinates for vegetation records along the first two axes of the second correspondence analysis and different environmental parameters; *n*, number of vegetation records available for correlations. For each axis, *r* Spearman correlation index was given as well as the significance of the correlation when r > 0.4: \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001

		<i>r</i> with significance			
Parameters	п	Axis 1	Axis 2		
Geography					
Latitude (decimal degrees)	141	+0.74***	_		
Longitude (decimal degrees)	141	+0.82***	_		
Climate					
Annual mean temperature (°C)	141	-0.80***	_		
Annual precipitation (P) (mm)	141	-0.51***	_		
Annual potential	141	-0.72***	_		
evapotranspiration (PET) (mm)					
P-PET (mm)	141	_	+0.55***		
Deposition					
NOx deposition (kg N $ha^{-1}$ yr <sup>-1</sup> )	141	+0.80***	_		
NHy deposition (kg N $ha^{-1}$ yr <sup>-1</sup> )	141	+0.43***	+0.55***		
N total deposition	141	+0.70***	_		
$(kg N ha^{-1} yr^{-1})$					
Soil					
рН	25	+0.52**	_		
Clay (%)	25	-0.62***	+0.41*		
Silt (%)	25	-0.47*	_		
Sand (%)	25	+0.60**	_		
C : N ratio	25	-0.73***	-		
N : P ratio	25	-0.62**	-		
$NO_3$ (mg kg <sup>-1</sup> dry soil)	25	+0.77***	-		
Al (mg kg $^{-1}$ dry soil)	25	-0.57**	+0.40*		
K (mg kg <sup><math>-1</math></sup> dry soil)	25	_	+0.57**		
Mg (mg kg <sup><math>-1</math></sup> dry soil)	25	_	+0.57**		
$SO_4$ (mg kg <sup>-1</sup> dry soil)	25	-0.50*	+0.45*		
Si (mg kg <sup>-1</sup> dry soil)	25	_	+0.59**		
Vegetation					
C (%)	21	_	-0.53*		
N (%)	21	+0.58**	_		
P (%)	21	+0.73***	_		
C : N ratio	21	-0.56**	_		
N : P ratio	21	-0.65**	_		

CT scale, precipitations in FEST sites were characterized by a reduction of 194 mm. During the same period, potential evapotranspiration showed stability for all sites (NARD) but presented significant changes for AGRO (+113 mm) and AVEN sites (+22 mm) (Fig. 2c). P-PET showed a dramatic decrease between 1980–1990 and 1995–2005 for all sites (NARD: –207 mm) including FEST sites (–223 mm) (Fig. 2d).

# *Species composition gradients and underlying ecological factors*

The first two ordination axes of the CA2 (210 species  $\times$  141 vegetation records) accounted for 14.2% of the total variation in the ordination (eigenvalues:  $\lambda_1 = 0.084$ ,  $\lambda_2 = 0.058$ ) (Fig. 3a, b). Species accounting for floristic gradients were, respectively, for Axis 1: Asphodelus albus, Euphorbia angulata and Gentiana pneumonanthe opposed to Anthyllis vulneraria, Koeleria macrantha and Leontodon autumnalis, and for Axis 2: Avenula pratensis, Genista sagitallis and Trifolium medium opposed to Arnica montana, Gentiana lutea and Stellaria holostea. Examining correlations existing between CA2 axes coordinates and environmental variables of sites (Table 3) can potentially provide hypotheses and evidence for the ecological significance of plant community variation. Axis 1 was related to wide regional ecological differences, as well as climatic and N deposition variables. It was positively correlated to latitude and longitude, which reflects a decrease in temperature and potential evapotranspiration. Axis 1 was related to biogeography and climate, which discriminated between the most Atlantic CTs (AGRO and PSEC) at the negative end and driest and continental CTs (AVEN and FEST) at the positive end of the gradient. The increase of nutrient availability (N and P) in soils and vegetation along Axis 1 was explained by the high correlation of oxidized and total N deposition along Axis 1. Indicators of acidification (pH and Al and SO<sub>4</sub> concentration) were negatively correlated to Axis 1, topsoil pH being more acid and Al and SO<sub>4</sub> concentration higher in the negative part of Axis 1. This increase in soil acidification was consistent with a low level of soil nitrate content in thermo-Atlantic communities. This impoverishment in nitrate was related to both the lowest level of N deposition and the highest precipitation amount which involves large leaching and therefore acidification. Axis 2 was positively correlated to a water availability (P-PET), reduced N deposition and soil texture and chemical content. Clay percentage of soils was related to water avaibility suggesting an increase in alteration processes in soils. At the same time, cation concentration increases especially for elements tightly related to the chemical composition of bedrocks (K, Mg, Si, Al) and/or involved in acidification processes (SO<sub>4</sub>, Al). C content of plants was negatively related to all these variables (Table 3).

# *Characterization of community changes and species trends*

At the FAD scale, species-rich NARD showed no difference in floristic composition between the two decades studied from the Axis 1 of CA2, whereas very significant differences were found for Axis 2 (Table 4). At the CT scale, consistent temporal trends were statistically significant along Axes 1 and 2 (Table 4). The magnitude of vegetation changes was

**Table 4** Comparison of mean coordinates between past and present (Pres) vegetation records for all vegetation records sampled in the French Atlantic Domain (NARD) and community type level. Differences in coordinates were calculated along the first two axes of the second correspondence cnalysis (i.e. without PSES records) accounting for 14.2% of total variation. Coordinates of past vegetation records were obtained by averaging coordinates of *n* randomly chosen vegetation records 10000-times, *n* being the number of present vegetation records in the corresponding community type. Present coordinates were expressed as the mean of the n vegetation records.

	Axis 1			Axis 2			
Community type	Hist	Pres	Difference	Hist	Pres	Difference	
NARD	0.013	-0.037	-0.050 ns	-0.061	-0.195	-0.134***	
AGRO	-0.198	-0.195	0.003 ns	0.086	-0.215	-0.301***	
AVEN	0.848	0.700	-0.148*	-1.096	-0.831	0.265 ns	
FEST	0.513	0.307	-0.207**	0.637	0.295	-0.342***	
PSEC	-1.040	-0.901	0.140 ns	-0.127	-0.112	0.014 ns	

Significance of the temporal variation in coordinates:

\*\*\*<<0.001;

ns: not significant.

<sup>\*&</sup>lt;0.05;

<sup>\*\*&</sup>lt;0.01;

**Table 5** Temporal change in species occurrence and abundance for all vegetation records sampled in the French Atlantic Domain (NARD) and community types level; *n*, total number of species detected in 1980s and 2007. Species occurrence was a frequency of occurrence in either present (Pres) or past vegetation records. Abundance variation was calculated as the difference of total cover of species between past and present vegetation records. Only the most common and variable species (i.e. occurrence  $\geq 20\%$  in past vegetation records and abundance  $\geq 2\%$ ) were considered (in bold, species in abundance variation  $\geq \pm 3\%$ )

Community type	п	Decreasing species	Distribution (%) Pres (Past)	Abundance variation (%)	Increasing species	Distribution (%) Pres (Past)	Abundance variation (%)
NARD	211	Deschampsia flexuosa	28 (28)	-2.80			
		Pteridium aquilinum	44 (46)	-2.62			
		Trifolium repens	32 (27)	-2.43			
		Anthoxanthum odoratum	76 (59)	-2.40			
		Festuca rubra	84 (70)	_2 33			
		Centaurea jacea vor	36 (39)	-2.30			
		nigra	50 (57)	2.01			
		Holcus lanatus	56 (48)	-2.24			
		Briza media	20 (38)	-2.08			
		Conopodium majus	16 (28)	-2.02			
AVEN	111	Avenula pratensis	0 (25)	-6.63	Nardus	20 (25)	7.30
		o 1	- ()		stricta		
		Genista sagittalis	0 (50)	-4.37	Luzula	100 (85)	4.38
		Consister uitere	0 (20)	2.50	campestris	90 (100)	2.25
		Genista pilosa	0 (30)	-3.76	festuca	80 (100)	3.37
		T	0 (00)	2.00	filiformis	100 (00)	2.27
		Festuca nigrescens	0 (90)	-3.00	Hieracium pilosella	100 (90)	2.27
		Rhinanthus minor	0 (30)	-2.77			
		Thesium linophyllon	0 (30)	-2.62			
		Euphrasia stricta	0 (45)	-2.52			
		Agrostis vinealis	0 (30)	-2.34			
		Stellaria graminea	0 (40)	-2.34			
		Stachys officinalis	0 (30)	-2.31			
		Succisa pratensis	0 (40)	-2.16			
		Molinia caerulea	0 (30)	-2.09			
		Campanula rotundifolia	0 (35)	-2.03			
		Calium numilum	0 (45)	-2.00			
AGRO	118	Festuca rubra	67 (73)	-6.07	Molinia	56 (45)	2.41
		Enica cinanaa	56 (27)	5.64	caerulea		
		Cincium	50 (27) 0 (27)	-5.04			
		filipendulum	0 (27)	-5.45			
		Agrostis canina	11 (27)	-5.22			
		Holcus mollis	11 (55)	-4.41			
		Veronica officinalis	22 (45)	-2.72			
		Lathyrus linifolius	11 (27)	-2.45			
		subsp. montanus					
		Galium saxatile	22 (73)	-2.34			
		Plantago lanceolata	56 (27)	-2.32			
		Wahlenbergia	11 (27)	-2.19			
		hederacea					
FEST	111	Gentiana lutea	0 (33)	-6.10	Danthonia decumbers	100 (29)	2.81
		Rhinanthus minor	0 (21)	-6.08	uccumpens		
		Amica montana	0(21)	-0.08			
		Amicu montana	0 (21)	-5.07			

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# Table 5 (continued)

Community type	п	Decreasing species	Distr (%)	ribution Pres (Past)	Abundance variation (%)	Increasing species	Dist (%)	ribution Pres (Past)	Abundance variation (%)
		Deschampsia	17	(38)	-4.15				
		flexuosa							
		Phyteuma spicatum	0	(31)	-3.00				
		Dactylorhiza maculata	0	(27)	-2.74				
		Briza media	50	(63)	-2.74				
		Trifolium repens	67	(38)	-2.62				
		Centaurea jacea var. nigra	50	(75)	-2.59				
		Conopodium majus	33	(58)	-2.40				
		Plantago lanceolata	67	(58)	-2.33				
		Achillea millefolium	83	(46)	-2.29				
		Stachys officinalis	50	(50)	-2.23				
PSEC	101	Asphodelus albus	0	(24)	-3.29				
		Brachypodium	40	(54)	-3.24				
		pinnatum							
		Festuca rubra	100	(84)	-3.20				
		Hieracium pilosella	100	(65)	-2.86				
		Solidago virgaurea	0	(38)	-2.79				
		Euphorbia angulata	0	(35)	-2.79				
		Gentiana meumonanthe	0	(24)	-2.78				
		Scilla verna	40	(38)	-2.25				
		Pteridium aauilinum	100 (	(100)	-2.15				
		Blechnum spicant	0	(22)	-2.05				

defined as the difference of mean coordinates along CA2 axis between 1980s and 2007 records. These changes were community-dependant, and when they were significant, they showed the same direction of change along CA2 axis (Table 4, Fig. 3a, b). CTs which showed the same directional changes to the negative part of the axes were AVEN and FEST for Axis 1 and AGRO and FEST for Axis 2.

During the last 25 years, many species have decreased at the FAD scale in occurrence and abundance whatever the functional type of species (forbs, legumes and grasses) (Table 5). At the CT scale, but not at the FAD scale (NARD), some species disappeared in the present dataset although they were originally common (more than 20%) in past vegetation records (present occurrence = 0; Table 5). Species that totally disappeared in the present dataset with the strongest decrease in abundance were mostly forbs (Cirsium filipendulum for AGRO; Arnica montana, Gentiana lutea, Rhinanthus minor and Phyteuma spicatum for FEST; Asphodelus albus for PSEC), and to a lesser extent legumes (Genista pilosa and Genista sagitallis for AVEN) and grasses (Avenula pratensis for AVEN). By contrast, some grasses were detected in the 2007 dataset while they were absent in past vegetation records at the CT scale,

like Deschampsia flexuosa for AGRO and AVEN, Luzula campestris for PSEC and Nardus stricta for AGRO (data not shown). Moreover, some species increased in occurrence and abundance, such as the grasses (Molinia caerulea for AGRO; Festuca filiformis, Luzula campestris and Nardus stricta for AVEN; Danthonia decumbens for FEST) and the forb: Hieracium pilosella for AVEN (Table 5).

# Discussion

This article attempts to detect any vegetation changes that have occurred in the French Atlantic acid grasslands in the last 25 years and uses correspondence analysis to identify the potential contribution of N deposition in the context of a changing climate. Achieving this objective was made difficult by two factors. Firstly, the time span of the study was relatively short (25 years at the most) compared to that generally considered in resampling approaches (40–70 years; e.g. Haveman & Janssen, 2008; Duprè *et al.*, 2010; McGovern *et al.*, 2011). Secondly, the level of N deposition (11–18 kg N ha<sup>-1</sup> yr<sup>-1</sup>) was moderately elevated above background levels in more pristine areas (1–5 kg N ha<sup>-1</sup> yr<sup>-1</sup>; Galloway *et al.*, 2004), whereas comparative studies performed at national or continental scale generally combine low and high levels (5–50 kg N ha<sup>-1</sup> yr<sup>-1</sup>; Maskell *et al.*, 2010; Stevens *et al.*, 2004, 2010). In the FAD, over the last 25 years, the general trend has been decreasing N deposition (Fagerli & Aas, 2008) as well as climatic changes with an increase of mean temperature and a decrease of water availability (IPCC, 2007). If these trends are detectable, then we expect that the effects on vegetation for the N driver should be strong where deposition levels have poorly decreased, i.e. when deposition is high and the most stable. Similarly, climatic impacts should be the strongest where climatic changes are the most pronounced.

# Temporal changes in both N deposition and climate

Both N deposition and climate changes are detected over the period of the study at the FAD scale. For atmospheric N deposition, the significant decrease of oxidized N deposition observed in our study is consistent with the oxidized N emissions reduction observed in France (Serveau *et al.*, 2010) and N deposition trends observed during a comparable period in the United Kingdom (Fowler *et al.*, 2005) and in Europe (Fagerli & Aas, 2008). For climatic variables, changes shown in our study agree with results from the literature (IPCC, 2007).

These time-scale variations throughout the FAD conceal spatial variations corresponding to local heterogeneities of N deposition and climate changes. Spatial variations in N deposition changes are especially important, as changes in total N and reduced N deposition are detected at a local scale but not at the FAD scale. Especially, in FEST and AVEN sites, significant decrease of total N deposition was recorded, mainly due to a decrease in oxidized N deposition during the period of the study (Fagerli & Aas, 2008). The level of N deposition observed was within the total range of the critical loads recommended for acid grasslands (10-15 kg N ha<sup>-1</sup> yr<sup>-1</sup>; Bobbink *et al.*, 2011), although the relevant range of the critical loads depends on abiotic factors (Achermann & Bobbink, 2002). For example, cold temperature and N limitation are likely to increase sensitivity of ecosystems to N deposition. As regards our data, FEST and AVEN sites are likely to be the most sensitive with a critical load ranging around 10 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Achermann & Bobbink, 2002), whereas the upper part of the critical loads range, i.e. around 15 kg N ha<sup>-1</sup> yr<sup>-1</sup>, is appropriate to the most thermo-Atlantic, and therefore the less sensitive, sites (AGRO and PSEC). Furthermore, the most sensitive sites have also experienced the highest loads of oxidized N deposition. Despite uncertainties for ammonia deposition stemming from the impacts of local sources (Sutton *et al.*, 1995; Erisman *et al.*, 2005, 2007), EMEP data reveal that FEST sites are the only ones to experience a decrease in reduced N deposition, although they are among the highest loads, probably due to local changes in agricultural management.

Spatial variation is also detectable in climatic variables. The southern and warmer sites sampled in the FAD, i.e. PSEC sites, are the only ones to avoid an increase in temperature. A significant decrease in water availability as a consequence of a decrease in precipitation may be detected in FEST sites only. Such spatial differences are likely to induce changes in the hierarchical balance of components of global change as regards geographical location. Therefore, the interaction between N deposition and climate change effects needs to be studied carefully to identify the key factors driving vegetation changes.

Among atmospheric variables, it is clear that climate changes are mainly detectable at the FAD scale, whereas N deposition changes are mainly dependant of local variation, affecting central and northern areas of French Atlantic acid grasslands.

# Spatial and temporal trends in vegetation patterns: what are the main potential drivers?

Overall floristic stability, with no significant species shift at the FAD scale, suggests similarity in the underlying ecological factors at work for both periods. This can be explained by the predominant biogeographical gradient, still perceptible, that has shaped species pools variations (Bensettiti *et al.*, 2005). This gradient is synthesized by geographical position (latitude and longitude) and by drivers such as climate variables (temperature and PET) and soil biogeochemistry (nutrient content and pH), all variables linked to the first axis of CA2.

However, species composition within CTs is not constant over time and differences in vegetation are especially noticeable at the local scale. The increasing gradient of available N, which is associated with a gradient of oxidized N deposition in our study, is consistent with a decrease of soil C : N ratio. Soil nitrate content can follow the gradient of N content in vascular plants (Carroll et al., 2003; Pilkington et al., 2005) which was not apparent at an European scale (Stevens et al., 2011 in press). In this study, the significant response of N content in plant tissues arises from A. capillaris, previously shown to be sensitive to N addition (Horswill et al., 2008). The second axis of CA2 is mostly related to water availability and reduced N deposition and also to soil base cations. Chronic summer drought, e.g. water availability, appears to be one of the major events to influence productivity and species richness in unfertile grasslands (Grime *et al.*, 2008). Moreover, ecosystems do not seem to be resilient to soil pH decrease as recovery in soil base saturation is less rapid and complete than soil pH recovery itself (McGovern *et al.*, 2011).

Differences in grasslands composition over time are supported by species turn-over examination. Our method, which considers only species that had an abundance of >20% in the past vegetation records, provides a cautious indication of present changes. Our results point out the decrease of forb cover in response to N deposition (Stevens et al., 2006, 2010). The decrease or absence of legumes such as Genista pilosa at local scale could mean that N deposition rather than biological N fixation is dominating N supply and could also explain the decrease of the hemi-parasite R. minor (Ameloot et al., 2008). The decreasing cover of R. minor in response to higher N avaibility (Smith et al., 2002) impacts the grassland ecosystem structure and function by allowing dominant grasses to proliferate and suppress forb cover, potentially decreasing the plant diversity (Press & Phoenix, 2005).

# *Footprints of N deposition and climate change on vegetation*

Effects of climate change can operate both at the FAD and local scale and may affect vegetation turn-over. The level of N deposition observed was within the range of the critical loads suggesting a weak impact of N deposition even if effects of low background deposition on acid grasslands are reported (Clark & Tilman, 2008; Stevens et al., 2010). Effects of N deposition are also dependent on local variation due to difference in N deposition form. Vegetation changes experienced in AVEN along Axis 1 are consistent with the vicinity of Vosges region with industrial activities in France and in neighbour countries, contributing to high oxidized N deposition (Fowler et al., 1998). Vegetation changes along Axis 2 in AGRO sites, corresponding mainly to Brittany, are consistent with a response to the highest reduced N deposition levels as Brittany region has one of the most intensive agriculture in France (Asman et al., 1998; Aneja et al., 2001). Differences in the scale effect between climate change and N deposition may induce a hierarchical effect of these components on ecosystems (Pan et al., 2009; Stevens et al., 2011a) and interactive effects at the same scale (Sala et al., 2000; Majdi & Ohrvik, 2004; Turner & Henry, 2009). N deposition and climate interactions are known to alter the carbon balance in terrestrial ecosystems (Churkina et al., 2010) or the dynamics of vegetation (Britton et al., 2001) but they can act independently for example on carbon dynamics (Pan et al., 2009) or on soil properties (Papanikolaou et al., 2010). Moreover, an N deposition signal was

detected, despite the short gradient and the short period initially described, probably because the sites that experience the highest loads were also likely to be the most sensitive, due to the interactive effects between N deposition and climate. The strongest change in vegetation (i.e. species turn-over along both Axis 1 and 2) was detected in the Festuca grasslands corresponding to sites that experienced the highest chronic N deposition and the highest increase in mean temperature. Conversely, the Pseudarrhenatherum grasslands, with no significant vegetation trend, are also the sites that are the more stable as regards climate with the lowest level of N deposition. The magnitude of vegetation responses to climate and atmospheric changes depends on local variation in N deposition and climatic changes, on the interaction between these factors. Indeed, climate changes and N deposition, are likely to act as drivers per se of plant communities, while climate may also influence ecosystem sensitivity to N deposition and vice versa. However, a hierarchy is hardly demonstrable without improved local models that are needed for calculations of these changes in the future (Zavaleta et al., 2003).

At the species level, N deposition is expected (i) to increase the potential for competition exclusion and shift selection pressure towards species able to capitalize on elevated N avaibility (Wedin & Tilman, 1993; Hautier et al., 2009) and (ii) to impact species by exceeding their physiological limits of acid tolerance and nutrient demand (Horswill et al., 2008). The species turn-over results show a general tendency to gain grasses at the expense of forbs (Duprè et al., 2010; Maskell et al., 2010). In our study, the extent of grasses such as Nardus strictae and Molinia caerulea is consistent with an increased level of N availability and loss of other grasslands species (Tomassen et al., 1999; Hartley & Mitchell, 2005; Kleijn et al., 2008). It has also been observed that rare species such as Arnica montana or Dactylorhiza maculata disappeared from these grasslands before grasses started to dominate over the vegetation because they are extremely sensitive to acidification and ammonium accumulation (van den Berg et al., 2005; Kleijn et al., 2008; De Graaf et al., 2009). Similarly, climate changes can disadvantage the less tolerant species, especially those species sensitive to drought and warmer conditions. Indeed, global warming is known to change species distribution and therefore biogeographical limits for species, particularly in mountain areas (Lenoir et al., 2008). Such changes could also be perceptible at lower altitude especially in grasslands sites with the least nutrient limitation (AVEN and FEST) where N deposition is not a confounding factor (Gilbert et al., 2003; Falkengren-Grerup et al., 2006). The species turn-over detected in these hilly communities indicates that endangered

mountains species, like *Arnica montana* and *Gentiana lutea*, are likely to be the affected in their distribution (Grabherr *et al.*, 1994). These results could provide more evidence of indirect effects of N deposition by increasing susceptibility of plants to frost (Caporn *et al.*, 2000; Sheppard & Leith, 2002). The vegetation appears to be sensitive to the cumulative amounts of N deposition especially in the sites where the loads of N are in the upper range of the critical loads for acid grasslands (Bobbink *et al.*, 2011) and where climate is cool (AVEN and FEST sites).

# Conclusion

Impact of global change on vegetation is complex because of the many ecological factors involved. By comparing vegetation data over a relatively short time span and in the context of a range of N deposition rates around the critical load range, this study provides evidence that the effects of cumulative N deposition on species composition are variable depending on spatial scale. This is because grasslands experiencing high oxidized or reduced N deposition levels respond locally with floristic and soil effects. However, these changes are always associated with climatic changes, suggesting that N deposition is a clear driver of grassland composition at more local scales whereas climate changes remain the predominant driver at the FAD scale. Climatic factors influence composition changes in grasslands at both local and FAD scales, especially in the most continental and hilly community sites where they are likely to explain community sensitivity to N deposition. Therefore, although it was not possible to disentangle the single effects of N deposition and climate change, this study allowed us to differentiate a scale effect and to suggest an interactive effect between both factors. To see the complete picture and understand processes at work, particularly N deposition and climate interactions, future research is needed to complement long-term and large scale comparatives studies with local studies associated to short-term and finegrain trends in contrasting bioclimatical areas. This is of particular importance to implement conservation policies and to provide meaningful management of valuable sites.

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# References

- Achermann B, Bobbink R (2002) Empirical Critical Loads for Nitrogen, Vol. 164. Swiss Agency for the Environment, Forests and Landscape, Berne.
- Ameloot E, Verlinden G, Boeckx P, Verheyen K, Hermy M (2008) Impact of hemiparasitic Rhinanthus angustifolius and R. minor on nitrogen availability in grasslands. Plant and Soil, 311, 255–268.
- Aneja VP, Roelle PA, Murray GC et al. (2001) Atmospheric nitrogen compounds II: emissions, transport, transformation, deposition and assessment. Atmospheric Environment, 35, 1903–1911.
- Asman WAH, Sutton MA, Schjerring JK (1998) Ammonia: emission, atmospheric transport and deposition. New Phytologist, 139, 27–48.
- Becker RA, Chambers JM, Wilks AR (1988) The New S Language. Wadsworth & Brooks/Cole, Pacific Grove, CA, USA.
- Bensettiti F, Boullet V, Chavaudret-Laborie C, Deniaud J (2005) Cahiers d'habitats Natura 2000. Connaissance et gestion des habitats et des espèces d'intérêt communautaire. Tome 4 – Habitats agropastoraux. La Documentation Française, Paris.
- Bobbink R, Hettelingh JP (2011) Review and revision of empirical critical loads and doseresponse relathionships. RIVM Report 680359002, Coordination Centre for Effects, National Institute for Public Health and the Environment (RIVM). Noordwijkerhout.
- Bobbink R, Hicks K, Galloway J et al. (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications, 20, 30–59.
- Botineau M, Descubes-Gouilly C, Ghestem A, Vilks A (1986) Les prairies montagnardes du Limousin; essai d'appréciation de leur valeur pastorale. *Documents Phytosociologiques*, **10**, 77–95.
- Braun-Blanquet J (1964) Pflanzensociologie: Grundzuge der Vegetationskunde, 3te aufl. Springer-Verlag, Wein.
- Braun-Blanquet J (1932) Plant Sociology (Transl. Fuller GD, Conrad HS). Mc Graw-Hill, New-York.
- Britton AJ, Pakeman RJ, Carey PD, Marrs RH (2001) Impacts of climate, management and nitrogen deposition on the dynamics of lowland heathland. *Journal of Vegetation Science*, 12, 797–806.
- Caporn SJM, Ashenden TW, Lee JA (2000) The effect of exposure to NO<sub>2</sub> and SO<sub>2</sub> on frost hardiness in *Calluna vulgaris*. *Environmental and Experimental Botany*, **43**, 111– 119.
- Carroll JA, Caporn SJM, Johnson D, Morecroft MD, Lee JA (2003) The interactions between plant growth, vegetation structure and soil processes in semi-natural acid and calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen deposition. Environmental Pollution, 121, 363–376.
- Chapman HD, Pratt PF (1985) Methods for Analysis of Soils, Plants and Waters. Agricultural Sciences Publications, Berkeley, CA.
- Churkina G, Zaehle S, Hughes J et al. (2010) Interactions between nitrogen deposition, land cover conversion, and climate change determine the contemporary carbon balance of Europe. *Biogeosciences*, 7, 2749–2764.
- Ciais P, Reichstein M, Viovy N et al. (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature, 437, 529–533.
- Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature*, 451, 712–715.
- Clément B (1978) Contribution à l'étude phytoécologique des Monts d'Arrée; organisation et cartographie des biocénoses, évolution et productivité des landes. University of Rennes, Rennes.
- Cunha A, Power SA, Ashmore MR, Green PRS, Haworth BJ, Bobbink R (2002) Whole Ecosystem Nitrogen Manipulation: An Update Review. JNCC, Peterborough.
- Day PR (1965) Particle fractionation and particle-size analysis. In: *Methods of Soil Anal-ysis*, Vol. 1 (ed. Black CA), pp. 545–567. American Society of Agronomy, Inc., Madison, WI.
- De Foucault B (1986) Données systémiques sur la végétation prairiale mésophile du pays Basque et des landes de Gascogne. *Documents Phytosociologiques*, **10**, 203– 219.
- De Foucault B (1993) Nouvelles recherches sur les pelouses de l'Agrostion curtisii et leur syndynamique dans l'Ouest et le Centre de la France. Bulletin de la Société Botanique du Centre-Ouest, Nouvelle Série, 24, 151–178.

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- De Graaf MCC, Bobbink R, Smits NAC, Van Diggelen R, Roelofs JGM (2009) Biodiversity, vegetation gradients and key biogeochemical processes in the heathland landscape. *Biological Conservation*, 142, 2191–2201.
- Dentener F, Stevenson D, Ellingsen K et al. (2006) The global atmospheric environment for the next generation. Environmental Science & Technology, 40, 3586–3594.
- de Vries W, Solberg S, Dobbertin M et al. (2009) The impact of nitrogen deposition on carbon sequestration by European forests and heathlands. Forest Ecology and Management, 258, 1814–1823.
- Dufrene M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*, 67, 345–366.
- Dullinger S, Dirnböck T, Greimler J, Grabherr G (2003) A resampling approach for evaluating effects of pasture abandonment on subalpine plant species diversity. *Journal of Vegetation Science*, 14, 243–252.
- Duprè C, Stevens CJ, Ranke T et al. (2010) Changes in species richness and composition in European acid grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. *Global Change Biology*, 16, 344–357.
- Erisman JW, Vermeulen A, Hensen A *et al.* (2005) Monitoring and modelling of biosphere/atmosphere exchange of gases and aerosols in Europe. *Environmental Pollution*, **133**, 403–413.
- Erisman JW, Bleeker A, Galloway J, Sutton MS (2007) Reduced nitrogen in ecology and the environment. *Environmental Pollution*, **150**, 140–149.
- Fagerli H, Aas W (2008) Trends of nitrogen in air and precipitation: model results and observations at EMEP sites in Europe, 1980-2003. Environmental Pollution, 154, 448–461.
- Fagerli H, Simpson D, Tsyro S (2004). Transboundary acidification, eutrophication and ground level ozone in Europe, EMEP status report 1/2004. The Norwegian Meteorological Institute, Oslo, Norway.
- Falkengren-Grerup U, ten Brink DJ, Brunet J (2006) Land use effects on soil N, P, C and pH persist over 40-80 years of forest growth on agricultural soils. *Forest Ecol*ogy and Management, 225, 74–81.
- Fowler D, Flechard C, Skiba U, Coyle M, Cape JN (1998) The atmospheric budget of oxidized nitrogen and its role in ozone formation and deposition. *New Phytologist*, 139, 11–23.
- Fowler D, Smith RI, Muller JBA, Hayman G, Vincent KJ (2005) Changes in the atmospheric deposition of acidifying compounds in the UK between 1986 and 2001. *Environmental Pollution*, 137, 15–25.
- Galloway JN (1998) The global nitrogen cycle: changes and consequences. Environmental Pollution, 102, 15–24.
- Galloway JN, Dentener FJ, Capone DG et al. (2004) Nitrogen cycles: past, present, and future. Biogeochemistry, 70, 153–226.
- Galloway JN, Townsend AR, Erisman JW et al. (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science*, 320, 889–892.
- Gilbert JC, Gowing DJG, Loveland P (2003) Chemical amelioration of high phosphorus availability in soil to aid the restoration of species-rich grassland. *Ecological Engineering*, 19, 297–304.
- Grabherr G, Gottfried M, Pauli H (1994) Climate effect on mountain plants. *Nature*, **369**, 448.
- Grime JP, Fridley JD, Askew AP, Thompson K, Hodgson JG, Bennett CR (2008) Long-term resistance to simulated climate change in an infertile grassland. Proceedings of the National Academy of Sciences of the United States of America, 105, 10028–10032.
- Haines-Young R, Barr CJ, Firbank LG et al. (2003) Changing landscapes, habitats and vegetation diversity across Great Britain. Journal of Environmental Management, 67, 267–281.
- Hannah L, Midgley GF, Lovejoy T et al. (2002) Conservation of biodiversity in a changing climate. Conservation Biology, 16, 264–268.
- Hanski I, Kouki J, Halkka A (1993) Three explanations of the positive relationship between distribution and abundance of species. In: *Community Diversity: Historical* and Geographical Perspectives (eds Ricklefs RE, Schluter D), pp. 108–116. Chicago University Press, Chicago, IL.
- Hartley SE, Mitchell RJ (2005) Manipulation of nutrients and grazing levels on heather moorland: changes in Calluna dominance and consequences for community composition. *Journal of Ecology*, 93, 990–1004.
- Hautier Y, Niklaus PA, Hector A (2009) Competition for light causes plant biodiversity loss after eutrophication. *Science*, 324, 636–638.
- Haveman R, Janssen JAM (2008) The analysis of long-term changes in plant communities using large databases: the effect of stratified resampling. *Journal of Vegetation Science*, 19, 355–362.
- Horswill P, O'Sullivan O, Phoenix GK, Lee JA, Leake JR (2008) Base cation depletion, eutrophication and acidification of species-rich grasslands in response to longterm simulated nitrogen deposition. *Environmental Pollution*, **155**, 336–349.

- IPCC (2007) Climate change and its impacts in the near and long term under different scenarios. In: Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (eds Core Writing Team Pachauri R K and Reisinger A), pp. 104, IPCC, Geneva, Switzerland.
- Kleijn D, Bekker RM, Bobbink R, De Graaf MCC, Roelofs JGM (2008) In search for key biogeochemical factors affecting plant species persistence in heathland and acidic grasslands: a comparison of common and rare species. *Journal of Applied Ecology*, 45, 680–687.
- Lenoir J, Gegout JC, Marquet PA, de Ruffray P, Brisse H (2008) A significant upward shift in plant species optimum elevation during the 20th century. *Science*, 320, 1768–1771.
- Lovett GM, Tear TH, Evers DC et al. (2009) Effects of air pollution on ecosystems and biological diversity in the eastern United States. Annals of the New York Academy of Sciences, 1162, 99–135.
- MAFF (1986) The Analysis of Agricultural Materials (3rd edn). Her Majesty's Stationary Office, London.
- Magnani F, Mencuccini M, Borghetti M et al. (2007) The human footprint in the carbon cycle of temperate and boreal forests. *Nature*, 447, 848–852.
- Majdi H, Ohrvik J (2004) Interactive effects of soil warming and fertilization on root production, mortality, and longevity in a Norway spruce stand in Northern Sweden. *Global Change Biology*, **10**, 182–188.
- Maskell LC, Smart SM, Bullock JM, Thompson K, Stevens CJ (2010) Nitrogen deposition causes widespread loss of species richness in British habitats. *Global Change Biology*, 16, 671–679.
- McGovern S, Evans CD, Dennis P, Walmsley C, McDonald MA (2011) Identifying drivers of species compositional change in a semi-natural upland grassland over a 40-year period. *Journal of Vegetation Science*, 22, 346–356.
- Moberg A, Sonechkin DM, Holmgren K, Datsenko NM, Karlen W (2005) Highly variable Northern Hemisphere temperatures reconstructed from low- and high-resolution proxy data. *Nature*, 433, 613–617.
- Muller S (1986) La végétation du pays de Bitche (Vosges du Nord) Analyse phytosociologique Application à l'étude synchronique des successions végétales. University of Paris-Sud, Paris.
- NEGTAP (2001) Transboundary Air Pollution: Acidification, Eutrophication and Ground-Level Ozone in the UK. CEH, Edinburgh.
- Osborn TJ, Briffa KR (2006) The spatial extent of 20th-century warmth in the context of the past 1200 years. *Science*, **311**, 841–844.
- Pan YD, Birdsey R, Hom J, McCullough K (2009) Separating effects of changes in atmospheric composition, climate and land-use on carbon sequestration of US Mid-Atlantic temperate forests. *Forest Ecology and Management*, 259, 151–164.
- Papanikolaou N, Britton AJ, Helliwell RC, Johnson D (2010) Nitrogen deposition, vegetation burning and climate warming act independently on microbial community structure and enzyme activity associated with decomposing litter in lowalpine heath. *Global Change Biology*, 16, 3120–3132.
- Pearson J, Stewart GR (1993) Tansley Review No. 56: the deposition of atmospheric ammonia and its effects on plants. *New Phytologist*, **125**, 283–305.
- Peñuelas J, Prieto P, Beier C et al. (2007) Response of plant species richness and primary productivity in shrublands along a north-south gradient in Europe to seven years of experimental warming and drought: reductions in primary productivity in the heat and drought year of 2003. Global Change Biology, 13, 2563–2581.
- Phoenix GK, Hicks WK, Cinderby S et al. (2006) Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. Global Change Biology, 12, 470–476.
- Pilkington MG, Caporn S, Carroll JA et al. (2005) Effects of increased deposition of atmospheric nitrogen on an upland moor: leaching of N species and soil solution chemistry. Environmental Pollution, 135, 29–40.
- Press MC, Phoenix GK (2005) Impacts of parasitic plants on natural communities. New Phytologist, 166, 737–751.
- Rich T, Redbane M, Fasham M, McMeechan F, Dobson D (2005) Ground and shrub vegetation. In: *Handbook of Biodiversity Methods: Survey, Evaluation and Monitoring* (eds Hill D, Fasham M, Tucker G, Shewry M, Shaw P), pp. 201–222. Cambridge University press, Cambridge.
- Rivas-Goday S, Rivas-Martinez S (1963) Estudio y clasificacion de los pastizales espanoles. Publication Ministerio de agricultura, 277, 269.
- Ryan J, Estefan G, Rashid A (2001) Soil and Plant Analysis Laboratory Manual (2nd edn). ICARDA, Aleppo, Syria.
- Sala OE, Chapin FS, Armesto JJ et al. (2000) Biodiversity global biodiversity scenarios for the year 2100. Science, 287, 1770–1774.
- Schwickerath M (1944) Das hohe Venn und seine Randgebiete: vegetation, Boden, und Landschaft. *Pflanzensoziologie*, 6, 1–278.

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- Secretariat of the Convention on Biological Diversity (2010) *Global Biodiversity Outlook* 3. CBD, Montréal.
- Serveau L, Allemand N, André JM et al. (2010) Inventaire des émissions de polluants atmosphériques en France – Séries sectorielles et analyses étendues. Centre Interprofessionnel Technique d'Etudes de la Pollution Atmosphérique (CITEPA), Paris.
- Sheppard LJ, Leith ID (2002) Effects of NH3 fumigation on the frost hardiness of Calluna – does N deposition increase winter damage by frost? *Phyton-Annales Rei Botanicae*, 42, 183–190.
- Simpson D, Fagerli H, Jonson JE, Tsyro S, Wind P, Tuovinen J-P (2003). Transboundary acidification, eutrophication and ground level ozone in Europe Unified EMEP Model Description, EMEP Status Report 1/ 2003 Part I, EMEP/MSC-W Report. The Norwegian Meteorological Institute, Oslo, Norway.
- Smith RS, Shiel RS, Millward D, Corkhill P, Sanderson RA (2002) Soil seed banks and the effects of meadow management on vegetation change in a 10-year meadow field trial. *Journal of Applied Ecology*, 39, 279–293.
- Stevens CJ, Duprè C, Dorland E et al. (2010) Nitrogen deposition threatens species richness of grasslands across Europe. Environmental Pollution, 158, 2940– 2945.
- Stevens CJ, Duprè C, Dorland E et al. (2011a) The impact of nitrogen deposition on acid grasslands in the Atlantic region of Europe. Environmental Pollution, doi: 10.1016/j.envpol.2010.11.026.
- Stevens CJ, Duprè C, Gaudnik C et al. (2011b) Changes in species composition of European acid grasslands observed along a gradient of nitrogen deposition. Journal of Vegetation Science, 22, 207–215.
- Stevens CJ, Dise NB, Gowing DJ, Mountford JO (2006) Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls. *Global Change Biology*, **12**, 1823–1833.
- Stevens CJ, Dise NB, Mountford JO, Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. *Science*, 303, 1876–1879.
- Stieperaere H (1990) De heischrale graslanden (Nardetea) van atlantisch Europa. University of Gent, Gent.
- Sutton MA, Place CJ, Eager M, Fowler D, Smith RI (1995) Assessment of the magnitude of ammonia emissions in the United-Kingdom. *Atmospheric Environment*, 29, 1393–1411.

- Throop HL, Holland EA, Parton WJ, Ojima DS, Keough CA (2004) Effects of nitrogen deposition and insect herbivory on patterns of ecosystem-level carbon and nitrogen dynamics: results from the CENTURY model. *Global Change Biology*, 10, 1092–1105.
- Tomassen HBM, Smolders AJP, Lamers LPM, Roelofs JGM (2003) Stimulated growth of *Betula pubescens* and *Molinia caerulea* on ombrotrophic bogs: role of high levels of atmospheric nitrogen deposition. *Journal of Ecology*, 91, 357–370.
- Tomassen H, Bobbink R, Peters R, Van der Ven P, Roelofs J (1999) Kritische stikstofdepositie in heischrale graslanden, droge duin graslanden en hoogvenen: op weg naar sekerheid. Eindrapport in het kader van het Stikstof Onderzoek Programma (STOP). Katholieke Universiteit Nijmegen en Universiteit Utrecht, Nijmegen and Utrecht, The Netherlands.
- Turner MM, Henry HAL (2009) Interactive effects of warming and increased nitrogen deposition on 15N tracer retention in a temperate old field: seasonal trends. *Global Change Biology*, 15, 2885–2893.
- van den Berg LJL, Dorland E, Vergeer P, Hart MAC, Bobbink R, Roelofs JGM (2005) Decline of acid-sensitive plant species in heathland can be attributed to ammonium toxicity in combination with low pH. New Phytologist, 166, 551–564.
- Vitousek PM, Aber JD, Howarth RW et al. (1997) Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications, 7, 737–750.
- Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea how can it occur. *Biogeochemistry*, 13, 87–115.
- Wamelink GWW, van Dobben HF, Berendse F (2009) Vegetation succession as affected by decreasing nitrogen deposition, soil characteristics and site management: a modelling approach. *Forest Ecology and Management*, 258, 1762–1773.
- Wedin D, Tilman D (1993) Competition among grasses along a nitrogen gradient: initial conditions and mechanisms of competition. *Ecological Monographs*, 63, 199–229.
- Zavaleta ES, Shaw MR, Chiariello NR, Mooney HA, Field CB (2003) Additive effects of simulated climate changes, elevated CO2, and nitrogen deposition on grassland diversity. Proceedings of the National Academy of Sciences of the United States of America, 100, 7650–7654.
- European Mapping and Emissions Programme (EMEP). Available at: www.emep.int. This site contains yearly model results of nitrogen deposition for the whole EMEP grid for the years 1980, 1985 and 1990–2007.

# Plant neighbours mediate bird predation effects on arthropod abundance and herbivory

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**Abstract.** 1. Tritrophic interactions among plants, herbivores and predators are expected to be influenced by the surrounding vegetation. Neighbouring plants can influence focal plant colonisation by herbivorous insects and the foraging behaviour of natural enemies, such as insectivorous birds.

2. The aim of the experiment was to disentangle the interactive effects of neighbouring plants and avian predation on arthropod abundance and insect leaf damage in oak tree seedlings, using exclusion cages and vegetation removal.

3. The presence or removal of surrounding herbaceous vegetation differentially mediated top-down effects of insectivorous birds on distinct arthropod guilds and herbivore damage in seedlings. Avian predation reduced sawfly larval abundance regardless of the presence of plant neighbours; lepidopteran larval abundance only when plant neighbours were removed; and spider abundance only when plant neighbours were left intact. The removal of plant neighbours increased prey accessibility for foraging insectivorous birds and decreased chewer damage on seedlings. The density of concealed-feeder insects (leaf miners) increased with plant neighbour removal and when seedlings were less damaged by chewer guild, suggesting intraguild competition.

4. These results highlight the strong indirect effects of neighbouring vegetation on tritrophic interactions involving a focal plant species, its associated herbivores and the upper trophic level of predators.

**Key words.** Avian predation, insect guilds, insect herbivory, neighbouring vegetation, predator removal, *Quercus robur*, trophic cascade

# Introduction

Predators can depress herbivore populations and thereby indirectly limit the consumption of primary producers (Hairston *et al.*, 1960). Birds are vertebrate predators that are likely to generate such a trophic cascade (Van Bael *et al.*, 2008; Mäntylä *et al.*, 2011). Insectivorous birds can limit damage to plants and facilitate plant growth by their predation on herbivorous insects (Whelan *et al.*, 2008; Mooney *et al.*, 2010; Mäntylä *et al.*, 2011). Previous studies have

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highlighted the importance of several factors likely to mediate this indirect effect, such as insect prey accessibility to avian predators (Marquis & Whelan, 1996; Hawkins *et al.*, 1997; Whelan, 2001), intraguild predation (Mooney *et al.*, 2010) and density-dependent predator-prey relationships (Bridgeland *et al.*, 2010).

Herbivorous insect guilds differ greatly in their sensitivity to bird predation. Concealed-feeding guilds, such as miners or gall-inducing insects, are subject to frequent parasitoid attacks, but low levels of predation by vertebrate or invertebrate predators (Hawkins *et al.*, 1997; Murakami, 1999). Conversely, caterpillars or hymenopteran larvae that feed on the plant externally are easily detected by predators and contribute a large proportion of prey to many insectivorous birds (Kristin & Patocka, 1997). Variation in insect prey density may also influence the magnitude of trophic cascades. The indirect effect of insectivorous birds on plants may be stronger when insect abundance and the associated damage level are high (Forkner & Hunter, 2000; Low & Connor, 2003; Bridgeland *et al.*, 2010). Top vertebrate predators, such as insectivorous birds, can also prey on intermediate predatory arthropods such as spiders (Gunnarsson, 2007; Mooney *et al.*, 2010). The exclusion of top predators may then release intraguild predation, i.e., favour predatory arthropods, and offset the effects of top predators on herbivory damage on plants (Finke & Denno, 2005).

Plant-insect-predator interactions also vary with plant neighbourhood. The surrounding vegetation may disrupt the colonisation and the consumption of a focal plant by insect herbivores, according to the resource concentration hypothesis (Root, 1973; Baraza et al., 2006). Neighbouring plants may either decrease resource availability and affect resistance traits or induced defence reaction of a focal plant species to herbivores (Dudt & Shure, 1994; Agrawal, 2004) or modify microclimate, which could affect herbivore communities and damage (Moore et al., 1988; Corcket et al., 2003; Savilaakso et al., 2009). Neighbouring vegetation can also enhance biological control of herbivores by predators through provision of alternative prey or complementary food resources, as predicted by the natural enemies hypothesis (Elton, 1958). Alternatively, it can reduce herbivore predation by decreasing the foraging effectiveness of natural enemies (Langellotto & Denno, 2004). For example, variation of branch and leaf architecture can impede prey accessibility for insectivorous birds (Gunnarsson, 1996; Whelan, 2001).

Studies that focused on the spatial variation of indirect effect of insectivorous birds on plants did not detect any effect, even when bird communities varied among sites (Mazía *et al.*, 2004; Barber & Marquis, 2009; Schwenk *et al.*, 2010). However, a considerable inter-site variation in avian predation has been previously observed (Gruner, 2004; Zehnder *et al.*, 2010), and could be related to the spatial variation in bird diversity between sites (Van Bael *et al.*, 2008). Plant–insect–predator interactions may occur over short spatial scales, such as amongst neighbouring plants or at the within-plant scale (Langellotto & Denno, 2004; Gripenberg & Roslin, 2007).

In this study, we manipulated both bird predation and understorey vegetation to assess their interactive effects on the abundance of arthropods and levels of herbivory on oak seedlings. We hypothesise that the presence of neighbouring vegetation will decrease both the density and apparency of target seedlings, thus resulting in lower insect herbivory. Conversely, the presence of neighbouring vegetation may disrupt bird predation on arthropods and consequently increase herbivory on target plants.

# Materials and methods

## Study system

This study was carried out in the Landes de Gascogne forest, in south-western France, a region covered by about one million hectares of maritime pine (*Pinus pinaster*) plantations. Pedunculate oak (*Quercus robur*) regenerates naturally within pine stands and may become co-dominant in older plantations. The understorey vegetation in pine plantations is composed of a low cover of saplings (*Q. robur*, *Betula pendula*) and shrubs (*Frangula alnus*, *Ulex europaeus* and *Ulex minor*) for c. 15% of the ground surface. Herbaceous cover–around 80% of the ground cover–is dominated by *Pteridium aquilinum* and *Molinia cærulea*.

Insect folivore guilds occurring on *Q. robur* seedlings include external feeders (chewers and skeletonisers) and concealed feeders (leaf miners). The chewer guild consists principally of lepidopteran larvae (from the families Geometridae, Lymantriidae and Noctuidae). Sawfly larvae (Hymenoptera Tenthredinidae) and grasshoppers (Orthoptera Tettigoniidae), together with first instars of lepidopteran species (particularly from the family Geometridae), are responsible for most of the skeletonising damage observed. Leaf miners associated with *Q. robur* are the larvae of small moths (Microlepidoptera Nepticulidae, Tischeriidae and Gracillariidae; see Giffard *et al.*, 2012b) and *Orchestes signifer* (Coleoptera Curculionidae).

The bird communities of the study area have been described in detail in previous studies (Barbaro & van Halder, 2009; Giffard *et al.*, 2012a). The predominant insectivorous bird species likely to forage on the understorey foliage of broadleaved tree species are Eurasian wren (*Troglodytes troglodytes*), great tit (*Parus major*), crested tit (*Lophophanes cristatus*), long-tailed tit (*Aegithalos caudatus*), common chiffchaff (*Phylloscopus collybita*), western Bonelli's warbler (*Phylloscopus bonelli*), Eurasian blackcap (*Sylvia atricapilla*), European robin (*Erithacus rubecula*), song thrush (*Turdus philomelos*) and blackbird (*Turdus merula*).

# Experimental design

We investigated the effects of bird exclusion in interaction with plant neighbour removal on arthropod abundance and insect herbivory in *Q. robur* seedlings planted in nine mixed forest stands of pine and pedunculate oak. These stands were located at least 0.5 km and at most 8 km far from each other. All pine stands were 40–45 years of age and established on sandy soil in similar bioclimatic conditions.

In February 2009, 12 seedlings of *Q. robur* were transplanted in each forest stand. Each stand was an even-aged pine plantation and included extensive midstorey and understorey vegetation, dominated by pedunculate oak (mature trees, saplings and seedlings). The 108 target seedlings (12 seedlings × nine stands) were 1 year old, came from nursery and all originated from the same area of south-western France. Using seedlings with similar growing conditions dampened the variability of size, number of leaves, defense compounds and storage amounts of seedlings in comparison with the growth in natural conditions. The mean ( $\pm$  SE) seedling height measured  $46 \pm 1$  cm and there were, on average,  $46 \pm 2$  leaves per seedlings.

In each of the nine forest stands, the 12 seedlings were split into four triplets in a two-factorial design in order to test the interactive effects of bird exclusion and plant neighbour removal. We assessed the effect of bird exclusion by protecting two of the four triplets of seedlings within cages and leaving

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the other two uncaged. Bird exclosures consisted of wire fences with a mesh size of  $15 \times 15$  mm fixed to metal stakes and a volume of 1 m<sup>3</sup>. These exclosures excluded all insectivorous birds, including the smallest species, T. troglodytes, but allowed access to seedlings to all foliage-dwelling arthropods. The exclosures had no effect on microclimate that would have affected insects or plant growth (Giffard et al., 2012a). We assessed the effect of plant neighbour removal by leaving the triplets of seedlings under two neighbourhood conditions. Above-ground vegetation was either left intact (one protected within cages and one uncaged) or cut over 1 m<sup>2</sup> around two triplets of seedlings (one protected within cages and one uncaged) before transplantation and was kept down by mowing throughout the growing season. Neighbouring vegetation was mostly composed of P. aquilinum and M. cærulea and the mean ( $\pm$  SE) vegetation height was  $77 \pm 3$  cm in control treatments. The four experimental units were located at least 10 m apart within a single stand, to lessen potential carryover effects of neighbouring treatments on bird foraging. Forest stands were at least 0.5 km apart, allowing different bird foraging areas.

#### Leaf area measurements

We counted the total number of leaves per focal seedling during two consecutive surveys in the early (mid-June 2009) and late (early September 2009) growing seasons. We estimated the total leaf area of each seedling by measuring, with a 1 cm<sup>2</sup> plastic grid, the area of 20 leaves sampled at regular intervals within the seedling foliage (e.g. every third leaf for a seedling with 60 leaves). In a preliminary test with 114 oak seedlings, we found a significant correlation (r = 0.985, P < 0.001) between total leaf area based on the measurement of all leaves and total leaf area estimated from a sample of 20 leaves. Moreover, leaf area estimated with the 1 cm<sup>2</sup> plastic grid was significantly correlated (r = 0.986, P < 0.001) with leaf area measured with a WinFolia planimeter (Pro 2007b, Regent Instruments, Quebec, Canada) on a subsample of 340 oak leaves.

#### Arthropod sampling

During two consecutive surveys in mid-June and early September 2009, arthropods were exhaustively counted on all seedlings and assigned to lepidopteran larvae, sawfly larvae, spiders or leaf miners. We also noted weevils (Coleoptera Curculionidae), grasshoppers (Orthoptera Tettigoniidae) and leaf rollers (Lepidoptera), but these groups were not sufficiently abundant for inclusion in the statistical analyses. Leaf miners are specialist herbivores and the species feeding on *Q. robur* are mostly small moths (Lepidoptera). We calculated the densities of total external arthropods, and of all the subgroups of external arthropods exposed to bird predation–lepidopteran larvae, sawfly larvae and spiders–as the number of individuals m<sup>-2</sup> of leaf area for each experimental unit. We calculated separately the densities of leaf mines m<sup>-2</sup> of leaf area (concealed from predation).

#### Insect herbivory measurements

Insect herbivory was assessed twice during the growing season, following arthropod sampling, and in a non-destructive manner by visual inspection of all the leaves on every seedling. Damaged leaf area was estimated with a 0.25 cm<sup>2</sup> transparent plastic grid. Leaf damage was classified on the basis of the feeding guild: chewers, skeletonisers (exposed to bird predation) or leaf miners (concealed from bird predation). We divided the total damaged leaf area by the total leaf area to calculate a percentage herbivory per guild in each seedling (LAR: Leaf Area Removed). Within a given experimental unit, observations performed on the three seedlings were not independent. We therefore used the mean herbivory or arthropod densities for further statistical analyses, which are the mean across the three seedlings of a given experimental unit.

#### Statistical analyses

We modelled percentage herbivory by chewers and skeletonisers in linear mixed models (LMMs with the NLME Rpackage, Pinheiro et al., 2011) with previous logit transformation (Warton & Hui, 2011) and using bird exclusion (BE), plant neighbour removal (PNR), and their interaction (BE × PNR) as fixed factors, and forest stands and date of measurement as a random grouping effect. Using these two nested random effects allows for the spatial correlation of model residuals for the four experimental treatments in the same forest stand, and the temporal correlation of model residuals of measures performed on the same seedlings, in June and September. We modelled the total densities of external arthropods (excluding the concealed guild of leaf miners), the densities of lepidopteran larvae, sawfly larvae, leaf miners and spiders, and the percentage herbivory by leaf miners in generalised linear mixed models (GLMMs with the LME4 R-package, Bates et al., 2011) fitted with a Poisson distribution and using a log link function and Laplace approximation for count data, with the same fixed and random factors as in the LMMs.

The significance of each fixed effect was tested by comparisons of model deviances fitted with and without treatment (Zuur et al., 2009). We removed the non-significant interaction term (BE  $\times$  PNR) of the models in order to test the simple effect of BE and PNR (P > 0.05). By contrast, a significant effect of the interaction term BE × PNR indicated the presence of an effect of BE on arthropod densities or percentage herbivory per guild that was different when plant neighbours were removed or not and/or an effect of the PNR on arthropod densities or percentage herbivory per guild that was different between exclusion cages or control plots with bird predation (see the 'Results' section). To identify treatment effects on arthropod densities, we tested the effect of BE separately for each condition of plant neighbourhood, and the effect of PNR separately for control and bird exclosures, using model contrasts. For significant effects in GLMMs, we set a more conservative significance level of 1%, as recommended by Zuur et al. (2009).

We finally investigated the potential relationship between damage from external insects and abundance of leaf miners,

**Table 1.** Results of generalised linear mixed models (GLMMs) and linear mixed models (LMMs) for testing the effect of bird exclusion (BE), plant neighbour removal (PNR) and their interaction on the density of total external arthropods (external-feeding and predatory arthropods), and subgroups analysed separately: lepidopteran larvae (external feeding), sawfly larvae (external feeding), and spiders (predatory arthropods), the density of leaf mines (concealed feeding), and percentage herbivory by chewers, skeletonisers and leaf miners, on *Quercus robur* seedlings.

	df	BE	PNR	$BE \times PNR$
Total external arthropods	1	<b>97.33</b> ***	0.75	0.36
Lepidopteran larvae	1	<sup>†</sup>	†	12.49***
Sawfly larvae	1	10.75*	57.43***	0.03
Spiders	1	†	†	30.96***
Internal (concealed) feeders				
Leaf miners	1	†	†	255.59***
Percentage herbivory by chewers	1, 51	†	†	$4.80^{*}$
Percentage herbivory by skeletonisers	1, 51	0.02	<b>9.94</b> **	0.16
Percentage herbivory by leaf miners	1	0.01	0.02	0.03

Significance of each treatment and their interaction were tested by deviance comparison between models fitted with and without the factors BE, PNR or BE × PNR for GLMM. Significant *F*- and  $\chi^2$  values are shown in bold (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001). †Corresponds to simple effects of BE and PNR that have not been tested because of a significant interactive effect BE × PNR (see the 'Results'

section and Tables S1 and S2 for details).

suggesting competitive interactions, using GLMM with percentage herbivory of external insects (skeletonisers and chewers) as the explanatory variable for leaf mine density (and forest stand and date of measurement as a random grouping effect, as in previous analyses).

#### Results

#### Arthropod densities

Only the BE factor significantly affected the density of total external arthropods-not PNR or the interaction term (Table 1). The mean density of total external arthropods was approximately 50% lower for uncaged seedlings ( $6.48 \pm 1.07$  individuals m<sup>-2</sup> of leaf area) than for seedlings protected by cages ( $12.85 \pm 1.19$  individuals m<sup>-2</sup> of leaf area).

Experimental treatments had different effects on the density of the individual subgroups of external arthropods exposed to bird predation and that of leaf miners. PNR and BE factors significantly impacted the densities of sawfly larvae, but not the interaction term (Table 1). The mean density of sawfly larvae significantly increased when plant neighbours were removed (compare right- and left-hand bars in Fig. 1b). Bird predation also significantly reduced sawfly larvae densities (compare white and black bars in Fig. 1b).

The interaction between the PNR and BE factors had a significant effect on the densities of lepidopteran larvae, spiders and leaf miners (Table 1). We therefore tested the effect of PNR separately for control and bird exclusion conditions, and the effect of BE separately in the presence of absence of plant neighbours.

In the presence of birds (control treatments), lepidopteran larval density decreased with PNR (Table S1 and white bars in Fig. 1a). When birds were excluded, we did not observe any effect of PNR on lepidopteran larval densities (Table S1, black bars on Fig. 1a). When plant neighbours were left intact, there was no effect of bird predation on lepidopteran larval density (Table S2 and right-hand bars in Fig. 1a). When plant neighbours were removed, lepidopteran larval density decreased with bird predation (Table S2 and right-hand bars in Fig. 1a).

In the presence of birds (control treatments), spider densities increased with PNR (Table S1 and white bars in Fig. 1d). When birds were excluded, we did not observe any effect of PNR on spider densities (Table S1 and black bars on Fig. 1d). Spider density decreased with bird predation only when plant neighbours were left intact (Table S2 and left-hand bars in Fig. 1d), but there was no effect of bird predation on spider density when plant neighbours were removed (Table S2 and right-hand bars in Fig. 1d).

In the presence of birds, leaf mine density increased significantly with PNR (Table S1 and white bars in Fig. 1c), whereas it decreased significantly with PNR within bird exclosures (Table S1 and black bars in Fig. 1c). Bird predation reduced leaf mine density when plant neighbours were left intact (Table S2 and left-hand bars in Fig. 1c), but increased leaf mine density when plant neighbours were removed (Table S2 and right-hand bars in Fig. 1c).

#### Herbivory

The interaction between PNR and BE also had a significant effect on herbivory by chewers (Table 1). In the presence of birds, chewer damage decreased with PNR (Table S1 and white bars in Fig. 2a). When birds were excluded, we did not observe any effect of plant neighbour removal on chewer damage (Table S1 and black bars in Fig. 2a). There was no effect of bird predation on chewer damage when the plant neighbours were left intact (Table S2 and right-hand bars in Fig. 2a), whereas percentage herbivory by chewers decreased with bird predation when the plant neighbours were removed (Table S2 and right-hand bars in Fig. 2a).

Only the PNR factor had a significant impact on percentage herbivory by skeletonisers-not BE or the interaction term

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 $\mathrm{m}^{-2}$ number Fig. 1. Mean density [mean leaf area  $(nb m^{-2} LA) + 1 SE$ ] of lepidopteran larvae (a), sawfly larvae (b), leaf mines (c) and spiders (d) on experimental oak seedlings. The plant icon with a cross through it indicates that plant neighbours were removed. The bird icon with a cross through it indicates that seedlings were caged, impeding bird predation. The significance of each treatment, bird exclusion (BE) and plant neighbour removal (PNR), and their interaction,  $BE \times PNR$ , is indicated (ns, non-significant, nt, non tested, see the 'Results' section and Tables S1 and S2 for details when the interaction is significant).



**Fig. 2.** Mean (% + 1 SE) percentage herbivory on experimental *Quercus robur* seedlings by chewers (a), skeletonisers (b) and leaf miners (c). The plant icon with a cross through it indicates that plant neighbours were removed. The bird icon with a cross through it indicates that seedlings were caged, impeding bird predation. The significance of each treatment, bird exclusion (BE) and plant neighbour removal (PNR), and their interaction, BE × PNR, is indicated (LAR, leaf area removed, ns, non-significant, nt, non tested, see the 'Results' section and Tables S1 and S2 for details when the interaction is significant).

(Table 1). Percentage herbivory by skeletonisers decreased when plant neighbours were removed (compare left- and right-hand bars in Fig. 2b).

On average, leaf miners caused only a small amount of damage and none of the experimental factors had a significant effect on percentage herbivory by leaf miners (Table 1, Fig. 2c). However, we observed a strong negative relationship between leaf mine density and percentage herbivory by external insects (chewers and skeletonisers together,  $\chi^2 = 49.76$ , z = -6.889, P < 0.001).

#### Discussion

#### Effect of plant neighbours on arthropod abundance and insect herbivory in uncaged oak seedlings

These results obtained for the control plots provide new experimental evidence that neighbouring vegetation affects the abundance of herbivorous insects and the damage they cause to a focal plant. Caterpillar abundance and damage due to external herbivorous insects (chewers and skeletonisers) were significantly higher on seedlings with plant neighbours. Higher insect abundance and greater damage in the presence of plant neighbours are consistent with the hypothesis of associational susceptibility: the presence of neighbouring vegetation increases the likelihood of colonisation by herbivores and associated damage to a focal plant (White & Whitham, 2000; Barbosa et al., 2009). Tree saplings may receive more herbivores and may therefore experience higher herbivory damage due to vertical transfer of insect herbivores from canopy trees or horizontal shifts from plant neighbours (White & Whitham, 2000; Pigot & Leather, 2008; Schuldt et al., 2010). In our study, the understorey vegetation may have the same negative impact, although we cannot confirm this hypothesis, as we did not survey insect herbivory on plant neighbours.

Plant neighbour removal may also have changed light and microclimate conditions around seedlings, which can affect insect herbivores directly or indirectly through leaf quality. Free-living insects, and especially young larvae, may be susceptible to desiccation or may prefer to feed within the concealed microhabitat provided by plant neighbours (Larsson *et al.*, 1997).

Conversely, the abundance of leaf miners was lower on seedlings with plant neighbours. Plant neighbours can limit the colonisation of a focal species by specialist insects through disruption of host-finding (Root, 1973; Barbosa *et al.*, 2009). The leaf miner guild is expected to be composed mainly of species specialising on pedunculate oak (Giffard *et al.*, 2012b) and unable to feed on neighbouring shrub species. Neighbouring plants may act as physical (Ross *et al.*, 1990) or chemical (Jactel *et al.*, 2011) barriers to tree colonisation by female moths prior to oviposition. However, some insect herbivores also prefer leaves grown in full light, which are higher in nitrogen content (Moore *et al.*, 1988).

## *Effect of bird predation on arthropod abundance and insect herbivory in oak seedlings*

The exclusion of insectivorous birds resulted in a higher density of total external arthropods, regardless of the presence or absence of plant neighbours. These results are consistent with previous experimental studies in which bird exclusion led to increased arthropod abundance (reviewed by Whelan *et al.*, 2008; Mooney *et al.*, 2010; Piñol *et al.*, 2010; Mäntylä *et al.*, 2011). However, in the present study, arthropod subgroups showed different responses to bird exclusion in the two plant neighbourhood conditions. Avian predation reduced spider densities on oak seedlings only in the presence of plant neighbours; reduced lepidopteran larval densities only in the absence of plant neighbours; and reduced sawfly larvae densities in both cases. Prey accessibility may account for these contrasting responses and is generally considered more important than prey abundance for microhabitat selection by foraging birds (see, e.g., Whelan, 2001; Romanowski & Zmihorski, 2008). When plant neighbours are taller than seedlings, they can impede bird foraging for lepidopteran larvae by reducing prey availability, especially for foliage-gleaning birds (Moorman et al., 2007). Conversely, understorey gleaners such as the Eurasian wren (T. troglodytes) prefer foraging for arachnids on dense patches of vegetation (Kristin & Patocka, 1997; Artman, 2003), which could explain why foraging on spiders has not been impeded by plant neighbours. Further studies on bird foraging intensity and predation behaviour are therefore required, notably through recording of foraging activities or avian insectivory (Barbaro et al., 2012).

As an alternative hypothesis, a release of intraguild predation may explain why lepidopteran larval abundance was not reduced by bird predation in the presence of plant neighbours. Predatory arthropods, such as spiders, like structurally complex vegetation and may therefore benefit from the absence of vertebrate predators (in caged seedlings) and then better control herbivorous insects (Mooney *et al.*, 2010). However, we observed no negative relationship between spider and lepidopteran larval densities, which does not support this hypothesis.

Consistent with the prey accessibility hypothesis, chewer damage was indirectly reduced by bird predation only when plant neighbours were removed. Insectivorous birds foraging on herbivorous larvae use a combination of olfactory and visual cues to locate their prey (Mäntylä *et al.*, 2008). The removal of neighbouring plants may have increased the probability of prey detection by insectivorous birds, resulting in higher rates of predation and a lower extent of herbivore damage.

Interestingly, we observed a consistent negative correlation between leaf mine density and external herbivory. This suggests that bird exclusion, allowing greater damage by external feeding insects, resulted in lower feeding resources (intact leaf area) or reduced leaf quality for leaf miners, thereby increasing resource or apparent competition between herbivores (Mazía et al., 2004; Tack et al., 2009). We also observed lower leaf miner abundance on seedlings with plant neighbours. The leaf miner guild is composed of species specialising on pedunculate oak and unable to feed on neighbouring shrub species. Plant neighbours can disrupt hostfinding behaviours, thereby limiting the colonisation of a focal species by specialist insects (Root, 1973; Barbosa et al., 2009). Neighbouring plants may act as physical or chemical barriers to tree colonisation by female moths prior to oviposition (reviewed by Barbosa et al., 2009).

#### Conclusion

Neighbouring plants clearly influence tritrophic interactions involving a focal plant, its herbivores and their vertebrate predators. The presence of plant neighbours increased the abundance of insect herbivores and herbivory damage on

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seedlings. This suggests horizontal transfers of damaging insects from neighbouring plants or a modification of oak leaf palatability due to increased competition with neighbouring plants. The small-scale removal of vegetation patches resulted in a higher intensity of bird predation on lepidopteran larvae and, indirectly, in lower levels of chewing damage on oak seedlings. By contrast, the presence of plant neighbours appeared to dampen the top-down effect of insectivorous birds, with the noticeable exception of spiders. Furthermore, our study provides experimental evidence that distinct guilds of insects associated with a focal plant species differ greatly in their sensitivity to bird predation and the presence of neighbouring plants. These results highlight the importance of neighbouring vegetation on multitrophic interactions with focal plants, herbivores and predators and support the use of manipulative experiments to disentangle top-down, bottom-up and horizontal interactions between species.

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#### **Supporting Information**

Additional Supporting Information may be found in the online version of this article under the DOI reference:

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Table S1. Results of GLMMs and LMM for testing the simple effect of plant neighbour removal when insectivorous birds were excluded by cages (bird exclosures) or not (control) on the densities of lepidopteran larvae (external feeding), leaf mines (concealed feeding) and spiders (predatory arthropods), and the percentage herbivory by chewers, on *Quercus robur* seedlings.

Table S2. Results of GLMMs and LMM for testing the simple effect of bird exclusion when plant neighbours were left intact or when plant neighbours were removed on the densities of lepidopteran larvae (external feeding), leaf mines (concealed feeding) and spiders (predatory arthropods), and the percentage herbivory by chewers, on *Quercus robur* seedlings.

#### References

- Agrawal, A.A. (2004) Resistance and susceptibility of milkweed: competition, root herbivory, and plant genetic variation. *Ecology*, 85, 2118–2133.
- Artman, V.L. (2003) Effects of commercial thinning on breeding bird populations in western hemlock forests. *The American Midland Naturalist*, 149, 225–232.

- Baraza, E., Zamora, R. & Hódar, J.A. (2006) Conditional outcomes in plant-herbivore interactions: neighbours matter. *Oikos*, **113**, 148–156.
- Barbaro, L. & van Halder, I. (2009) Linking bird, carabid beetle and butterfly life-history traits to habitat fragmentation in mosaic landscapes. *Ecography*, **32**, 321–333.
- Barbaro, L., Brockerhoff, E.G., Giffard, B. & van Halder, I. (2012) Edge and area effects on avian assemblages and insectivory in fragmented native forests. *Landscape Ecology*, 27, 1451–1463.
- Barber, N.A. & Marquis, R.J. (2009) Spatial variation in top-down direct and indirect effects on white oak (*Quercus alba L.*). *The American Midland Naturalist*, **162**, 169–179.
- Barbosa, P., Hines, J., Kaplan, I., Martinson, H., Szczepaniec, A. & Szendrei, Z. (2009) Associational resistance and associational susceptibility: Having right or wrong neighbors. *Annual Review of Ecology, Evolution, and Systematics*, 40, 1–20.
- Bates, D., Maechler, M. & Bolker, B. (2011) Ime4: Linear mixedeffects models using S4 classes. R package version 0.999375-39.
- Bridgeland, W.T., Beier, P., Kolb, T. & Whitham, T.G. (2010) A conditional trophic cascade: birds benefit faster growing trees with strong links between predators and plants. *Ecology*, **91**, 73–84.
- Corcket, E., Callaway, R.M. & Michalet, R. (2003) Insect herbivory and grass competition in a calcareous grassland: results from a plant removal experiment. *Acta Oecologica*, 24, 139–146.
- Dudt, J.F. & Shure, D.J. (1994) The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology*, **75**, 86–98.
- Elton, C.S. (1958) *The Ecology of Invasions by Animal and Plants*. Methuen, London,U.K.
- Finke, D.L. & Denno, R.F. (2005) Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecology Letters*, 8, 1299–1306.
- Forkner, R.E. & Hunter, M.D. (2000) What goes up must come down? Nutrient addition and predation pressure on oak herbivores. *Ecology*, 81, 1588–1600.
- Giffard, B., Corcket, E., Barbaro, L. & Jactel, H. (2012a) Bird predation enhances tree seedling resistance to insect herbivores in contrasting forest habitats. *Oecologia*, **168**, 415–424.
- Giffard, B., Jactel, H., Corcket, E. & Barbaro, L. (2012b) Influence of surrounding vegetation on insect herbivory: a matter of spatial scale and herbivore specialisation. *Basic and Applied Ecology*, 13, 458–465.
- Gripenberg, S. & Roslin, T. (2007) Up or down in space? Uniting the bottom-up versus top-down paradigm and spatial ecology. *Oikos*, 116, 181–188.
- Gruner, D.S. (2004) Attenuation of top-down and bottom-up forces in a complex terrestrial community. *Ecology*, **85**, 3010–3022.
- Gunnarsson, B. (1996) Bird predation and vegetation structure affecting spruce-living arthropods in a temperate forest. *Journal of Animal Ecology*, **65**, 389–397.
- Gunnarsson, B. (2007) Bird predation on spiders: ecological mechanisms and evolutionary consequences. *Journal of Arachnology*, 35, 509–529.
- Hairston, N.G., Smith, F.E. & Slobodkin, L.B. (1960) Community structure, population control, and competition. *The American Naturalist*, 94, 421–425.
- Hawkins, B.A., Cornell, H.V. & Hochberg, M.E. (1997) Predators, parasitoids, and pathogens as mortality agents in phytophagous insect populations. *Ecology*, **78**, 2145–2152.
- Jactel, H., Birgersson, G., Andersson, S. & Schlyter, F. (2011) Non-host volatiles mediate associational resistance to the pine processionary moth. *Oecologia*, **166**, 703–711.
- Kristin, A. & Patocka, J. (1997) Birds as predators of lepidoptera: selected examples. *Biologia*, **52**, 319–326.

- Langellotto, G.A. & Denno, R.F. (2004) Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. *Oecologia*, **139**, 1–10.
- Larsson, S., Häggström, H.E. & Denno, R.F. (1997) Preference for protected feeding sites by larvae of the willow-feeding leaf beetle *Galerucella lineola*. *Ecological Entomology*, 22, 445–452.
- Low, C. & Connor, E.F. (2003) Birds have no impact on folivorous insect guilds on a montane willow. *Oikos*, **103**, 579–589.
- Mäntylä, E., Alessio, G.A., Blande, J.D., Heijari, J., Holopainen, J.K., Laaksonen, T., *et al.* (2008) From plants to birds: higher avian predation rates in trees responding to insect herbivory. *PLoS ONE*, **3**, e2832.
- Mäntylä, E., Klemola, T. & Laaksonen, T. (2011) Birds help plants: a meta-analysis of top-down trophic cascades caused by avian predators. *Oecologia*, 165, 143–151.
- Marquis, R.J. & Whelan, C. (1996) Plant morphology and recruitment of the third trophic level: subtle and little-recognized defenses? *Oikos*, **75**, 330–334.
- Mazía, C.N., Kitzberger, T. & Chaneton, E.J. (2004) Interannual changes in folivory and bird insectivory along a natural productivity gradient in northern Patagonian forests. *Ecography*, 27, 29–40.
- Mooney, K.A., Gruner, D.S., Barber, N.A., Van Bael, S.A., Philpott, S.M. & Greenberg, R. (2010) Interactions among predators and the cascading effects of vertebrate insectivores on arthropod communities and plants. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 7335–7340.
- Moore, L.V., Myers, J.H. & Eng, R. (1988) Western tent caterpillars prefer the sunny side of the tree, but why? *Oikos*, 51, 321–326.
- Moorman, C.E., Bowen, L.T., Kilgo, J.C., Sorenson, C.E., Hanula, J.L., Horn, S., *et al.* (2007) Seasonal diets of insectivorous birds using canopy gaps in a bottomland forest. *Journal of Field Ornithologist*, 78, 11–20.
- Murakami, M. (1999) Effect of avian predation on survival of leafrolling lepidopterous larvae. *Research on Population Ecology*, **41**, 135–138.
- Pigot, A.L. & Leather, S.R. (2008) Invertebrate predators drive distance-dependent patterns of seedling mortality in a temperate tree *Acer pseudoplatanus*. *Oikos*, **117**, 521–530.
- Pinheiro, J., Bates, D., Debroy, S. & Sarkar, D., The R Development Core Team (2011) *nlme:linear and nonlinear mixed effects models*. R package version 3.1-100.
- Piñol, J., Espadeler, X., Cañellas, N., Martínez-Vilalta, J., Barrientos, J.A. & Sol, D. (2010) Ant versus bird exclusion effects on the arthropod assemblage of an organic citrus grove. *Ecological Entomology*, **35**, 367–376.

- Romanowski, J. & Zmihorski, M. (2008) Selection of foraging habitat by grassland birds: effect of prey abundance or availability? *Polish Journal of Ecology*, **56**, 365–370.
- Root, R.B. (1973) Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica* oleracea). Ecological Monographs, 43, 95–124.
- Ross, D.W., Berisford, C.W. & Godbee, J.F.J. (1990) Pine tip moth, *Rhyacionia* spp., response to herbaceous vegetation control in an intensively site-prepared loblolly pine plantation. *Forest Science*, **36**, 1105–1118.
- Savilaakso, S., Koivisto, J., Veteli, T.O. & Roininen, H. (2009) Microclimate and tree community linked to differences in lepidopteran larval communities between forest fragments and continuous forest. *Diversity and Distributions*, **15**, 356–365.
- Schuldt, A., Baruffol, M., Böhnke, M., Bruelheide, H., Härdtle, W., Lang, A.C., *et al.* (2010) Tree diversity promotes insect herbivory in subtropical forests of south-east China. *Journal of Ecology*, 98, 917–926.
- Schwenk, W.S., Strong, A.M. & Sillett, T.S. (2010) Effects of bird predation on arthropod abundance and tree growth across an elevational gradient. *Journal of Avian Biology*, 41, 367–377.
- Tack, A.J.M., Ovaskainen, O., Harrison, P.J. & Roslin, T. (2009) Competition as a structuring force in leaf miner communities. *Oikos*, 118, 809–818.
- Van Bael, S.A., Philpott, S.M., Greenberg, R., Bichier, P., Barber, N.A., Mooney, K.A., *et al.* (2008) Birds as predators in tropical agroforestry systems. *Ecology*, **89**, 928–934.
- Warton, D.I. & Hui, F.K.C. (2011) The arcsine is asinine: the analysis of proportions in ecology. *Ecology*, **92**, 3–10.
- Whelan, C.J. (2001) Foliage structure influences foraging of insectivorous forest birds: an experimental study. *Ecology*, 82, 219–231.
- Whelan, C.J., Weeny, D.G. & Marquis, R.J. (2008) Ecosystem services provided by birds. Annuals of the New York Academy of Sciences, 1134, 25–60.
- White, J.A. & Whitham, T.G. (2000) Associational susceptibility of cottonwood to a box elder herbivore. *Ecology*, 81, 1795–1803.
- Zehnder, C.B., Stodola, K.W., Cooper, R.J. & Hunter, M.D. (2010) Spatial heterogeneity in the relative impacts of foliar quality and predation pressure on red oak, *Quercus rubra*, arthropod communities. *Oecologia*, **164**, 1017–1027.
- Zuur, A.F., Ieno, E.F., Walker, N.J., Saveliev, A.A. & Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology With R*. Springer, Berlin, Germany.

Accepted 15 March 2013 First published online 5 June 2013 PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

### Bird predation enhances tree seedling resistance to insect herbivores in contrasting forest habitats

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Abstract According to the associational resistance hypothesis, neighbouring plants are expected to influence both the insect herbivore communities and their natural enemies. However, this has rarely been tested for the effects of canopy trees on herbivory of seedlings. One possible mechanism responsible for associational resistance is the indirect impact of natural enemies on insect herbivory, such as insectivorous birds. But it remains unclear to what extent such trophic cascades are influenced by the composition of plant associations (i.e. identity of 'associated' plants). Here, we compared the effect of bird exclusion on insect leaf damage for seedlings of three broadleaved tree species in three different forest habitats. Exclusion of insectivorous birds affected insect herbivory in a species-specific manner: leaf damage increased on Betula pendula seedlings whereas bird exclusion had no effect for two oaks (Ouercus robur and O. ilex). Forest habitat influenced both the extent of insect herbivory and the effect of bird exclusion. Broadleaved seedlings had lower overall leaf damage within pine plantations than within broadleaved stands, consistent with the resource concentration hypothesis. The indirect effect of bird exclusion on leaf damage was only significant in pine

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plantations, but not in exotic and native broadleaved woodlands. Our results support the enemies hypothesis, which predicts that the effects of insectivorous birds on insect herbivory on seedlings are greater beneath noncongeneric canopy trees. Although bird species richness and abundance were greater in broadleaved woodlands, birds were unable to regulate insect herbivory on seedlings in forests of more closely related tree species.

**Keywords** Tritrophic interactions · Insectivorous birds · Resource concentration hypothesis · Natural enemies hypothesis · Seedling herbivory

#### Introduction

The relative importance of different mechanisms regulating patterns of insect herbivory in plant communities is still a matter of debate. In particular, insect herbivory is thought to depend on intrinsic plant resistance traits, as a given plant species may experience different rates of herbivory depending on the identity and concentration of neighbouring plants. This is one of the principles of the "associational resistance" theory (Barbosa et al. 2009). Two main hypotheses have been proposed to explain associational resistance: the resource concentration hypothesis and the natural enemies hypothesis (Root 1973; Russell 1989). The resource concentration hypothesis states that the likelihood of a plant being located by herbivores, i.e. its apparency (Karban 2010), is influenced by the relative abundance and the nature of neighbouring plants. In more diverse plant communities, a given host plant is proportionally less frequent and can be hidden by neighbouring non-host plants. The natural enemies hypothesis suggests that richer plant assemblages offer a greater array of complementary food and habitat resources that benefit natural enemies which can, in turn, control herbivores more effectively (Root 1973; Russell 1989).

It has been suggested that the resource concentration hypothesis may account for a significant part in the reduction of damage from specialised herbivores within diverse plant communities (Finch and Collier 2000; Björkman et al. 2010). In particular, the association of noncongeneric plant species with contrasting morphological or semiochemical traits can greatly reduce the ability of hostspecific herbivores to locate, colonise and exploit their host plants (Jactel and Brockerhoff 2007). However, while some studies observed clearly reduced insect herbivory in more diverse forests (Jactel and Brockerhoff 2007), others found no clear evidence for this (Vehviläinen et al. 2006). Depending on the presence of host tree species in mixed forest, there can even be an opposite effect (Vehviläinen et al. 2007) that may be attributable to associational susceptibility (Barbosa et al. 2009).

Most previous studies on this subject focussed on canopy trees, but the same issues apply to seedlings and saplings which represent a critical developmental stage for forest regeneration. Interestingly, similarly varying responses of insect herbivory were observed on young trees along tree diversity gradients or underneath other adult tree species, with examples of more (White and Whitham 2000; Schuldt et al. 2010) or less damage (Maetô and Fukuyama 1997; Sobek et al. 2009). Tree seedlings can be expected to experience a higher risk of insect herbivore spillover from conspecific canopy trees (Maetô and Fukuyama 1997; Pigot and Leather 2008), according to the Janzen–Connell hypothesis (Norghauer et al. 2010).

Surprisingly few studies have investigated the role of natural enemies in controlling insect herbivory on tree seedlings along ecological gradients (Riihimäki et al. 2005; Kaitaniemi et al. 2007; Sobek et al. 2009). These provide some support for the occurrence of top-down control of forest insects by natural enemies, which may be tree species-dependent. However, these studies only focused on predatory insects. Vertebrates predators, such as birds (Bock et al. 1992), lizards (Dial and Roughgarden 1995) and bats (Kalka et al. 2008), can significantly reduce populations of herbivorous arthropods and indirectly limit damage and/or promote vegetation growth (Mooney et al. 2010; Mäntylä et al. 2011). However, the indirect effect of predators on plant biomass via a trophic cascade is highly variable due to compensation effects at the herbivore or plant levels (Pace et al. 1999).

In temperate forests, insectivorous birds are among the most important predators of herbivorous insects, especially Lepidoptera and Hymenoptera (Holmes et al. 1979; Glen 2004). Several studies have reported an increase of leaf damage when insectivorous birds were experimentally

excluded using caged plants (Atlegrim 1989; Marquis and Whelan 1994; Sipura 1999; Mazía et al. 2004; Van Bael et al. 2008), whereas others reported more limited effects (Forkner and Hunter 2000; Low and Connor 2003). Insectivorous birds are considered as an element of indirect plant defences (Price et al. 1980) with many documented top-down effects on insect herbivores (Whelan et al. 2008).

The identity of the focal plant species may be important to explain both the influence of natural enemies (Vehviläinen et al. 2008), such as insectivorous birds, and the effect of host concentration on insect herbivory. Plants differ in their physical characteristics (e.g. architecture, foliage structure), which provides insectivorous birds with a wide variety of distinct foraging substrates, which, in turn, may influence trophic cascades (Marquis and Whelan 1996; Whelan 2001). Plant species also present different constitutive defences (Sipura 1999) or volatile organic compounds (Mäntylä et al. 2008) that have been demonstrated to affect both insect herbivory and insectivorous bird foraging.

In this study, we investigated the importance of bird predation and host resource concentration as mechanisms affecting insect herbivory. We designed a manipulative experiment in the Landes de Gascogne forest, the largest pine plantation forest in Europe. Previous studies in such forests have shown that deciduous trees at the understorey level or as remnants of natural forests provide key resources or habitat to several insect and bird taxa in this context of conifer plantations (Barbaro et al. 2005; Brockerhoff et al. 2008; van Halder et al. 2008). To improve the management of planted forests for the benefit of biodiversity, it is therefore important to know more about how stand composition may affect the impact of insect damage on these broadleaved species at their most susceptible developmental stages, i.e. seedlings and saplings (Vásquez et al. 2007).

We tested the effect of the identity of the plant species by comparing the level of insect herbivory on seedlings of three native broadleaved species: silver birch (Betula pendula), pedunculate oak (Quercus robur) and holm oak (O. ilex). We then examined the resource concentration hypothesis, and, more specifically, the importance of taxonomic similarity between seedlings and canopy tree species, by experimentally planting seedlings of these native broadleaved trees within three forest habitats: native broadleaved, exotic broadleaved, and native conifer habitat. We hypothesised that deciduous seedlings planted within stands composed of conspecific deciduous tree species would experience higher leaf damage than when they are planted into conifer stands. To test the enemies hypothesis, we estimated the effect of removing bird predation on insect herbivory by planting seedlings within bird exclosure cages. We hypothesised that bird predation

would be higher in pine stands where deciduous seedlings represent sources of alternative prey.

#### Materials and methods

#### Study site

The study was carried out in the Landes de Gascogne forest, south-western France, a region covered by ca 10,000 km<sup>2</sup> of planted forests of native maritime pine Pinus pinaster. The climate is thermo-Atlantic and soils are podzols established on several meters of sandy deposit. The study sites are located ca 40 km south of Bordeaux (44°44'N, 00°46'W) near the field research centre of the French National Institute for Agricultural Research (INRA). This area is dominated by a mosaic of maritime pine plantations of different ages with small deciduous forest remnants. These woodlands are dominated by native pedunculate oak Quercus robur and silver birch Betula pendula with scattered holm oaks Q. ilex. A few plantations of exotic oak species (red oak Q. rubra and Bartram's oak Q. heterophylla) also occur in the area. The understorey vegetation is relatively species-poor, due to both nutrient-poor acidic soils and intensive stand management. Dominant understorey species are common bracken (Pteridium aquilinum), various herbaceous species such as Molinia cærulea and Pseudarrhenaterum longifolium, dwarf and common gorse (Ulex minor, U. europaeus), heather species (Erica scoparia, E. cinerea, Calluna vulgaris), glossy buckthorn (Frangula alnus) and European honeysuckle (Lonicera periclymenum).

#### Experimental design

To test for an effect of contrasting forest habitats, we selected 20 stands comprising 6 stands of maritime pine, 6 stands of exotic oaks (Q. rubra or Q. heterophylla) and 8 native woodlands dominated by Q. robur and B. pendula. In these 20 stands, we established two experimental units to compare two treatments: control and bird exclusion. Both treatments were located in the same stand but 5-10 m from each other to avoid any potential disturbance by birds foraging in control areas. The bird exclosure was a cage measuring of  $100 \times 100 \times 100$  cm fenced off with wire netting  $(15 \times 15 \text{ mm mesh})$ . This treatment allowed insects to access seedlings while excluding all insectivorous birds including the smallest species recorded from the experimental plots (wren, Troglodytes troglodytes, and firecrest, Regulus ignicapillus). In the control treatment, seedlings were left uncaged so that herbivorous insects were left exposed to bird predation. A comparison of the microclimatic conditions between treatments was undertaken at the beginning of the experiment (using Hobo; Onset Computer, Bourne, USA). This showed that exclusion cages had no significant effect on soil humidity, air temperature and relative humidity (Wilcoxon matched-pairs signed-ranks tests).

In March 2007, two seedlings each of silver birch *B. pendula*, *Q. robur* and *Q. ilex* were transplanted together as an experimental unit of six seedlings. Seedlings were 1 year old and had the same south-western France origin. They were planted ca. 40 cm apart from each other and the position of each species was randomly assigned. Experimental units were protected by an exclusion cage (cage treatment) or left uncaged (control). The same paired design was replicated in the 20 experimental forest stands, for a total of 240 transplanted seedlings (2 seedlings  $\times$  3 species  $\times$  2 experimental units  $\times$  20 stands).

#### Herbivory measures

Nondestructive assessments of insect herbivory were performed by visual inspection of all leaves on every seedling. Damaged leaf area was estimated using transparent plastic grids of two different mesh sizes  $(0.25 \text{ and } 1 \text{ cm}^2)$ . The smaller grid was used on leaves smaller than  $6 \text{ cm}^2$  for greater accuracy. A percentage of leaf area removed (LAR) was calculated for each leaf and averaged per seedling. Intact leaves were recorded as 0% LAR. Within the same unit, the two seedlings of the same species were considered as pseudo-replicates and their herbivory rates were averaged. The response variable was therefore calculated as the percent LAR per species for each experimental unit. Herbivory measures were performed during two growth seasons (2007 and 2008), with assessments in May (early season) and July (mid-season) and a final assessment in September 2008.

#### Bird censuses

Bird communities were censused in nine of the sampled stands, providing three representative replicates of the three forest habitats. We used the point-count method with two visits during early and late breeding seasons of 2007. We recorded all birds heard and seen during the two 20-min visits and used the maximal abundance across the two visits for a given species to obtain species richness and abundance for each stand (see Barbaro et al. 2005). We classified all the species recorded according to their diet and foraging guild (Barbaro and van Halder 2009), and kept for further analyses only the insectivorous species likely to forage on the experimental seedlings (Electronic Supplemental Material Table S1).

#### Statistical analyses

To compare herbivory rates between seedling species and forest habitats, linear mixed-model analyses (Pinheiro and Bates 2004) were performed with the procedure *lme* (R software; R Development Core Team 2008) using LAR estimated in the control treatment at each period of assessment as response variables. We used fixed effects for seedling species, forest habitat and their interaction and a random effect for forest stand within forest habitat.

To test the effect of bird exclusion on herbivory rates according to forest habitats, we performed a second set of linear mixed-model analyses. We used fixed effects for forest habitat, bird exclusion treatment and their interaction and a random effect for forest stand within forest habitat. Analyses were performed separately on LAR values for each seedling species and each date of assessment. Repeated-measures analysis was not used to avoid confounding within-year and between-year repeated measures.

In all linear mixed-models, LAR data were arc-sine transformed to meet the assumptions of normality and homoscedasticity, which were verified by graphical analyses and Shapiro–Wilk tests on model residuals. Multiple comparisons were conducted to test for difference between seedling species or forest habitats using Tukey's HSD test.

Insectivorous bird richness and abundance were analysed by Kruskal–Wallis rank sum tests followed by Behrens–Fisher multiple comparisons to test for difference between the three forest habitats.

Ultimately, we estimated the magnitude of the effect of bird exclusion on LAR in seedlings throughout the 2 years of assessments (i.e. the difference in LAR between control and exclusion cage treatments). We tested how this magnitude varied with seedling species and forest habitat (Nakagawa and Cuthill 2007) by calculating Hedge's effect size (d) for each combination of seedling species  $\times$  forest habitat, using stands within habitat types as replicates. All censuses were pooled following the method proposed by Borenstein et al. (2009) for repeated measurements within a study. For each combination of seedling species  $\times$  forest habitat, mean LAR values were averaged across the five census dates. The averaged standard deviation was calculated as the mean standard deviations in each date weighted by the correlation coefficients between dates. The effect size was calculated as:

$$d = J[(X_{BE} - X_{CTL})/S]$$

with  $X_{BE}$  representing the mean LAR per species and forest habitat in the bird exclusion treatment,  $X_{CTL}$  the mean LAR for the control treatment, *S* the pooled standard deviation and *J* a factor that corrects for potential bias due to small sample sizes (Hedges and Olkin 1985). An effect size was considered as significantly different from zero if its bias-corrected bootstrap confidence interval did not bracket the null value (based on 9,999 iterations). Metaanalyses were carried out using METAWIN 2.0 software (Rosenberg et al. 2000).

#### Results

Seedling species response to insect herbivory

The three seedling species exhibited significantly different defoliation levels (LAR) in the control treatment for all the five assessment periods over the two study years (F = 18.64, 45.74, 16.82, 10.26 and 21.34, respectively, P < 0.01). *Quercus ilex* seedlings were less damaged ( $7.5 \pm 0.6\%$  mean LAR  $\pm$  SE) than the deciduous species, *Q. robur* (17.8  $\pm$  1.1%) and *B. pendula* (20.2  $\pm$  1.7%). The two deciduous species did not show any significant difference in LAR, except in July 2007 when *B. pendula* seedlings were more damaged than *Q. robur* seedlings (|z| = 3.17, P = 0.005) and in May 2008 when *Q. robur* seedlings were more damaged than *B. pendula* (|z| = 3.78, P = 0.001). Due to the strong seedling species effect on insect herbivory, further analyses on the effects of forest habitat and bird exclusion were therefore conducted separately for each species.

Effect of forest habitat on insect herbivory

Forest habitat had a significant effect on insect herbivory in the control treatment where birds were not excluded, but this effect differed between the three seedling species and the five periods of assessment. LAR in B. pendula seedlings was significantly lower in pine plantations than in exotic and native oak and birch woodlands only in May 2007 (Table 1; Fig. 1). For Q. ilex seedlings, no effect of forest habitat on herbivory was detected in 2007, but in May and September 2008 insect herbivory was significantly lower within pine plantations than within native oak and birch woodlands (Fig. 1; Table 1). Herbivory was intermediate within exotic oak woodlands. Forest habitat had a strong effect on herbivory for Q. robur seedlings in 2007, LAR being consistently lower within pine plantations than within native oak woodlands (Fig. 1; Table 1). Again herbivory rates were intermediate within exotic oak woodlands. These trends persisted in May and July 2008, but differences were not significant because of large between-stand variations.

# Effect of forest habitat on insectivorous bird communities

Forest habitat had a significant effect on insectivorous bird richness and abundance (Kruskal–Wallis H = 6.83,

Table 1 Results of Tukey's

HSD tests of the effects of forest habitat on leaf area removed

(LAR %) by herbivorous insects for three seedling species

Forest	habitat	Betula pendula	Quercus robur	Quercus ilex
Pine v	ersus native	0.55	2.54*	0.15
Pine v	ersus exotic	1.49	1.80	1.26
Exotic	versus native	1.05	0.62	1.50
Pine v	versus native	0.80	2.54*	1.86

0.43

1.26

1.64

1.38

0.46

0.82

1.65

0.72

0.83

2.59\*\*

3.96\*\*\*

Pine versus exotic Exotic versus native

Pine versus native

Pine versus exotic

Pine versus native

Pine versus exotic

Pine versus native

Pine versus exotic

Exotic versus native

Exotic versus native

Exotic versus native

May 2008Values show |z|-statistics for<br/>each comparisonPine pine plantations, Native<br/>native deciduous woodlands,<br/>Exotic exotic deciduous<br/>woodlands\* P < 0.05\*\* P < 0.01\*\*\* P < 0.001

Date

May 2007

July 2007

P = 0.033 and H = 6.54, P = 0.038, respectively). More bird species and individuals occurred in exotic and native deciduous woodlands than in pine plantations (multiple comparisons all significant, P < 0.001). Native deciduous woodlands had the highest mean richness ( $R \pm SE$ ) and abundance ( $A \pm SE$ ) of insectivorous birds ( $R = 14.7 \pm$ 0.7 and  $A = 29.0 \pm 2.0$ ), exotic deciduous woodlands were intermediate ( $R = 13.3 \pm 0.3$  and  $A = 23.3 \pm 1.3$ ), whereas pine plantations had the lowest richness and abundance ( $R = 9.7 \pm 0.3$  and  $A = 19.5 \pm 0.6$ ).

#### Effect of bird exclusion on insect herbivory

For B. pendula seedlings, mixed-models analyses showed that herbivory rates in the bird exclusion treatment were significantly higher than in the control treatment in July 2007 and May 2008 (Table 2). Values of LAR were about 50% higher in July 2007 and twice as high in May 2008, and, although non-significantly, apparently higher in the bird exclusion treatment at the other assessment periods (Fig. 2). For none of the five periods of herbivory measure was the bird exclosure × forest habitat interaction term significant, indicating that these two effects operate independently of each other. Bird predation, therefore, contributed to reduced insect herbivory in birch seedlings. In contrast, we never observed any significant effect of bird exclusion on insect herbivory in Q. robur and Q. ilex seedlings (Table 2), except in May 2007 on Q. robur seedlings. The bird exclosure  $\times$  forest habitat interaction term was significant due to a significant effect of forest habitat on herbivory in the control treatment (see Table 1) with no significant effect of bird exclosure.

1.68

0.06

2.44\*

0.41

2.00

2.29

1.48

0.71

1.28

1.87

3.23\*\*

Overall effect of forest habitat on bird insectivory

Insect herbivory on the three seedling species showed different responses to bird exclusion when data for all assessment periods were pooled (Fig. 3a). In *B. pendula* seedlings, LAR was significantly higher in bird exclusion cages than in control treatment ( $d_+ = 0.55$  with bias CI = 0.34–0.69) indicating that bird predation reduced insect herbivory throughout the 2 years of measures. For the two oak species, there was no significant effect of bird exclusion on LAR ( $d_+ = -0.02$  with bias CI = -0.31 to 0.51 for *Q. robur* and  $d_+ = 0.16$  with bias CI = -0.24 to 0.35 for *Q. ilex*).

2.09

0.30

2.11

1.92

0.07

2.00

1.70

0.17

0.34

0.11

0.22

Forest habitat also influenced the effect of bird exclusion on insect herbivory in a similar way for the three seedling species as they shared the same gradient of response with higher bird insectivory within pine plantations than within native and exotic deciduous stands (Fig. 3b). Within pine plantations, bird exclusion had a significant effect on insect herbivory with higher damage in bird exclusion cages irrespective of the seedling species ( $d_+ = 0.56$  with bias CI = 0.40–0.77). Conversely, there was no significant effect of bird exclusion in native ( $d_+ = 0.24$  with bias CI = -0.05 to 0.55) or exotic deciduous woodlands ( $d_+ = 0.14$  with bias CI = -0.49 to 0.33), irrespective of the seedling species (Fig. 3b).

#### Discussion

The resource concentration hypothesis

Our results provide new experimental evidence for the importance of resource concentration in the regulation of



**Fig. 1** Mean (+SE) percent leaf area removed (% *LAR*) by insects in control treatment on **a** *Betula pendula*, **b** *Quercus robur* and **c** *Quercus ilex* seedlings planted in pine plantations (*white bars*), exotic woodlands (*grey bars*) and native woodlands (*black bars*) at the five assessment dates. Analyses were performed separately for each seedling species and each assessment date and different *letters above bars* indicate significant differences between forest habitats (at P < 0.05)

insect herbivory, a mechanism of associational resistance (Root 1973). For the three broadleaved species tested, seedlings under non-broadleaved canopy trees (maritime pines) were less damaged than those planted within stands of broadleaved tree species. These results are consistent with previous observations of lower insect damage on seedlings or saplings grown in mixed stands than in pure stands of the same canopy species (Batzer et al. 1987; Keenan et al. 1995), and with decreased insect damage on beech and Acer saplings with higher abundance of conspecific adult trees (Maetô and Fukuyama 1997; Pigot and Leather 2008; Sobek et al. 2009). The Janzen–Connell hypothesis predicts that seedlings growing close to mother trees suffer from more damage or higher mortality than seedlings growing farther away (Lemen 1981; Norghauer et al. 2010). The resource concentration hypothesis also predicts that specialised herbivores are more likely to find a host plant in stands where this particular species is more abundant (Root 1973). Our results are consistent with both hypotheses: insect herbivory on seedlings was higher beneath a canopy of congeneric trees (i.e. on oak seedlings within native deciduous woodlands dominated by oaks) and lower when the host plant abundance decreased (i.e. on oak seedlings within pine plantations).

It is noteworthy that we observed the most significant associational resistance in Quercus seedlings which is a genus abundant in the canopy of deciduous woodlands. By contrast, associational susceptibility was the least in B. pendula, a species much less frequent in the study area. Seedlings of this rare species probably recruited mainly generalist herbivores, which could increase the risk of associational susceptibility (White and Whitham 2000). Associational susceptibility seems to be most prevalent when the herbivore species is highly polyphagous because generalist herbivores may benefit from mixed forests with multiple host tree species, whereas specialist herbivores are limited by the amount of suitable host trees (Jactel and Brockerhoff 2007). Oak seedlings were more likely to be colonised by specialised oak feeders, which could have led to increased associational resistance.

#### The natural enemies hypothesis

Associational resistance may also be provided by natural enemy populations which benefit from greater resources provided by plant associations and can therefore provide control of herbivores more effectively (Russell 1989; Barbosa et al. 2009). Changes in the composition of dominant canopy species assemblages or along tree diversity gradients have been shown to increase species richness and abundance of natural enemies such as predatory ants (Riihimäki et al. 2005), staphylinids (Vehviläinen et al. 2008) or birds (Greenberg et al. 1997). In tropical agroecosystems, arthropod abundance decreased when bird species richness increased (Philpott et al. 2009), and bird predation is enhanced by higher floristic diversity (Perfecto et al. 2004). Our study also provides experimental evidence of the natural enemies hypothesis since the more diversified association between seedlings and canopy species (broadleaved tree seedlings within pine stands) resulted in more intense bird predation. Surprisingly, in our experiment, pine plantations had lower insectivorous bird richness and abundance compared to deciduous woodlands, although we found a higher effect of bird predation in pine plantations than in deciduous woodlands. Some authors found no difference in indirect effect of insectivorous birds on plant damage within different forest stand types while bird abundance varied significantly between stands (Greenberg et al. 2000; Barber and Marquis 2009).

<b>Table 2</b> Mixed-model analyses           for the effect of bird exclusion	Date	Treatment	Betula pendula	Quercus robur	Quercus ilex
(BE) and forest habitat $(FH)$ on	May 2007	FH	1.25	2.83	1.04
leaf area removed (LAR %) by		BE	0.45	0.23	2.80
seedling species ( <i>B. pendula</i> ,		$FH \times BE$	0.02	3.67*	0.99
Q. robur and Q. ilex)	July 2007	FH	2.43	2.06	4.11*
		BE	6.32*	2.24	0.04
		$FH \times BE$	1.20	0.44	1.24
May 2008 July 2008	May 2008	FH	2.97	3.47	7.11**
		BE	8.65**	0.01	0.83
		$FH \times BE$	0.56	0.16	0.05
	July 2008	FH	1.59	1.61	5.10*
	-	BE	0.03	1.79	0.49
		$FH \times BE$	0.75	0.75	0.13
Values show F-statistics for	September 2008	FH	8.10**	0.05	5.14*
each model * $P < 0.05$		BE	0.02	0.04	0.79

 $FH \times BE$ 

2.42

\*\* P < 0.01



Fig. 2 Mean ( $\pm$ SE) percent leaf area removed (% *LAR*) by insects in control (white bars) and bird exclusion (grey bars) treatments on Betula pendula seedlings, irrespective of the forest habitat. Asterisks above bars indicate significant differences between treatments (\*\*P < 0.01, \*P < 0.05)

Conversely, González-Gómez et al. (2006) found that bird insectivory on seedlings, as measured by larval predation, was similar in exotic pine plantations and native forest fragments although bird abundance was lower in plantations. We hypothesised that broadleaved tree seedlings may have provided new feeding resources for insectivorous birds in the pine stands. Conversely, in deciduous woodlands where insect herbivores are likely to be more abundant, the effect of bird predation may have been weakened by a dilution process.

#### Effect of seedling identity on trophic cascades

In our study, the effects of insectivorous birds affected insect herbivory in the three seedling species differently,

and irrespective of the observed effect of contrasting forest habitat. Bird exclusion led to a significant increase of insect herbivory on B. pendula seedlings whereas no effect was detected for the two oak species, Q. robur and Q. ilex. The identity of the plant species is therefore important as it influences the indirect effect of bird predation. Bird predation decreased insect leaf damage on many woody species such as Vaccinium myrtillus (Atlegrim 1989), Quercus alba (Marquis and Whelan 1994) or Nothofagus pumilio (Mazía et al. 2004). Conversely, no effect of bird predation on insect herbivory was detected on Quercus prinus and Q. rubra (Forkner and Hunter 2000) or Salix lemonii (Low and Connor 2003). To explain these differences, it has been proposed that bird predation effects would be negligible for plant species that show low average herbivore load. Insectivorous birds seem to prefer foraging on trees where arthropods are more abundant (Bridgeland et al. 2010) and then respond in a density-dependent way to herbivore population dynamics (Sipura 1999). Our results are consistent with this hypothesis: bird predation effect is stronger on B. pendula seedlings which were on average more damaged than the oak species. Antiherbivore defences may also dampen the indirect effect of predators: the effect of bird insectivory was stronger on Salix phylicifolia than on S. myrsinifolia, a willow species with higher concentrations of phenolic glycosides and hence lower insect abundance (Sipura 1999). In our study, the evergreen species Q. ilex was less damaged than the deciduous species. Species with long-lifespan leaves are less palatable because of important allocation to antiherbivore defences (Crawley 1989). On the contrary, pioneer species such as B. pendula are char-

acterised by low investment in constitutive plant defences

(Coley et al. 1985). Mäntylä et al. (2008) also showed that

0.31

0.30



Fig. 3 Mean ( $\pm$ bias-corrected bootstrap confidence interval) effect size of bird exclusion on leaf area removed (*LAR*), **a** by seedling species irrespective of the forest habitat and **b** by forest habitat irrespective of the seedling species. A positive effect size indicates higher *LAR* in the bird exclusion treatment than in control (i.e. true bird predation effect) and was considered significant if the confidence interval did not bracket zero

leaves of *Betula pubescens* can release volatile organic compounds and have lower light reflectance as result of insect damage. These olfactory and visual cues are used by insectivorous birds and may explain why they prefer foraging on highly damaged plants. Lastly, birch seedlings are taller than the two oak species, with longer and less dense branches. This crown architecture may have provided insectivorous birds with easier access to insect prey since avian foraging intensity is highly dependent on foliage structure (Robinson and Holmes 1984; Marquis and Whelan 1996; Whelan 2001).

Bird exclusion clearly increased defoliation on *B. pendula* seedlings when all the herbivory assessments were pooled. However, when the five assessments were analysed separately, it appeared that bird exclusion effect showed an important temporal variation. For both years, significant bird exclusion effect was mainly observed during earlyand mid-season of vegetation (July 2007 and May 2008). Again, this might be a density-dependent response since, during this period, the abundance peak of insect larvae matched with the bird breeding season when food demand for fledglings is the highest (Holmes 1990). On the other hand, *Betula pendula* leaves are known to be subjected to early leaf abscission when severely damaged (Giertych et al. 2006), which suggests a lack of accuracy in damage assessment in autumn.

Our study is one of the first providing experimental evidence of associational resistance to insect herbivory in tree seedlings. It gives support to both the natural enemies hypothesis, since bird predation was more effective in the more diverse association of seedling and canopy species (broadleaved seedlings under conifers), and the resource concentration hypothesis, since the lowest insect herbivory level was observed in seedling species planted under non-congeneric canopy species. Furthermore, our results underline the importance of considering both the identity of focal plant species and the composition of habitats as determinants of the occurrence of trophic cascades.

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#### References

- Atlegrim O (1989) Exclusion of birds from bilberry stands—impact on insect larval density and damage to the bilberry. Oecologia 79:136–139
- Barbaro L, Pontcharraud L, Vetillard F, Guyon D, Jactel H (2005) Comparative responses of bird, carabid, and spider assemblages to stand and landscape diversity in maritime pine plantation forests. Ecoscience 12:110–121
- Barbaro L, van Halder I (2009) Linking bird, carabid beetle and butterfly life-history traits to habitat fragmentation in mosaic landscapes. Ecography 32:321–333
- Barber NA, Marquis RJ (2009) Spatial variation in top-down direct and indirect effects on white oak (*Quercus alba* L.). Am Midl Nat 162:169–179
- Barbosa P, Hines J, Kaplan I, Martinson H, Szczepaniec A, Szendrei Z (2009) Associational resistance and associational susceptibility: having right or wrong neighbors. Annu Rev Ecol Evol Syst 40:1–20
- Batzer HO, Benzie JW, Popp MP (1987) Spruce budworm damage in aspen/balsam fir stands affected by cutting methods. North J Appl For 4:73–75

- Björkman M, Hambäck PA, Hopkins RJ, Rämert B (2010) Evaluating the enemies hypothesis in a clover-cabbage intercrop: effects of generalist and specialist natural enemies on the turnip root fly (*Delia floralis*). Agric For Entomol 12:123–132
- Bock CE, Bock JH, Grant MC (1992) Effects of bird predation on grasshopper densities in an Arizona grassland. Ecology 73:1706–1717
- Borenstein M, Hedges LV, Higgins JPT, Rothstein HR (2009) Introduction to meta-analysis, 1st edn. Wiley, Chichester
- Bridgeland WT, Beier P, Kolb T, Whitham TG (2010) A conditional trophic cascade: birds benefit faster growing trees with strong links between predators and plants. Ecology 91:73–84
- Brockerhoff EG, Jactel H, Parrota JA, Quine CP, Sayer J (2008) Plantation forests and biodiversity: oxymoron or opportunity? Biodivers Conserv 17:925–951
- Coley PD, Bryant JP, Chapin FS III (1985) Resource availability and plant antiherbivore defense. Science 230:895–899
- Crawley MJ (1989) Insect herbivores and plant-population dynamics. Annu Rev Entomol 34:531–564
- Dial R, Roughgarden J (1995) Experimental removal of insectivores from rain forest canopy: direct and indirect effects. Ecology 76:1821–1834
- Finch S, Collier RH (2000) Host-plant selection by insects—a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. Entomol Exp Appl 96:91–102
- Forkner RE, Hunter MD (2000) What goes up must come down? Nutrient addition and predation pressure on oak herbivores. Ecology 81:1588–1600
- Giertych MJ, Karolewski P, Zytkowiak R, Oleksyn J (2006) Differences in defence strategies against herbivores between two pioneer tree species: *Alnus glutinosa* (L.) Gaertn. and *Betula pendula*. Roth. Pol J Ecol 54:181–187
- Glen DM (2004) Birds as predators of lepidopterous larvae. In: Van Emden HF, Rothschild M (eds) Insect and Bird Interactions. Intercept, Andover, pp 89–108
- González-Gómez P, Estades CF, Simonetti JA (2006) Strengthened insectivory in a temperate fragmented forest. Oecologia 148:137–143
- Greenberg R, Bichier P, Angon AC, MacVean C, Perez R, Cano E (2000) The impact of avian insectivory on arthropods and leaf damage in some Guatemalan coffee plantations. Ecology 81:1750–1755
- Greenberg R, Bichier P, Angon AC, Reitsma R (1997) Bird populations in shade and sun coffee plantations in Central Guatemala. Conserv Biol 11:448–459
- Hedges LV, Olkin I (1985) Statistical methods for meta-analysis. Academic, New York
- Holmes RT (1990) Ecological and evolutionary impacts of bird predation on forest insects: an overview. Stud Avian Biol 13:6–13
- Holmes RT, Schultz JC, Nothnagle P (1979) Bird predation on forest insects: an exclosure experiment. Science 206:462–463
- Jactel H, Brockerhoff EG (2007) Tree diversity reduces herbivory by forest insects. Ecol Lett 10:835–848
- Kaitaniemi P, Riihimaki J, Koricheva J, Vehvilainen H (2007) Experimental evidence for associational resistance against the European pine sawfly in mixed tree stands. Silva Fenn 41:259–268
- Kalka MB, Smith AR, Kalko EKV (2008) Bats limit arthropods and herbivory in a tropical forest. Science 320:71
- Karban R (2010) Neighbors affect resistance to herbivory—a new mechanism. New Phytol 186:564–566
- Keenan R, Lamb D, Sexton G (1995) Experience with mixed species rainforest plantations in North Queensland. Commonw For Rev 74:315–321
- Lemen C (1981) Elm trees and elm leaf beetles—patterns of herbivory. Oikos 36:65–67

- Low C, Connor EF (2003) Birds have no impact on folivorous insect guilds on a montane willow. Oikos 103:579–589
- Maetô K, Fukuyama K (1997) Mature tree effect of Acer mono on seedling mortality due to insect herbivory. Ecol Res 12:337–343
- Mäntylä E, Alessio GA, Blande JD, Heijari J, Holopainen JK, Laaksonen T, Piirtola P, Klemola T (2008) From plants to birds: higher avian predation rates in trees responding to insect herbivory. Plos ONE 3:e2832
- Mäntylä E, Klemola T, Laaksonen T (2011) Birds help plants: a metaanalysis of top-down trophic cascades caused by avian predators. Oecologia 165:143–151
- Marquis RJ, Whelan CJ (1994) Insectivorous birds increase growth of white oak through consumption of leaf-chewing insects. Ecology 75:2007–2014
- Marquis RJ, Whelan CJ (1996) Plant morphology and recruitment of the third trophic level: subtle and little-recognized defenses? Oikos 75:330–334
- Mazía CN, Thomas K, Enrique JC (2004) Interannual changes in folivory and bird insectivory along a natural productivity gradient in northern Patagonian forests. Ecography 27:29–40
- Mooney KA, Gruner DS, Barber NA, Van Bael SA, Philpott SM, Greenberg R (2010) Interactions among predators and the cascading effects of vertebrate insectivores on arthropod communities and plants. Proc Natl Acad Sci USA 107:7335–7340
- Nakagawa S, Cuthill IC (2007) Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol Rev 82:591–605
- Norghauer JM, Grogan J, Malcolm JR, Felfili JM (2010) Longdistance dispersal helps germinating mahogany seedlings escape defoliation by a specialist caterpillar. Oecologia 162:405–412
- Pace ML, Cole JJ, Carpenter SR, Kitchell JF (1999) Trophic cascades revealed in diverse ecosystems. Trends Ecol Evol 14:483–488
- Perfecto I, Vandermeer JH, Bautista GL, Nuñez GI, Greenberg R, Bichier P, Langridge S (2004) Greater predation in shaded coffee farms: the role of resident neotropical birds. Ecology 85:2677–2681
- Philpott SM, Soong O, Lowenstein JH, Pulido AL, Lopez DT, Flynn DFB, DeClerck F (2009) Functional richness and ecosystem services: bird predation on arthropods in tropical agroecosystems. Ecol Appl 19:1858–1867
- Pigot AL, Leather SR (2008) Invertebrate predators drive distancedependent patterns of seedling mortality in a temperate tree Acer pseudoplatanus. Oikos 117:521–530
- Pinheiro J, Bates D (2004) Mixed-effects models in S and S-PLUS. Springer, New York
- Price PW, Bouton CE, Gross P, McPheron BA, Thompson JN, Weis AE (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annu Rev Ecol Syst 11:41–65
- R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing. V, Austria, ISBN:3-900051-07-0, http://www.R-project.org
- Riihimäki J, Kaitaniemi P, Koricheva J, Vehviläinen H (2005) Testing the enemies hypothesis in forest stands: the important role of tree species composition. Oecologia 142:90–97
- Robinson SK, Holmes RT (1984) Effects of plant-species and foliage structure on the foraging behavior of forest birds. Auk 101:672–684
- Root RB (1973) Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica* oleracea). Ecol Monogr 43:95–124
- Rosenberg MS, Adams DC, Gurevitch J (2000) Metawin: statistical software for meta-analysis, version 2.0. Sinauer, Sunderland
- Russell EP (1989) Enemies hypothesis: a review of the effect of vegetational diversity on predatory insects and parasitoids. Environ Entomol 18:590–599

- Schuldt A, Baruffol M, Böhnke M, Bruelheide H, Härdtle W, Lang AC, Nadrowski K, Von Oheimb G, Voigt W, Zhou H, Assmann T (2010) Tree diversity promotes insect herbivory in subtropical forests of south-east China. J Ecol 98:917–926
- Sipura M (1999) Tritrophic interactions: willows, herbivorous insects and insectivorous birds. Oecologia 121:537–545
- Sobek S, Scherber C, Steffan-Dewenter I, Tscharntke T (2009) Sapling herbivory, invertebrate herbivores and predators across a natural tree diversity gradient in Germany's largest connected deciduous forest. Oecologia 160:279–288
- Van Bael SA, Philpott SM, Greenberg R, Bichier P, Barber NA, Mooney KA, Gruner DS (2008) Birds as predators in tropical agroforestery systems. Ecology 89:928–934
- van Halder I, Barbaro L, Corcket E, Jactel H (2008) Importance of semi-natural habitats for the conservation of butterfly communities in landscapes dominated by pine plantations. Biodivers Conserv 17:1149–1169
- Vásquez PA, Grez AA, Bustamante RO, Simonetti JA (2007) Herbivory, foliar survival and shoot growth in fragmented populations of *Aristotelia chilensis*. Acta Oecol 31:48–53

- Vehviläinen H, Koricheva J, Ruohomäki K (2007) Tree species diversity influences herbivore abundance and damage: metaanalysis of long-term forest experiments. Oecologia 152:287– 298
- Vehviläinen H, Koricheva J, Ruohomäki K (2008) Effects of stand tree species composition and diversity on abundance of predatory arthropods. Oikos 117:935–943
- Vehviläinen H, Koricheva J, Ruohomäki K, Johansson T, Valkonen S (2006) Effects of tree stand species composition on insect herbivory of silver birch in boreal forests. Basic Appl Ecol 7:1–11
- Whelan CJ (2001) Foliage structure influences foraging of insectivorous forest birds: an experimental study. Ecology 82:219–231
- Whelan CJ, Wenny DG, Marquis RJ (2008) Ecosystem services provided by birds. Ann NY Acad Sci 1134:25–60
- White JA, Whitham TG (2000) Associational susceptibility of cottonwood to a box elder herbivore. Ecology 81:1795–1803







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# Influence of surrounding vegetation on insect herbivory: A matter of spatial scale and herbivore specialisation

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#### Abstract

The diversity of surrounding vegetation is thought to modify the interactions between a focal plant and its herbivores, disrupting (associational resistance) or enhancing (associational susceptibility) host plant location and colonisation. We compared the effects of host plant concentration on herbivory by generalist and specialist insects feeding on oak seedlings by increasing local concentration of seedlings. We also assessed the effects of the composition and structure of surrounding vegetation, both at stand and local levels. The damage caused by generalist leaf-feeding insects depended on the structure of plant communities at stand level, and increased with tree cover. By contrast, infestation by specialist leaf miners was affected by local understorey vegetation surrounding oak seedlings, and decreased with increasing shrub cover and stratification diversity. Leaf mine abundance was higher at higher oak seedling density, giving support to the host concentration hypothesis. However, the abundance of these specialist herbivores was also negatively correlated with damage caused by the generalist external leaf-feeders, suggesting competitive interactions.

#### Zusammenfassung

Es wird angenommen, dass die Diversität der umgebenden Vegetation die Interaktionen zwischen einer Pflanze und ihren Herbivoren beeinflusst, indem Wirtsfindung und -kolonisation gestört (associational resistance) oder gefördert (associational susceptibility) werden. Wir verglichen die Effekte der Wirtspflanzenkonzentration auf generalistische und spezialisierte Insekten, die an jungen Eichenpflanzen fraßen, indem wir die lokale Konzentration der Pflanzen erhöhten. Wir bestimmten außerdem die Effekte von Zusammensetzung und Struktur der umgebenden Vegetation für die Bestandsebene und im lokalen Maßstab. Der von generalistischen Blattfressern verursachte Fraßschaden hing von der Struktur der Pflanzengemeinschaft auf Bestandsebene ab und nahm mit dem Deckungsgrad der Bäume zu. Im Gegensatz dazu wurde der Befall durch die spezialisierten Blattminierer durch den Unterwuchs, der die Eichenpflanzen lokal umgab, beeinflusst. Der Befall nahm mit zunehmendem Deckungsgrad der Sträucher und zunehmender Diversität der Vegetationsschichten ab. Die Abundanz der Minen war höher bei höherer Dichte der Eichenpflanzen, was die Wirtskonzentrationshypothese unterstützt. Allerdings war die Abundanz dieser spezialisierten Herbivoren auch negativ mit den von generalistischen externen Blattfressern verursachten Fraßschäden korreliert, was auf Konkurrenz-Beziehungen hindeutet.

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Keywords: Specialist; Generalist; Insect herbivory; Plant neighbours; Host plant concentration; Biodiversity

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#### Introduction

Interactions between plants and herbivores have long been viewed as binary relationships between resistance traits and herbivore communities, but the importance of the surrounding vegetation is increasingly recognised (Barbosa et al. 2009). The diversity of the surrounding vegetation has been reported to reduce herbivore damage to a focal plant, by providing associational resistance (Root 1973) in many ecosystems, such as crops (Tonhasca & Byrne 1994), forests (Jactel & Brockerhoff 2007; Vehviläinen, Koricheva, & Ruohomäki 2007) and grasslands (Unsicker et al. 2006). However, some studies have failed to confirm this relationship (Scherber et al. 2006; Vehviläinen, Koricheva, Ruohomäki, Johansson, & Valkonen 2006), or even reported the opposite response: associational susceptibility with higher herbivory in more diverse plant communities (Schuldt et al. 2010; Vehviläinen et al. 2007).

Resource concentration may account for a significant proportion of the decrease in damage due to specialist herbivores within diverse plant communities (Root 1973). The relative abundance and nature of the neighbouring plants determine the probability of a plant being colonised by herbivores (Barbosa et al. 2009). In more diverse plant communities, host plants are proportionally less frequent and non-host neighbouring plants can provide chemical or physical barriers to host plant location (Hambäck & Beckerman 2003; Jactel, Birgersson, Andersson, & Schlyter 2011; Randlkofer, Obermaier, Hilker, & Meiners 2010). For example, unpalatable or spiny shrubs may provide a physical barrier protecting tree seedling against mammalian grazers (Baraza, Zamora, & Hódar 2006).

These plant–herbivore interactions may also vary with the spatial scale at which they occur (Banks 1998; Gunton & Kunin 2007). The effect of plant neighbours on herbivory depends on the distance between a focal plant and its neighbours (Barbosa et al. 2009). Many studies have reported a significant influence of plant diversity on herbivores in small plots, whereas experiments over large spatial scales have tended to show no such benefits of plant diversity (Bommarco & Banks 2003).

Finally, the magnitude and direction of the relationship between plant diversity and insect herbivory seems to vary with the mobility of herbivores and their diet breadth (Banks 1998). Associational susceptibility would be expected to be most prevalent with polyphagous herbivores, because they are able to feed on multiple host plant species (Unsicker, Oswald, Koehler, & Weisser 2008), whereas specialist herbivores are limited by the numbers of suitable hosts (Bertheau, Brockerhoff, Roux-Morabito, Lieutier, & Jactel 2010). Plant neighbours may disrupt host finding by specialist insects, whereas generalist insects may simply shift from one host plant to another.

The objective of this study was therefore to investigate the effect of host concentration on insect herbivory on pedunculate oak *Quercus robur* seedlings, by manipulating the local

concentration of focal host plants. We also investigated the influence of the composition (species diversity) and structure (cover and stratification) of the surrounding vegetation of oak seedlings at local and stand levels. We hypothesised that specialist and generalist herbivores associated with oak seedlings would not be influenced in the same direction and magnitude by surrounding vegetation. We compared the effect of neighbouring plants on specialist leaf miners and on generalist external feeders.

#### Methods

#### Study system

This study was conducted in the Landes de Gascogne forest, in south-western France, a region covered by one million hectares of native maritime pine plantations (*Pinus pinaster*). Pedunculate oak (*Q. robur*) regenerates naturally within pine stands and becomes co-dominant in older plantations. Other broadleaf species present are: chestnut (*Castanea sativa*), European holly (*Ilex aquifolium*), red and Holm oaks (*Q. rubra* and *Q. ilex*), and silver birch (*Betula pendula*). The predominant understorey species are common bracken (*Pteridium aquilinum*), moor grass (*Molinia caerulea*), dwarf and common gorse (*Ulex europaeus*, *U. minor*), heather species (*Erica scoparia*, *E. cinerea*, *Calluna vulgaris*), glossy buckthorn (*Frangula alnus*), blackberry (*Rubus* spp.) and European honeysuckle (*Lonicera periclymenum*).

#### **Experimental design**

We investigated the effects of host plant concentration and surrounding vegetation on insect herbivory in eight mixed stands of Q. robur and P. pinaster (40-45 years of age). We established two experimental plots within each stand (Fig. 1). In the low-host concentration treatment ("low HC plot"), three oak seedlings were planted about 60 cm apart in a  $2.25 \text{ m}^2$  ( $1.5 \text{ m} \times 1.5 \text{ m}$ ) plot. In the high-host concentration treatment ("high HC plot"), we planted six additional seedlings around the three target seedlings, reducing the distance between seedlings to about 30 cm in a  $2.25 \text{ m}^2$  plot (Fig. 1). In total, 96 one-year-old Q. robur seedlings were randomly transplanted in February 2009: 48 target seedlings (3 replicate seedlings  $\times$  2 plots  $\times$  8 stands) and 48 additional seedlings (6 replicates  $\times$  1 high HC plot  $\times$  8 stands). The oak seedlings had been grown in nursery for one year and were about  $45 \pm 2$  cm high (mean  $\pm$  SE) and had  $47 \pm 3$  leaves.

#### **Insect herbivory on focal species**

Most of the insect damage occurring on *Q. robur* leaves is caused by generalist external leaf-feeders (chewer and skeletoniser guilds), principally lepidopteran larvae and grasshoppers, which are able to feed on several plant species.



**Fig. 1.** Experimental design for assessing the effect of host plant concentration and surrounding vegetation on insect herbivory on oak seedlings. Stand vegetation was sampled over  $400 \text{ m}^2$  around the two experimental plots. The low-host concentration plot consisted of 3 focal oak seedlings (grey squares, top-right), and the high-host concentration plot consisted of 3 focal oak seedlings (grey squares) and 6 additional oak seedlings (solid circles, bottom-right). The vegetation at the plot level was sampled over 2.25 m<sup>2</sup>.

A few lepidopteran species are specialist feeders (Appendix A Table 1), but these species had very low abundance in the area studied and caused little damage (Giffard, Corcket, Barbaro, & Jactel 2012, unpublished data). All external insect damage was pooled and considered to correspond to damage caused by external leaf-feeders. Leaf miners are specialist herbivores and the species feeding on *Q. robur* are mostly small moths (Lepidoptera). We therefore used the abundance of leaf mines (presence of mines in leaves) as a proxy for herbivory.

Herbivory by external leaf-feeders and the abundance of leaf mines were assessed by visual inspection of all leaves in each of the 48 target oak seedlings. The leaf area removed (LAR) by generalist insects was estimated with transparent 0.25-cm<sup>2</sup> grid and divided by the total leaf area (estimated with a 1 cm<sup>2</sup> grid) to calculate a percentage LAR. Insect herbivory was assessed twice during a single growing season, in June and September 2009, as well as the number of the main insect herbivore species (Appendix A Table 1). Within a given plot, LAR and leaf mine abundance were averaged over the three seedling replicates.

#### Stand and plot vegetation sampling

The vegetation present in the eight stands and the 16 plots was sampled in August 2009. We recorded all vascular plants and their cover in an area of  $400 \text{ m}^2$  around the two experimental plots in each forest stand (Fig. 1). Species covers were assessed using the Braun-Blanquet semi-quantitative

scale (Sutherland 1996). The vegetation layers considered were the tree layer (trees higher than 7 m), the shrub layer (woody species 2–7 m high), and the herbaceous layer (all plants less than 2 m high). Plant cover was assessed visually for each vegetation layer, regardless of species composition. We also estimated mean Q. *robur* cover in the herbaceous layer (recruitment) and in both the tree and shrub layers (saplings and mature trees).

At the plot level, we recorded all plant species in the herbaceous layer (<2 m high) and estimated shrub and herbaceous cover (Fig. 1). We also estimated the *Q. robur* cover in the herbaceous layer. We then calculated plant species richness and Shannon's index of diversity, in each layer at both stand and plot levels. We also calculated an index of stratification diversity at both stand and plot levels (Prodon & Lebreton 1981). At the plot level, stratification diversity increased with decreasing herbaceous cover and increasing shrub cover. All vegetation variables are listed in Appendix A Table 2.

#### Statistical analyses

Because vegetation variables were correlated, we first performed principal component analyses (PCA), at stand and plot levels separately, to select a minimum set of meaningful variables capturing most of the variation in the complete data set. Then we selected the original variable best correlated to each of the first two axes (Appendix A Figs. 1 and 2) even though other non-selected variables might have been also relevant to insect herbivory. Oak cover and tree cover were retained for the stand level (17 and 23% contribution to axes 1 and 2, respectively) and stratification diversity and herbaceous diversity for the plot level (31 and 34% contribution to axes 1 and 2, respectively).

Next, linear mixed model analyses (LMM) were performed on LAR by external leaf-feeders, after logit transformation for proportion data (Warton & Hui 2011). Generalised linear mixed model analyses (GLMM) were performed on the abundance of leaf mines, with Poisson distribution fitting and the use of a log link function and Laplace approximation for count data (Zuur, Ieno, Walker, Saveliev, & Smith 2009).

In all models, we considered "stand" as random effect to account for the spatial non-independence of the two plots within a given forest stand (high and low HC plots), and "plot" as random effect to account for the two repeated measurements conducted in a each plot. We investigated the fixed effects of host concentration, season, selected vegetation variables (oak and tree covers at stand level, and stratification and herbaceous diversities at plot level) and their interactions in two complete models, for LAR by external leaf-feeders and leaf mine abundance separately.

For the two complete models, likelihood-ratio tests were then performed in each model to assess the significant effects of predictive variables and their interactions on insect herbivory. Non-significant variables were successively removed until the minimal model best explaining the data was obtained



**Fig. 2.** Mean (+SE) percent leaf area removed (%LAR) by external leaf-feeding insects (white bars) and mean (+SE) leaf mine number (black bars) on oak seedlings planted in low-host concentration plots (low HC) and high-host concentration plots (high HC), in June and September (increase from June to September for leaf mine number and LAR by external-feeding insects, P < 0.001, and increase of leaf mine number in high HC plots, P = 0.03).

(Zuur et al. 2009). When significant three-way interactions were found (season × host concentration × vegetation variable), we made two separated models (for each season) in order to highlight the effects of the experimental treatments. We carried out analyses with maximum likelihood methods, to compare models with different fixed effect structures. We checked the normality and homoscedasticity of the residuals of all minimal models. Finally, we performed GLMM to investigate the effect of LAR by external leaf-feeders on the abundance of leaf mines. For both GLMMs and LMMS, the likelihood ratio  $R^2$  was estimated if a continuous variable was found to have a significant effect (Kramer 2005) and we used a more conservative *P*-value of 1% as the significance threshold for *z*-values for GLMMs (Zuur et al. 2009).

Analyses were performed with the ade4 package for multivariate analyses (Dray & Dufour 2007), the nlme package for LMM analyses (Pinheiro, Bates, Debroy, & Sarkar 2011) and lme4 for GLMM analyses (Bates, Maechler, & Bolker 2011) with R software (R Development Core Team 2011). Intercepts and slopes and their standard errors were estimated with the AICcmodavg package (Mazerolle 2011) and then exponentially transformed to represent raw data on figures.

#### Results

#### Effect of season

The abundance of leaf mines  $(n=32, \chi^2=13.4, df=1, P < 0.001)$  and LAR by external leaf-feeders  $(F_{1,15}=81.83, P < 0.001)$  increased significantly from June to September (Fig. 2). Treatment effects, i.e. host concentration and continuous vegetation variables, were consistent regardless of season for LAR by external leaf-feeders, indicated by non-significant interaction terms (no significant three-way



Fig. 3. Relationship between the number of leaf mines and percent leaf area removed by external leaf-feeding insects (%LAR), for oak seedlings in September. Open and closed circles represent low- and high-host concentration plots, respectively. For the sake of clarity, the graph shows raw point data and exponential fitting curves (the shaded area represents  $\pm 1$  SE) since GLMMs were done using log-link functions.

interactions). By contrast, analyses of leaf mine abundance showed significant interactive effects of vegetation variables with season and host concentration treatments (significant three-way interactions) and we then tested vegetation effects and their interactions with host concentration separately for each date.

In June, there was no significant relationship between LAR by external leaf-feeders and leaf mine abundance whereas the leaf mine abundance decreased with increasing LAR by external leaf-feeders in September, regardless of host plant concentration (n = 16,  $\chi^2 = 24.45$ , df = 1, P < 0.001) (Fig. 3).

#### Effect of host plant concentration

The addition of conspecific seedlings had different effects on damage, according to host plant specialisation of insect herbivores (Fig. 2). Regardless of the season, the abundance of leaf mines on oak seedlings was significantly higher in high-HC plots (n=32,  $\chi^2=4.60$ , df=1, P=0.03), but the HC effect was weak on each date (n=16,  $\chi^2=3.42$ , df=1, P=0.06 and n=16,  $\chi^2=2.80$ , df=1, P=0.09, for June and September, respectively). Conversely, damage by external leaf-feeders tended to be lower in high HC plots, if nonsignificantly ( $F_{1,7}=1.91$ , P=0.20).

#### Effects of plot diversity on insect herbivory

LAR by external leaf-feeders was not significantly affected by vegetation variables estimated at the local level (all P > 0.10).



**Fig. 4.** Relationship between the abundance of leaf mines (number per seedling) and the stratification diversity index at plot level in September (2009). Open and closed circles represent low- and high-host concentration plots, respectively. Note that stratification diversity increased as herbaceous cover decreased and shrub cover increased. For the sake of clarity, the graph shows raw point data and exponential fitting curves (the shaded area represents  $\pm 1$  SE) since GLMMs were done using log-link functions.

The structure of the surrounding vegetation significantly influenced herbivory by specialist insects in September  $(n=16, \chi^2 = 11.31, df = 1, P < 0.001)$  but not in June  $(n = 16, \chi^2 = 0.05, df = 1, P = 0.82)$ . The abundance of leaf mines significantly decreased with increasing stratification diversity (Fig. 4).

#### Effects of stand diversity on insect herbivory

Effects of stand vegetation variables were not consistent among host plant concentration treatments for LAR by external leaf-feeders. We found a significant interaction term between host concentration and tree cover ( $F_{1,5} = 6.8$ , P = 0.05), so we performed separate analyses for each level of HC (high and low HC plots). The structure and composition of the tree layer significantly influenced LAR by external leaf-feeders on oak seedlings only in plots with low HC (Fig. 5). LAR significantly increased with increasing tree cover ( $F_{1,6} = 7.70$ , P = 0.03), whereas no significant effect was found in plots with high HC ( $F_{1,6} = 0.10$ , P = 0.77).

The abundance of leaf mines was not significantly affected by vegetation variables estimated at the stand level in both June and September (all P > 0.10). We did not find any significant effect of oak cover on LAR by external leaf-feeders or leaf mine abundance.

#### Discussion

These results provide new experimental evidence that neighbouring plants can affect insect herbivory on a focal plant. However, we observed considerable variation in the direction and magnitude of the influence of surrounding vegetation. The diet breadth of insect herbivores and the spatial scale over which neighbouring plants are considered emerged as two key factors explaining damage response to plant diversity or structure.

We found that damage caused by generalist leaf-feeding insect herbivores increased with increasing vegetation diversity (associational susceptibility), whereas the abundance of specialist insects decreased (associational resistance). Herbivory by generalist insects was best explained by tree cover and by structural complexity of surrounding vegetation for specialist insects. However, we found strong covariation



**Fig. 5.** Effects of tree cover at the stand level on percent leaf area removed (%LAR) by external leaf-feeding insects from oak seedlings in (A) low-host concentration plots and (B) high-host concentration plots. Triangles and circles indicate the results for June and September, respectively. For the sake of clarity, the graphs show raw point data and an exponential fitting curve (the shaded area represents  $\pm 1$  SE) since statistical analyses were done using logit transformations.

between structural (cover or stratification diversity) and compositional (richness and diversity) vegetation variables. For example tree cover positively covaried with shrub and herbaceous diversities (Appendix A Fig. 1) and stratification diversity was correlated to shrub oak cover (Appendix A Fig. 2). It was therefore difficult to identify which of these variables were the most relevant for explaining insect herbivory patterns.

The effect of the surrounding vegetation on herbivory was stronger over short distances for specialist insects (at the plot level) and stronger over larger distances for generalist insects (at the stand level).

According to the resource concentration hypothesis, the likelihood of specialised herbivores locating their host plants is lower in more diverse plant communities (Root 1973). Here we found new evidence to support this hypothesis since leaf miner abundance decreased with increasing stratification diversity of vegetation around oak seedlings, and also when oak seedlings were less concentrated. Host plants are proportionally less frequent and are chemically or visually hidden from insects by non-host neighbours in plant communities more diverse in terms of structure or composition (Hambäck & Beckerman 2003). By contrast, generalist herbivorous insects attacking oak seedlings, mostly composed of external leaf-feeders (Appendix A Table 1), may benefit from richer plant communities, which provide access to multiple host species, enabling insects to increase the size of their populations and/or their fitness (Unsicker et al. 2008), as they can easily shift from one host species to another (Bertheau et al. 2010; Jactel & Brockerhoff 2007). Many examples of these opposite patterns of response have been reported, with greater damage due to polyphagous herbivores (Massey, Massey, Press, & Hartley 2006; Pigot & Leather 2008; Scherber et al. 2006; Schuldt et al. 2010), or lower levels of damage due to oligophagous herbivores (Giffard et al. 2012; Otway, Hector, & Lawton 2005) in more diverse plant communities.

High concentration of host plants facilitated host location, whereas the presence of non-host plants may deter insect herbivores, particularly if those plants are unpalatable (Massey et al. 2006; Otway et al. 2005). However, some studies have suggested that individual host plants may be less likely to be colonised in larger populations, due to a dilution effect (Bañuelos & Kollmann 2011; Otway et al. 2005). In our experiment, planting conspecific neighbours artificially increased host plant concentration and resulted in higher levels of leaf miner damage on focal oak seedlings. However, we did not observe an increase in abundance of leaf mines when oak density increased at the stand level. This would have been observed if the abundance of leaf miners had been high enough to exploit most of the food resources provided by canopy oak trees and then requiring a spill over onto other potential hosts such as oak seedlings. At the plot level, we found that the abundance of specialist insects was affected by the stratification diversity, which was also correlated to shrub oak cover (Appendix A Fig. 2). We can thus hypothesise a local effect of host plant concentration, as focal oak seedlings diluted amongst a natural regeneration of oaks were less likely to be colonised by leaf miners.

Another mechanism underlying associational resistance to specialist herbivores involves the disruption of host plant location, with neighbouring plants hindering the detection of visual or olfactory cues by female moths before oviposition (Jactel et al. 2011; Ross, Berisford, & Godbee 1990). Vegetation diversity results in a complexity of plant chemical and structural cues, which may disturb arthropod orientation and mobility (Randlkofer et al. 2010). Such disruption is more likely to occur in close proximity of focal plants than at a larger scale, potentially accounting for the effect of vegetation complexity on the abundance of leaf miners on oak seedlings being limited at the plot level. Furthermore, the surrounding vegetation can conceal the host plant even if it is not particularly diverse (Hambäck & Beckerman 2003).

By contrast, generalist herbivores display a remarkable ability to shift between diverse host plants (Bertheau et al. 2010) and may not be influenced by local sparseness of a particular plant species. This may account for the lack of a significant effect of host plant concentration on damage to oak seedlings caused by generalist herbivores. On the other hand, tree seedlings may suffer higher levels of damage than other plants, due to the vertical transfer of generalist insect herbivores from canopy trees (Pigot & Leather 2008; White & Whitham 2000). This is consistent with our observation that damage to oak seedlings due to external feeders increased with increasing tree cover. However, tree, shrub and herbaceous covers positively covaried with tree, shrub and herbaceous diversities at stand level (Appendix A Fig. 1). Not only the amount but also the diversity of feeding resources may have then benefited generalist herbivores before they spill over onto oak seedlings but our experimental design did not allow disentangling the two mechanisms.

The vegetation around tree seedlings may modify light and microclimate conditions, and this may also account for the effect of neighbouring plants on herbivory in focal plants (Barbosa et al. 2009). Leaves exposed to the sun generally have higher concentrations of defence compounds and lower nitrogen and water contents than shaded leaves (Dudt & Shure 1994), which may render them less attractive to insect herbivores. Our results about herbivory by generalist insects support this hypothesis, since we found that their damage decreased with decreasing tree cover, i.e. with increasing light availability. However, we found the opposite to be true, with higher abundance of leaf mines in seedlings surrounded by low shrub cover and, hence, more exposed to light. Barber and Marquis (2011) also reported higher levels of herbivory on saplings previously exposed to high light intensity and argued that the search for oviposition sites may have led females to choose saplings with more foliage.

Insect herbivory may also be affected by top-down regulation. The natural enemies hypothesis predicts higher predator species richness and abundance in more diverse plant communities, resulting in lower levels of herbivory (Langellotto & Denno 2004; Root 1973). At the plot level, we observed a negative correlation between stratification diversity and abundance of leaf mines, which might have been due to higher levels of regulation by natural enemies. However, we found the opposite pattern at the stand scale, with higher levels of damage by generalist insects in stands with higher tree cover. Foraging by insectivorous birds or predatory arthropods may also be hindered by greater structural complexity (Sanders, Nickel, Grützner, & Platner 2008), resulting in larger populations of their prey, which then cause more extensive damage to plants (Giffard et al. 2012).

Finally, we found that damage due to specialist insects was inversely correlated with damage due to generalist insects at the end of the growing season. This is consistent with previous findings of negative interactions between herbivore guilds (Kaplan & Denno 2007), particularly between exophytic and endophytic insects. In addition, generalist insect damage early in the season may have indirectly induced stronger chemical defences in oak leaves, thus decreasing their quality as oviposition sites for leaf miners.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.baae.2012.08.004.

#### References

- Banks, J. E. (1998). The scale of landscape fragmentation affects herbivore response to vegetation heterogeneity. *Oecologia*, 117, 239–246.
- Bañuelos, M. J., & Kollmann, J. (2011). Effects of host-plant population size and plant sex on a specialist leaf-miner. *Acta Oecologica*, 37, 58–64.
- Baraza, E., Zamora, R., & Hódar, J. A. (2006). Conditional outcomes in plant-herbivore interactions: Neighbours matter. *Oikos*, 113, 148–156.
- Barber, N. A., & Marquis, R. J. (2011). Light environment and the impacts of foliage quality on herbivorous insect attack and bird predation. *Oecologia*, 166, 401–409.

- Barbosa, P., Hines, J., Kaplan, I., Martinson, H., Szczepaniec, A., & Szendrei, Z. (2009). Associational resistance and associational susceptibility: Having right or wrong neighbors. *Annual Review* of Ecology Evolution and Systematics, 40, 1–20.
- Bates, D., Maechler, M., & Bolker, B. (2011). *lme4: Linear mixed-effects models using S4 classes*. R package version 0. 999375-39.
- Bertheau, C., Brockerhoff, E. G., Roux-Morabito, G., Lieutier, F., & Jactel, H. (2010). Novel insect-tree associations resulting from accidental and intentional biological 'invasions': A meta-analysis of effects on insect fitness. *Ecology Letters*, 13, 506–515.
- Bommarco, R., & Banks, J. E. (2003). Scale as modifier in vegetation diversity experiments: Effects on herbivores and predators. *Oikos*, 102, 440–448.
- Dray, S., & Dufour, A. B. (2007). The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22, 1–20.
- Dudt, J. F., & Shure, D. J. (1994). The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology*, 75, 86–98.
- Giffard, B., Corcket, E., Barbaro, L., & Jactel, H. (2012). Bird predation enhances tree seedling resistance to insect herbivores in contrasting forest habitats. *Oecologia*, 168, 415–424.
- Gunton, R. M., & Kunin, W. E. (2007). Density effects at multiple scales in an experimental plant population. *Journal of Ecology*, 95, 435–445.
- Hambäck, P. A., & Beckerman, A. P. (2003). Herbivory and plant resource competition: A review of two interacting interactions. *Oikos*, 101, 26–37.
- Jactel, H., Birgersson, G., Andersson, S., & Schlyter, F. (2011). Non-host volatiles mediate associational resistance to the pine processionary moth. *Oecologia*, 166, 703–711.
- Jactel, H., & Brockerhoff, E. G. (2007). Tree diversity reduces herbivory by forest insects. *Ecology Letters*, 10, 835–848.
- Kaplan, I., & Denno, R. F. (2007). Interspecific interactions in phytophagous insects revisited: A quantitative assessment of competition theory. *Ecology Letters*, 10, 977–994.
- Kramer, M. (2005). R2 statistics for mixed models. In *Proceedings* of the 17th conference on applied statistics in agriculture Kansas State University,
- Langellotto, G. A., & Denno, R. F. (2004). Responses of invertebrate natural enemies to complex-structured habitats: A meta-analytical synthesis. *Oecologia*, 139, 1–10.
- Massey, F. P., Massey, K., Press, M. C., & Hartley, S. E. (2006). Neighbourhood composition determines growth, architecture and herbivory in tropical rain forest tree seedlings. *Journal of Ecology*, 94, 646–655.
- Mazerolle, M. J. (2011). AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 1.15.
- Otway, S. J., Hector, A., & Lawton, J. H. (2005). Resource dilution effects on specialist insect herbivores in a grassland biodiversity experiment. *Journal of Animal Ecology*, 74, 234–240.
- Pigot, A. L., & Leather, S. R. (2008). Invertebrate predators drive distance-dependent patterns of seedling mortality in a temperate tree *Acer pseudoplatanus*. *Oikos*, 117, 521–530.
- Pinheiro, J., Bates, D., Debroy, S., & Sarkar, D. (2011). nlme: Linear and Nonlinear Mixed Effects Models (pp. 1–100). R package version 3.
- Prodon, R., & Lebreton, J. D. (1981). Breeding avifauna of a Mediterranean succession – The holm oak and cork oak series in

the eastern Pyrenees. 1. Analysis and modelling of the structure gradient. *Oikos*, *37*, 21–38.

- R Development Core Team (2011). *R: A language and environment for statistical computing*. Vienna, Austria, ISBN: 3-900051-07-0.
- Randlkofer, B., Obermaier, E., Hilker, M., & Meiners, T. (2010). Vegetation complexity – The influence of plant species diversity and plant structures on plant chemical complexity and arthropods. *Basic and Applied Ecology*, *11*, 383–395.
- Root, R. B. (1973). Organization of a plant-arthropod association in simple and diverse habitats: The fauna of collards (*Brassica oleracea*). *Ecological Monographs*, 43, 95–124.
- Ross, D. W., Berisford, C. W., & Godbee, J. F. (1990). Pine tip moth, *Rhycionia* spp. response to herbaceous vegetation control in an intensively site-prepared loblolly-pine plantation. *Forest Science*, *36*, 1105–1118.
- Sanders, D., Nickel, H., Grützner, T., & Platner, C. (2008). Habitat structure mediates top-down effects of spiders and ants on herbivores. *Basic and Applied Ecology*, 9, 152–160.
- Scherber, C., Mwangi, P. N., Temperton, V. M., Roscher, C., Schumacher, J., Schmid, B., et al. (2006). Effects of plant diversity on invertebrate herbivory in experimental grassland. *Oecologia*, *147*, 489–500.
- Schuldt, A., Baruffol, M., Bohnke, M., Bruelheide, H., Härdtle, W., Lang, A. C., et al. (2010). Tree diversity promotes insect herbivory in subtropical forests of south-east China. *Journal of Ecology*, 98, 917–926.
- Sutherland, W. J. (1996). Ecological census techniques: A handbook. Cambridge: Cambridge University Press.

- Tonhasca, A. J., & Byrne, D. N. (1994). The effects of crop diversification on herbivorous insects: A meta-analysis approach. *Ecological Entomology*, 19, 239–244.
- Unsicker, S. B., Baer, N., Kahmen, A., Wagner, M., Buchmann, N., & Weisser, W. W. (2006). Invertebrate herbivory along a gradient of plant species diversity in extensively managed grasslands. *Oecologia*, 150, 233–246.
- Unsicker, S. B., Oswald, A., Koehler, G., & Weisser, W. W. (2008). Complementarity effects through dietary mixing enhance the performance of a generalist insect herbivore. *Oecologia*, 156, 313–324.
- Vehviläinen, H., Koricheva, J., & Ruohomäki, K. (2007). Tree species diversity influences herbivore abundance and damage: Meta-analysis of long-term forest experiments. *Oecologia*, 152, 287–298.
- Vehviläinen, H., Koricheva, J., Ruohomäki, K., Johansson, T., & Valkonen, S. (2006). Effects of tree stand species composition on insect herbivory of silver beach in boreal forests. *Basic and Applied Ecology*, 7, 1–11.
- Warton, D. I., & Hui, F. K. C. (2011). The arcsine is asinine: The analysis of proportions in ecology. *Ecology*, 92, 3–10.
- White, J. A., & Whitham, T. G. (2000). Associational susceptibility of cottonwood to a box elder herbivore. *Ecology*, 81, 1795–1803.
- Zuur, A. F., Ieno, I. E., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. Berlin: Springer.

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## Stress tolerance abilities and competitive responses in a watering and fertilization field experiment

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#### Abstract

**Question:** Do water gradients produce patterns of responses to stress and competition similar to those induced by nutrient gradients?

Location: French Alps.

**Methods**: We established a split-plot design in a calcareous grassland, with watering and fertilization as main plot treatments and competition as subplot treatment. We followed individual and competitive responses of transplants of the three potential dominant grass species: *Bromus erectus*, *Brachypodium rupestre* and *Arrhenatherum elatius*, in all plots during two growing seasons. Changes in natural relative abundances of the three grass species were also monitored.

**Results:** The growth and the relative abundance of *A. elatius* were primarily stimulated by nutrient addition and those of *B. rupestre* by water addition, whereas *B. erectus* decreased in abundance and had a very low flexibility with enhanced resource supply. Competition intensity increased for all species with both watering and fertilization and the ranking in competitive responses did not change with treatments: *A. elatius* > *B. rupestre* > *B. erectus*.

**Conclusions:** Patterns of dominance were efficiently explained by stress tolerance abilities and competitive responses for dry and poor sites, and wet and rich sites for *B. erectus* and *A. elatius* respectively, whereas competitive responses were poor predictors of dominance for *B. rupestre* in wet and nutrient-poor sites. Further studies are needed to assess the potential role of other processes, such as increasing competitive effect on light with increasing age as well as interference, to explain the dominance of this conservative competitor type of species in wet and nutrient-poor sites.

Keywords: Community response; Nutrient; Water.

**Abbreviations:** C = Control plot; F = Fertilized plot; PAR = Photosynthetically active radiation; RNE = Relative Neighbour Effect index; W = Watered plot; WF = Watered and Fertilized plot.

Nomenclature: Tutin et al. (1964-1980).

#### Introduction

Stress tolerance and competition may be considered as the two main mechanisms driving variations in dominance along productivity gradients in low disturbance herbaceous communities. The 'CSR model' of Grime (1974) considers that dominance is driven by competition in fertile conditions but emphasizes the importance of stress tolerance in unproductive environments. In contrast, the 'resource ratio hypothesis' of Tilman (1982) proposes that competition is important at both ends of the fertility gradient with the dominance of a lightcompetitor type of species in fertile conditions and the dominance of a nutrient-competitor type of species in nutrient-poor conditions. During the past two decades there has been substantial debate between plant ecologists about the potential of each model to explain patterns of dominance in plant communities (Grubb 1985; Thompson 1987; Tilman 1987; Thompson & Grime 1988; Grace 1991; Grubb 1998; Wilson & Lee 2000). We suggest that the conflicting results obtained by a number of authors who tested these models along natural or experimental gradients (see reviews of Gurevitch et al. 1992; Goldberg & Barton 1992; Goldberg & Novoplansky 1997; Corcket et al. 2003) are evidence that the mechanisms driving dominance in herbaceous communities are too complex to be summarized by only one of these two models. The predictive power of each model may be limited to particular environments (Goldberg & Novoplansky 1997) and particular target species (Twolan-Strutt & Keddy 1996; Grubb 1998; Liancourt et al. 2005).

Because there was an increasing number of field experimental evidences indicating that competition intensity increases along water gradients (e.g. Kadmon 1995) but remains stable along nutrient gradients (Wilson & Tilman 1991), Goldberg & Novoplansky (1997) argued that Grime's productivity gradients are natural gradients mainly driven by water (xeric vs mesic environments), whereas Tilman's productivity gradients are experimental gradients mainly driven by nutrients (fertile vs infertile). However, this prediction was not supported at this time by their review and they concluded that too few studies were conducted along water gradients to adequately test this hypothesis. Additionally, Grubb (1998) reassessed the stress tolerance strategy by distinguishing three types of plant species occurring in sites characterized by a shortage of resources, without relating them to specific resources shortage (e.g. water, nutrient or light). The stress tolerant type of species of Grime (1974) should be restricted to a 'low flexibility' strategy (strong ability to tolerate resources shortage and a constant low growth rate and size). Two other strategies with no equivalence in the CSR model were distinguished, the 'gearing down' and the 'switching' strategies for species which also tolerate resource shortages but which may have a high growth rate and size in optimal conditions (Grubb 1998).

European lowland calcareous communities are dominated by three different perennial grass species with contrasted traits (Ryser & Urbas 2000; Ryser & Wahl 2001) under varying soil resource conditions: the short, slow growing *Bromus erectus* in dry and nutrient-poor sites, the large, slow growing *Brachypodium rupestre* in mesic and nutrient-poor sites and the tall, fast growing *Arrhenatherum elatius* in mesic and nutrient-rich sites (Royer 1987; Corcket et al. 2003). Our main objective is to assess differences in stress tolerance and competitive abilities among those three grasses, naturally dominating the emblematic species-rich European calcareous grasslands under contrasting water and nutrient conditions.

#### **Material and Methods**

#### Study site and target species

The experiment was performed in a meso-xeric calcareous grassland, 'Liche Petet', close to Grenoble, France (700 m a.s.l., see Corcket et al. 2003). In Grenoble (210 m a.s.l.) mean extreme temperatures vary from -2 °C in January to 27 °C in July and mean precipitation is 300 mm in summer (June-August) (Anon. 2001, 2002). The soil is poor in nitrogen (1.84 mg available N per kg dry soil) and uniform throughout the slope of our experimental site (Corcket et al. 2003). As in many other European mesoxeric calcareous grasslands (Royer 1987) the two dominant grass species are *B. erectus* (55-60% cover) and *B.* rupestre (10-15% cover). Nitrogen demanding grasses, such as A. elatius, Dactylis glomerata and Holcus lanatus have very low cover (< 5%). Other species (25-30%) cover) are sedges: Carex flacca and C. caryophyllea; forbs: Euphorbia cyparissias, Teucrium chamaedrys,

#### *Thymus serpyllum, Origanum vulgare* and legumes: *Lotus corniculatus, Medicago falcata* and *M. lupulina*.

The three target species are known to dominate herbaceous communities in different habitats and require different management regimes (Royer 1987; Grime et al. 1996; Ryser & Urbas 2000), B. erectus and B. rupestre mainly occur in nutrient-poor and poorly managed grasslands and A. elatius in mowed nutrient and water-rich meadows (Willems 1980; Bobbink 1991; Buckland et al. 2001). B. erectus is the dominant species in the driest sites, on steep slopes with a south exposure (Rover 1987) and, in particular, in grasslands moderately grazed by sheep (Barbaro & Cozic 1998). B. rupestre encroaches into, and dominates, grasslands after abandonment (Barbaro & Cozic 1998; Moog et al. 2002) and forest edges on mesic soils (Corcket et al. 2003). Both species are slow growing (Ryser & Wahl 2001) but Grime et al. (1996) consider B. erectus as the most stress-tolerant species and B. rupestre as a species with an intermediate strategy between the typical stresstolerant and competitive strategies. A. elatius is a fast growing species, classified as a competitor by Grime et al. (1996).

#### The experiment

In April 2001 we established a split-plot design surrounded by a fence, to exclude the main vertebrate grazers (deer, sheep and rabbits). The main treatments were environmental manipulations (watering and fertilization) applied as a factorial combination: control (C), watered (W), fertilized (F) and watered and fertilized (WF), in 15-m<sup>2</sup> plots (28 plots, seven replicates of each treatment). Within these main plots, competition and species were applied as subplot treatments. Because watering and fertilization treatments were assigned randomly to the plots and to avoid a slope-induced contamination, the 28 plots were located on two elevation lines with a 5-m buffer zone between upper and lower plots. Half of the plots (14) were watered using a permanent irrigation system, from April to September 2001 and 2002, whereas the 14 unwatered plots received only natural precipitation. The total amount of water received by the control plots during the summer was 348 mm the first year and 268 mm the second year, whereas the watered plots received the equivalents of ca. 800 mm each summer. Half of the irrigated and unirrigated plots were fertilized using commercial fertilizer tablets (NPK 12:12:17 + micro-elements). The amount applied was 80 g.m<sup>-2</sup> (96 kg-N.ha<sup>-1</sup>). In the first year fertilizer was applied twice, in May at the beginning of the growing season and in August. In the second year, fertilizer was applied only once at the end of May.

Seeds of the three target species collected in the field

were germinated on petri plates in a greenhouse at the beginning of March 2001. Seedlings were transplanted into a 1:3 field site mixture of soil and grey river sand in 0.24 l plastic pots containing one seedling per pot. They were grown for one month in the greenhouse and then one month in the common garden of the University and were watered every day until field transplantation.

For the species and competition treatments, we identified 24 circular 50-cm diameter subplots (three species × two competition levels × four replicates) within each of the 28 environmental plots. Above-ground vegetation of half of the randomly chosen subplots (12 no neighbours subplots) was removed using chemical, non-selective herbicide (glyphosate) two weeks before target transplantations. Glyphosate is a systemic herbicide, taken-up via leaves and is known to be quickly decomposed in soils (Peltzer & Köchy 2001). Dead vegetation was removed by hand and we used a knife to dig a 20 cm deep trench around expanding roots at the edges of the neighbour removal areas. The neighbour removal subplots were kept free from vegetation by periodic hand weeding. We randomly selected individuals of the three target species, which were at the same life stage (from three to five leaves), and transplanted them into the centre of each subplot (four replicates per species) between 20th May and 5th June 2001. Individuals which died were replaced within the following three weeks.

#### Measurements

#### Soil resources

Soil moisture and total soil available N (the sum of N-NH<sub>4</sub><sup>+</sup> and N-NO<sub>3</sub><sup>-</sup>) were measured in early August 2001 and 2002 in four plots randomly chosen from the seven replicates of each combination of the two environmental treatments. Three soil samples per plot were collected from a depth of 5-10 cm, mixed and stored at 4 °C before analysis. N-NH<sub>4</sub><sup>+</sup> was determined (after extraction in 1 M KCl) with the blue indophenol method (Dorich & Nelson 1983) and N-NO<sub>3</sub><sup>-</sup> was determined (after extraction in water) by ion chromatography (Dionex 4500i, Dionex Corporation, Sunnyvale, California). Total soil available N was expressed in mg N per kg dried soil. Soil moisture was determined by drying samples at 105 °C for 72 h and was expressed as percentage of fresh soil mass.

#### Total biomass and relative biomass of the dominants

Above-ground vegetation was clipped at ground level in early June 2002 in three plots randomly chosen from the seven replicates of each combination of the two environmental treatments. We sampled two  $0.25 \text{ m} \times 0.5$ m quadrats per plot, pooled the samples and dried them at 70 °C for 72h. Above-ground biomass of each species was separated and weighed. Relative biomass values (expressed in % of total biomass) were calculated separately for *B. erectus*, *B. rupestre*, while the fast growing grasses, *A. elatius*, *D. glomerata* and *H. lanatus*, were pooled in one functional group. The fast growing grasses were pooled because each of them had a low cover in the control plots at the beginning of the experiment (particularly *A. elatius*) and they are known to have very similar strategies (Grime et al. 1996; Ryser & Urbas 2000). However, we also analysed changes in relative abundance of *A. elatius* alone, and results were very similar to those obtained with the pooled group (data not shown).

#### Individual responses and competitive responses

Survival of all transplants was recorded in late September 2001. Because the effect of fertilization on survival was very strong at the end of the first growing season, with a high mortality for transplants grown with neighbours in the F and WF plots, we decided to harvest all transplants in these plots for above-ground biomass measurements. Transplants from unfertilized plots (C and W) were left intact for a second year of treatment, so we also counted the number of leaves of all transplants in late September 2001 to provide a common growth measurement to all treatments after the first growing season. The relationship between this growth measurement and above-ground biomass was tested using Pearson correlations for transplants grown in the F and WF plots. Because leaf number was strongly correlated with above-ground biomass of transplants for the three species (Table 1 and see also Corcket et al. 2003 for the same species in unfertilized plots), biomass data were not shown for the first year of the experiment. For the second year of experiment only survival and above-ground biomass of transplants were measured in late September 2002 in the C and W plots. To summarize, survival and leaf number of transplants were used to assess individual and competitive responses

**Table 1.** Pearson correlation coefficients (*r*) between leaf number and above-ground biomass of transplants of *Bromus erectus*, *B. rupestre* and *Arrhenatherum elatius* in the fertilized plots (F) and the fertilized and watered plots (WF) after one year of experimentation.

	F			WF
	Ν	r	Ν	r
Bromus erectus	25	0.957***	25	0.901***
Bromus rupestre	26	0.834***	28	0.932***
Arrhneatherum elatius	31	0.841***	21	0.76***

of the three species after one year of experiment in all plots, whereas survival and above-ground biomass were used to assess individual and competitive responses of the three species after two years of experiment in the C and W plots only. Above-ground biomass of transplants was calculated by clipping living shoots at ground level and drying them at 70 °C for 72 h before weighing.

The performance of transplants grown without neighbours were used to assess individual responses to our environmental treatments, whereas competitive responses were assessed by comparing the performances of transplants with and without neighbours, using the Relative Neighbour Effect index (RNE) defined by Markham & Chanway (1996):

$$RNE = (P_{-N} - P_{+N})/x$$
 (1)

where  $P_{-N}$  is the performance (survival or growth) of transplants without neighbours,  $P_{+N}$  the performance with neighbours and *x* is the greater absolute value between  $P_{-N}$  and  $P_{+N}$ . This index ranges from -1 to 1, with negative values indicating facilitation, positive values indicating competition and a value of zero indicating that neighbours have no effect on the performance of transplants.

#### Data analyses

The effects of our environmental treatments on soil moisture, soil available N and total above-ground biomass were analysed using Kruskal-Wallis H non-parametric tests and Mann-Whitney U-tests to contrast group means among soil resource treatments. For individual responses (survival, leaf number and above-ground biomass) and competitive responses (RNE for survival, leaf number and above-ground biomass) of transplants, as well as relative biomass, we used a split-plot ANOVA model. Water and nutrients were treated as a main plot effect and species as a subplot effect. Sphericity assumptions on transformed data were tested by Mauchly's test. We used Huynh-Feldt  $\varepsilon$  when the sphericity assumption appeared to be violated Quinn & Keough 2002).

Survival was calculated in % per plot for each species and each competition treatment, whilst the mean growth of the four replicate seedlings of each species in each plot for each competition treatment was used to avoid pseudoreplication (Hulbert 1984). RNE (survival and growth) was then calculated for each plot. Size variables (biomass and leaf number) were log-transformed and proportion variables (survival and relative biomass) were arcsine square root-transformed prior to analysis to reduce heteroscedasticity (Sokal & Rohlf 1995). *Post hoc* comparisons within species were tested with Tukey's btest. All analyses were carried out with SPSS 11.0 for Windows.

#### Results

## Changes in nutrient and water availability and total above-ground biomass

Because results were similar in 2001 and 2002, only 2001 data are presented. Our treatments significantly affected soil resource availability (Kruskal-Wallis test: soil nitrogen,  $\chi^2 = 8.66$ , df = 3, P = 0.015; soil moisture,  $\chi^2 = 11.25$ , df = 3, P < 0.001). Soil moisture in early August increased significantly with the irrigation treatment from 18% soil humidity in the unwatered plots, C and F, to 28% in the watered plots, W and WF (Mann-Whitney U-test: P < 0.001). Soil available N also increased significantly with the fertilization treatment from 2.2 mg-N.kg<sup>-1</sup> dry soil in the unfertilized plots, F and WF (Mann-Whitney U-test: P < 0.001).

Treatments had a significant effect on above-ground biomass (Kruskal-Wallis test,  $\chi^2 = 9.75$ , df = 3, P = 0.006). Plant biomass was 48% higher in W plots than in C plots (U-test: P = 0.014) and 94% higher in the F plots than in C plots (Mann-Whitney U-test: P = 0.029). There were no significant differences between F and WF plots (U-test: P = 0.171).

#### Individual responses (without neighbours)

#### First year of experiment

The two environmental treatments had no significant effect on plant survival (data not shown). Fertilization had an overall significant effect on plant growth, increasing transplant leaf number (fertilization:  $F_{1,24}$  = 9.37, P = 0.005). However, species differed in their responses to nutrients (species  $\times$  nutrients:  $F_{2.48} = 9.86$ , P<0.001; Fig. 1). B. erectus was not responsive, whereas A. elatius produced more leaves with nutrient addition. This effect was only significant in the unwatered (F) plots (Fig. 1, Tukey test P < 0.05). In contrast, the increase in leaf number for *B. rupestre* in the fertilized (F vs C and WF vs W) plots was not significant. This shows that nutrient availability was limiting for growth in the control plots for A. elatius, but not for the two other species and in particular B. erectus. Differences in species responses to water improvement were marginally significant (species × water:  $F_{2.48} = 2.5$ , P = 0.092) with no response for B. erectus, a significant positive effect for A. elatius only in the unfertilized plots and a tendency for B. rupestre to produce more leaves in the watered (W and WF) plots than in the unwatered (C and F) plots (Fig. 1).

#### Second year of experiment

Water addition had a positive effect on the survival of the three species (water:  $F_{1,12} = 24.72, P < 0.001$ , Fig. 2a) but this effect was much stronger for A. elatius which had a low survivorship in the C plots (species  $\times$ water:  $F_{2,24} = 6.84$ , P = 0.004, Fig. 2a). For all three species, target biomass was significantly higher in W than in C plots (water:  $F_{1,11} = 15.08$ , P = 0.003, Fig. 2b). Although the species  $\times$  water interaction was only marginally significant ( $F_{1.95,21,42} = 3.07$ , P =0.068), B. rupestre was significantly, and by far, the largest species in the W plots and A. elatius significantly the smallest species in the C plots (Tukey test P < 0.005, Fig. 2b). In summary, A. *elatius* was the least drought tolerant, as indicated by the survival data, whereas B. rupestre was the largest species in the watered plots.



**Fig. 2.** The effects of watering on (**a**) survival and (**b**) aboveground biomass of each transplanted species *Bromus erectus*, *B. rupestre* and *Arrhenatherum elatius* without neighbours after two years of experimentation (means  $\pm$  SE). Letters indicate significant means contrasts among species within treatments. Lowercase letters refer to control plots, capital letters refer to watered plots. Bars within treatments that share letters were not different at *P* < 0.05 (post-ANOVA Tukey test).



**Fig. 1.** The effects of watering and fertilization on the leaf number (means  $\pm$  SE) of each transplanted species *Bromus erectus*, *B. rupestre* and *Arrhenatherum elatius* without neighbours after one year of experimentation. Letters indicate significant means contrasts among treatments within species. Bars within species that share letters were not different at *P* < 0.05 (post-ANOVA Tukey test).

#### Competitive responses

#### First year of experiment

For survival, fertilization had the strongest effect on competition intensity, which was almost nil in the unfertilized (C and W) plots (nutrients: RNE survival:  $F_{1,24} = 39.67, P < 0.001$ , Fig. 3). There was also, for all three species, an increase in RNE survival with improved water supply: RNE survival:  $F_{1,24} = 9.06, P =$ 0.006). This effect was mainly due to the decrease in facilitation with water improvement for *A. elatius* and



**Fig. 3.** The effects of watering and fertilization on the Relative Neighbour Effect index (RNE) for survival of each transplanted species *Bromus erectus*, *B. rupestre* and *Arrhenatherum elatius* after one year of experimentation. Letters indicate significant means contrasts among treatments within species. Bars within species that share letters were not different at P < 0.05 (post-ANOVA Tukey test).



**Fig. 4.** The effects of watering on the Relative Neighbourhood Effect index (RNE) for (**a**) survival and (**b**) above-ground biomass for each transplanted species: *Bromus erectus*, *B. rupestre* and *Arrhenatherum elatius* after two years of experimentation. Letters indicate significant means contrasts among species within treatments. Lowercase letters refer to control plots, capital letters refer to watered plots. Bars within treatments that share letters were not different at P < 0.05 (post-ANOVA Tukey test).

*B. rupestre* in the unfertilized plots (C vs W), and to an increase in competition in the fertilized (F vs WF) plots for the three species. Species were not similarly affected by neighbours (species: RNE <sub>survival</sub>:  $F_{2,48} = 7.9, P = 0.001$ ): *A. elatius* had the lowest RNE values, *B. erectus* the highest values and *B. rupestre* was intermediate. The non-significant interaction between species and resources for RNE <sub>survival</sub> suggests that our environmental treatments did not modify the ranking in competitive responses among species.

RNE leaf number was high in all plots with weak differences among species and treatments (data not shown).



**Fig. 5.** The effects of watering and fertilization on relative biomass (%) of *Bromus erectus*, *B. rupestre* and fast growing species (naturally occurring populations) after two years of experimentation. Letters indicate significant means contrasts among treatments within species. Bars within species that share letters were not different at P < 0.05 (post-ANOVA Tukey test).

#### Second year of experiment

After two years, results for RNE survival were very similar to those obtained after one year of experiment. We observed the same ranking in competitive responses among species (species: RNE <sub>survival</sub>:  $F_{2,24} = 14.19$ , P < 0.001), a stability of this ranking with watering (no species × water interaction) and an overall increase in RNE with water improvement (water: RNE <sub>survival</sub>:  $F_{1,12} = 11.23$ , P = 0.006). For growth, RNE values were lower for *A. elatius* than for the two other species (species: RNE <sub>biomass</sub>:  $F_{1.15,12.66} = 15.62$ , P = 0.001, Fig. 4b) and this ranking was not affected by the water treatment (water and species × water non significant).

#### Changes in relative biomass of dominant species

Both treatments negatively affected the relative biomass of *B. erectus* although these effects were significant only in the WF plots (Fig. 5). Watering increased significantly the relative biomass of *B. rupestre* (species × water:  $F_{2,16} = 4.88$ , P = 0.022) in low nutrient plots (W) but not in fertilized plots (WF) (Fig. 5). Fertilization strongly increased the relative biomass of fast growing grasses (species × nutrients:  $F_{2,16} = 14.41$ , P < 0.001) but this effect was significant only in the watered plots (WF) (Fig. 5). As a consequence, *B. rupestre* and *B. erectus* were both dominants in the watered unfertilized (W) plots, where fast growing grasses had a very low relative biomass. In contrast, fast growing grasses *B. erectus* were both dominants in the WF plots.

#### Discussion

#### Stress tolerance abilities

Following Grubb (1998), we will examine both the tolerance of resource shortage with data on survival, and the flexibility in growth response to resource improvement. Grubb (1998) argued that the tolerance of resource shortage was inherently associated with low growth flexibility in the model of Grime (1974), because of the long leaf life span and low growth rate of the stress tolerators of Grime (1974). For survival, only A. elatius was strongly negatively affected by low water availability of the control plots, whereas no differences were observed between the two other species. However, Corcket et al. (2003) showed that B. rupestre was negatively affected in its survival by an experimental drought conducted in the same community, but B. erectus was not. This indicates that water availability was not low enough in our control plots to test for the tolerance of B. rupestre to drought. Our results, and those of Corcket et al. (2003), suggest that only B. erectus can be considered as a typical stress tolerant species sensu Grime, A. elatius as a non-tolerant species and B. rupestre as an intermediate species. There were no significant differences in responses to nutrient shortage for the survival of the three species which was consistent with Goldberg & Novoplansky (1997) who have argued that nutrient availability primarily affects growth but not survival. This suggests that survival responses to physical constraints (e.g. water shortage and cold) are likely to be the best predictors of the stress tolerance ability of a species.

The high growth increase observed for A. elatius and B. rupestre with the addition of nutrient and water respectively revealed their strong flexibility and specific resource requirements. In contrast, B. erectus did not show any flexibility. B. erectus belongs to the 'low flexibility' strategy of Grubb (1998), because of both its high tolerance to drought and its low flexibility to resource improvement. B. rupestre may rather belong to the 'gearing down' strategy of Grubb (1998), because of its high flexibility with water improvement and low mortality in the control plots. Because of its high mortality in the control plots, A. elatius cannot be classified in any of these categories and cannot be considered as stress tolerant, both in Grime's and Grubb's terminologies. We suggest that Grubb's conception of stress tolerance may refine the real strategy of species such as B. rupestre which are only considered as intermediate species between the S and C strategies in Grime's model. The growth flexibility with resources improvement and the large increase in biomass observed in the watered plots may confer a competitive advantage to B. rupestre to dominate in water-rich but nutrient-poor conditions.

#### Competitive responses abilities

Our results showed that the ranking in competitive responses among the three target species was not altered by our environmental treatments. B. erectus was always the most sensitive to competition, A. elatius was always the least sensitive to competition and B. rupestre was intermediate. Consistent with Mahmoud & Grime (1976), Wilson & Keddy (1986) and Keddy et al. (2002), but in opposition to McGraw & Chapin (1989), Aerts et al. (1990) and Novoplansky & Goldberg (2001), there was no reversal in competitive responses in our experiment, either along the water or the nutrient gradients. This result does not support Tilman's argument that species from nutrient-poor sites (i.e. nutrient competitors) may dominate because of their high competitive responses. According to Tilman's definition, to behave as nutrient competitors, the slow growing B. erectus and B. rupestre should have been less affected by competition in the unfertilized plots than the fast growing A. elatius. For Tilman (1982, 1990), tolerance and competitive ability are conferred by the same traits; competitors are species which show the best ability to reduce the limiting resource to a low level (R\*) and to tolerate those low levels.

The lower competitive response of *B. rupestre* in the watered and unfertilized plots (W), compared to *A. elatius*, does not support our prediction that the growth flexibility with resources improvement (in particular water) may confer a competitive advantage to *B. rupestre* to dominate in water-rich but nutrient-poor conditions. This result supports Grime's (1974) model, which classified this species as intermediate between the true stress tolerant and competitive strategies. However, another alternative may be that competitive responses are not the best component of the competitive ability to predict patterns of dominance in plant communities.

#### Community responses

The two environmental treatments significantly increased the above-ground biomass of our calcareous community, which shows that both water and nutrients were limiting resources. This result is consistent with other experiments, which have shown that the productivity of European calcareous communities is primarily limited by these two resources (Grime & Curtis 1976; Thompson & Grime 1988; Grime et al. 2000; Thompson et al. 2001). The increase in biomass was two times greater with nutrients than with water addition. We suggest that this is due primarily to a greater limitation by nutrient than by water in our control plots rather than a larger experimental supply of nutrients than water. The studied community belongs to the mesic or mesoxeric type of calcareous grasslands (*Mesobromion* type of the phytosociological classification, see Royer 1987), which is moderately limited by drought cf. the xeric type (*Xerobromion* type of the phytosociological classification). Corcket et al. (2003) observed a significant decrease in biomass in the same field following an experimental drought, also observed by Grime et al. (2000) in a similar mesic type of calcareous grassland.

We observed contrasted qualitative responses to our environmental treatments, which were highly consistent with individual responses. The watering significantly increased the relative biomass of B. rupestre in low nutrient plots only, whereas the increase in relative biomass for A. elatius and other fast growing species was significant with fertilization, but only when this treatment was associated with watering. In contrast, relative biomass of B. erectus decreased in all resource rich plots. The increase in dominance of fast growing species, and in particular A. elatius with fertilization, is a result commonly observed in mesic calcareous communities (Grime & Curtis 1976; Bobbink 1991; Buckland et al. 2001; Thompson et al. 2001) and in mixtures grown in common garden experiments (Berendse et al. 1992; Campbell & Grime 1992). Corcket et al. (2003) observed a similar increase in those species with an improvement of both water and nutrient availability under experimental shade (50%). To our knowledge, our experiment is the first one showing an increase in biomass of B. rupestre with watering, although Corcket et al. (2003) observed a decrease in the cover of B. rupestre following an experimental drought in the same site.

The contrasted qualitative responses observed for these three naturally occurring species to our environmental manipulations demonstrate in a more general perspective that this European calcareous community behaved as a set of co-occurring species or group of species with specific functional strategies (see also Chapin & Shaver 1985). Each species or group of species were either subordinated and stressed due to limitations of a particular resource (nutrient or water) or dominant and not stressed because these limitations prevented their competitive exclusions (Weiher 2004; Lortie et al. 2004). In opposition to Körner's arguments (Körner 2004), our results are evidence that the species-based concept of stress is not useless and, because communities do not react to environmental changes as single organisms (Chapin & Shaver 1985), there is no reason to consider that this concept may or may not be useless at the community level.

#### Conclusions

The ranking in competitive responses and the stress tolerance abilities (sensu Grime) observed in our experiment were consistent with Grime's model (1974), but does not indicate that B. rupestre should have a competitive advantage over A. elatius in mesic nutrient-poor conditions, and neither supports the hypothesis of Goldberg & Novoplansky (1997) that water gradients are different from nutrient gradients. However, this ranking was not consistent with natural patterns of dominance and community responses, which showed in particular that B. rupestre increased in abundance with watering during our experiment. These latter results supported our prediction that the strong flexibility (sensu Grubb) of B. rupestre transplants with resource improvements and the large increase in biomass observed in the watered plots should confer a competitive advantage for this species in water-rich but nutrient-poor conditions, through a biomass accumulation process.

A number of plant ecologists agree that this biomass accumulation is the primary mechanism explaining the maintenance of slow growing species in nutrient-poor sites (Aerts 1995; Ryser & Lambers 1995; Craine et al. 2002). This slow increase in biomass with time could give them a competitive advantage by reversing the competitive asymmetry with fast growing species (Keddy et al. 2000; Köchy & Wilson 2000). The competitive ability of a species is determined by its ability to tolerate suppressive effects of neighbours - its competitive response - and by its ability to negatively affect its neighbours - its competitive effect (Goldberg & Fleetwood 1987; Goldberg 1990; Keddy et al. 1994). Dominance of large, slow growing species such as *B*. rupestre may, overall, be controlled by competitive effects rather than by competitive response abilities (Peltzer & Köchy 2001; see also Mitchley & Grubb 1986), which have been shown to be poorly indicative of plant dominance (Suding et al. 2003). Mitchley & Willems (1995) have shown that the competitive effect of B. pinnatum in dense Dutch mesic grassland was due to its suppression of most other growth forms by reducing PAR (Photosynthetically active radiation) in the lower canopy. Competition for light may not be the primary driver of these changes in dominance and other mechanisms such as allelopathy and accumulation of recalcitrant litter have been shown to determine the competitive success of such large conservative species in nutrient-poor, low disturbance habitats (Grime 1974; Wardle et al. 1998; Foster 1999).

Our main objective was to assess differences in stress tolerance and competitive abilities for species naturally dominating communities with contrasted water and nutrient availabilities. In water and nutrient-

poor conditions, as well as in water and nutrient-rich conditions, our results supported the Grime's model (1974) that stress tolerance and competitive abilities (inferred from observed competitive responses) efficiently explain natural patterns of dominance. In contrast, in water-rich but nutrient-poor conditions results were more confusing for B. rupestre, and in particular competitive responses appeared to be poor predictors of natural patterns of dominance. However, community and individual responses suggested that this conservative species might be able to tolerate resources shortage but to acquire a strong competitive effect with time, in particular, in water-rich but nutrient-poor communities. Other experimental studies are needed to test the hypothesis that the success of this conservative competitor type of species is due to its competitive effect, and in particular to the depletion of light due to increasing size with biomass accumulation or to interference processes.

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#### References

- Anon. 2001-2002. Bulletin climatologique annuel de l'Isère. Météo-France, Grenoble/Saint-Martin d'Hères.
- Aerts, R. 1995. The advantages of being evergreen. *Trends Ecol. Evol.* 10: 402-415.
- Aerts, R., Berendse, F., De Caluwe, H. & Schmitz, M. 1990. Competition in heathland along an experimental gradient of nutrient availability. *Oikos* 57: 310-318.
- Barbaro, L. & Cozic, P. 1998. Organisation agro-écologique des pelouses et landes calcicoles du Parc Naturel Régional du Vercors. *Ecologie* 29: 443-457.
- Berendse, F., Elberse, W.Th. & Geerts, R.H.E.M. 1992. Competition and nitrogen loss from plants in grassland ecosystems. *Ecology* 73: 46-53.
- Bobbink, R. 1991. Effects of nutrient enrichment in Dutch chalk grassland. J. Appl. Ecol. 28: 28-41.
- Buckland, S.M., Thompson, K., Hodgson, J.G. & Grime, J.P. 2001. Grassland invasions: effects of manipulations of climate and management. J. Appl. Ecol. 38: 301-309.
- Campbell, B.D. & Grime, J.P. 1992. An experimental test of plant strategy theory. *Ecology* 73: 15-29.
- Chapin III, F.S. & Shaver, G.R. 1985. Individualistic growth response of tundra plant species to environmental manipulations in the field. *Ecology* 66: 564-576.

- Corcket, E., Liancourt, P., Callaway, R.M. & Michalet, R. 2003. The relative importance of competition for two dominant grass species, as affected by environmental manipulations in the field. *Ecoscience* 10: 186-194.
- Craine, J.M., Tilman, D., Wedin, D., Reich, P., Tjoelker, M. & Knops, J. 2002. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. *Funct. Ecol.* 16: 563-574.
- Dorich, R.A & Nelson, D.W. 1983. Ammonium measurement. Soil Sci. Soc. Am. 47: 833-836.
- Foster, B.L. 1999. Establishment, competition and the distribution of native grasses among Michigan old-fields. *J. Ecol.* 87: 476-489.
- Goldberg, D.E. 1990. Components of resource competition in plant communities. In: Grace, J.B. & Tilman, D. (eds.) *Perspectives in plant competition*, pp. 357-364. Academic Press, San Diego, CA, US.
- Goldberg, D.E. & Barton, A.M. 1992. Patterns and consequences of interspecific competition in natural communities: a review of field experiments with plants. *Am. Nat.* 139: 771-801.
- Goldberg, D.E. & Fleetwood, L. 1987. Competitive effect and response in four annual plants. J. Ecol. 75: 1131-1143.
- Goldberg, D.E. & Novoplansky, A. 1997. On the relative importance of competition in unproductive environments. *J. Ecol.* 85: 409-418.
- Grace, J.B. 1991. A clarification of the debate between Grime and Tilman. *Funct. Ecol.* 5: 585-587.
- Grime, J.P. 1974. Vegetation classification by reference to strategies. *Nature* 250: 26-31.
- Grime, J.P. & Curtis, A.V. 1976. The interaction of drought and mineral nutrients stress in calcareous grassland. *J. Ecol.* 64: 976-998.
- Grime, J.P., Hodgson, J.G. & Hunt, R. 1996. Comparative plant ecology: a functional approach to common British species. Unwin Hyman, London, UK.
- Grime, J.P., Brown, V.K., Thompson, K., Masters, G.J., Hillier, S.H., Clarke, I.P., Askew, A.P., Corker, D. & Kielty, J.P. 2000. The response of two contrasting limestone grasslands to simulated climate change. *Science* 289: 762-765.
- Grubb, P.J. 1985. Plant populations and vegetation in relation to habitat, disturbance and competition: problems of generalization. In: White, J. (ed.) *The population structure of vegetation*, pp. 595-611. Junk, Dordrecht, NL.
- Grubb, P.J. 1998. A reassessment of the strategies of plants which cope with shortages of resources. *Perspect. Plant. Ecol. Evol. Syst.* 1: 3-31.
- Gurevitch, J., Morrow, L.L., Wallace, A. & Walsh, J.S. 1992. A meta-analysis of competition in field experiments. *Am. Nat.* 140: 539-572.
- Hulbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* 54: 187-211.
- Kadmon, R. 1995. Plant competition along soil moisture gradients: a field experiment with the desert annual *Stipa capensis*. J. Ecol. 83: 253-262.
- Keddy, P.A., Twolan-Strutt, L. & Wisheu, I.C. 1994. Competitive effect and response ranking in 20 wetland plants: are they consistent across three environments? *J. Ecol.* 82: 635-643.

- Keddy, P.A., Gaudet, C. & Fraser, L.H. 2000. Effects of low and high nutrients on the competitive hierarchy of 26 shoreline plants. J. Ecol. 88: 413-423.
- Keddy, P., Nielsen, K., Weiher, E. & Lawson, R. 2002. Relative competitive performance of 63 species of terrestrial herbaceous plants. J. Veg. Sci. 13: 5-16.
- Köchy, M. & Wilson, S.D. 2000. Competitive effects of shrubs and grasses in prairie. *Oikos* 91: 385-395.
- Körner, C. 2004. Individuals have limitations, not communities – A response to Marrs, Weiher & Lortie et al. J. Veg. Sci. 15: 581-582.
- Liancourt, P., Callaway, R.M. & Michalet, R. 2005. Stress tolerance and competitive response ability determine the outcome of biotic interactions. *Ecology* 86: 1611-1618.
- Lortie, C.J., Brooker, R.W., Kikvidze, Z. & Callaway, R.M. 2004. The value of stress and limitation in an imperfect world. *J. Veg. Sci.* 15: 577-580.
- Mahmoud, A. & Grime, J.P. 1976. An analysis of competitive ability in three perennial grasses. *New Phytol*. 77: 431-435.
- Markham, J.H. & Chanway, C.P. 1996. Measuring plant neighbour effects. *Funct. Ecol.* 10: 548-549.
- McGraw, J.B. & Chapin, F.S. III. 1989. Competitive ability and adaptation to fertile and infertile soils in two *Eriophorum* species. *Ecology* 70: 736-749.
- Mitchley, J. & Grubb, P.J. 1986. Control of relative abundance of perennials in chalk grassland in southern England. I. Constancy of rank order and results of pot- and fieldexperiments on the role of interference. J. Ecol. 74: 1139-1166.
- Mitchley, J. & Willems, J.H. 1995. Vertical canopy structure of Dutch chalk grasslands in relation to their management. *Vegetatio* 117: 17-27.
- Moog, D., Poschlod. P., Kahmen, S. & Schreiber, K.F. 2002. Comparison of species composition between different grassland management treatments after 25 years. *Appl. Veg. Sci.* 5: 99-106.
- Novoplansky, A. & Goldberg, D.E. 2001. Effects of water pulsing on individual performance and competitive hierarchies in plants. J. Veg. Sci. 12: 199-208.
- Peltzer, D.A. & Köchy, M. 2001. Competitive effects of grasses and woody plants in mixed-grass prairie. J. Ecol. 89: 519-527.
- Quinn G.P., Keough, M.J. 2002. Experimental design and data analysis for biologists. University Press, Cambridge, UK.
- Royer, J.M. 1987. Les pelouses des Festuco-Brometea. D'un exemple régional à une vision eurosibérienne: étude phytosociologique et phytogéographique. Thèse d'état. Université de Franche-Comté, Besançon, FR.
- Ryser, P. & Lambers, H. 1995. Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. *Plant Soil* 170: 251-265.
- Ryser, P. & Urbas, P. 2000. Ecological significance of life span among Central European grass species. *Oikos* 91: 41-50.
- Ryser, P. & Wahl, S. 2001. Interspecific variation in RGR and the underlying traits among 24 grass species grown in full

daylight. Plant Biol. 3: 426-436.

- Sokal, R.R. & Rohlf, F.J. 1995. *Biometry: the principles and practice of statistics in biological research*. 3rd ed. Freeman, New York, NY, US.
- Suding, K.N., Goldberg, D. & Hartman, K.M. 2003. Relationships among species traits: separating levels of response and identifying linkages to abundance. *Ecology* 84: 1-16.
- Thompson, K. 1987. The resource ratio hypothesis and the meaning of competition. *Funct. Ecol.* 1: 297-303.
- Thompson, K. & Grime, J.P. 1988. Competition reconsidered a reply to Tilman. *Funct. Ecol.* 2: 114-116.
- Thompson, K., Hodgson, J.G., Grime, J.P. & Burke, M.J.W. 2001. Plant traits and temporal scale; evidence from a five-year invasion experiment using native species. J. Ecol. 89: 1054-1060.
- Tilman, D. 1982. *Resource competition and community structure*. Princeton University Press, Princeton, NJ, US.
- Tilman, D. 1987. On the meaning of competition and the mechanisms of competitive superiority. *Funct. Ecol.* 1: 304-315.
- Tilman, D. 1990. Constraints and tradeoffs: toward a predictive theory of competition and succession. *Oikos* 58: 3-15.
- Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M. & Webb, D.A. 1964-1980. *Flora Europaea*. Vols. 1-5. Cambridge University Press, Cambridge, UK.
- Twolan-Strutt, L. & Keddy, P.A. 1996. Above- and belowground competition intensity in two contrasting wetland plant communities. *Ecology* 77: 259-270.
- Wardle, D.A., Nilsson, M.C., Gallet, C. & Zackrisson, O. 1998. An ecosystem-level perspective of allelopathy. *Biol. Rev.* 73: 305-319.
- Weiher, E. 2004. Why should we constrain stress and limitation? Why conceptual terms deserve broad definitions? J. Veg. Sci. 15: 569-572.
- Willems, J.H. 1980. Observations on north-west European limestone grassland communities. *Proc. K. Ned. Akad. Wet. Ser. C* 83: 279-306.
- Wilson, J.B. & Lee, W.G. 2000. C-S-R triangle theory: community-level predictions, tests, evaluation of criticisms, and relation to other theories. *Oikos* 91: 77-96.
- Wilson, S.D. & Keddy, P.A. 1986. Species competitive ability and position along a natural stress/disturbance gradient. *Ecology* 67: 1236-1242.
- Wilson, S.D. & Tilman, D. 1991. Components of plant competition along an experimental gradient of nitrogen availability. *Ecology* 72: 1050-1065.

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## Impact of nitrogen deposition at the species level

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In Europe and, increasingly, the rest of the world, the key policy tool for the control of air pollution is the critical load, a level of pollution below which there are no known significant harmful effects on the environment. Critical loads are used to map sensitive regions and habitats, permit individual polluting activities, and frame international negotiations on transboundary air pollution. Despite their fundamental importance in environmental science and policy, there has been no systematic attempt to verify a critical load with field survey data. Here, we use a large dataset of European grasslands along a gradient of nitrogen (N) deposition to show statistically significant declines in the abundance of species from the lowest level of N deposition at which it is possible to identify a change. Approximately 60% of species change points occur at or below the range of the currently established critical load. If this result is found more widely, the underlying principle of no harm in pollution policy may need to be modified to one of informed decisions on how much harm is acceptable. Our results highlight the importance of protecting currently unpolluted areas from new pollution sources, because we cannot rule out ecological impacts from even relatively small increases in reactive N deposition.

plant ecology | Threshold Indicator Taxon Analysis | gradient survey

**S** ince the 1980s, the key policy tool for the control of pollution in Europe has been the critical load (1). A critical load is defined as a "quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge" (1). Empirical critical load values are currently set for pollutants and habitats based primarily on pollution addition experiments and expert judgment, and they were most recently revised in 2010 (2). Critical loads have a central role in pollution management and are used for mapping pollution impacts, controlling and permitting individual pollution sources, and framing international negotiations on transboundary air pollution. They have recently been applied in the United States (3) and Canada (4), and they are under active consideration and development in many parts of the developing world (5).

Embedded in the critical load concept is the idea that it is possible to define a level of pollution that does not harm the natural environment. We test this concept for the deposition of reactive nitrogen (N). N deposition is recognized as one of the most serious pollution threats to global ecosystems (6), and it is ranked among the top five drivers of global biodiversity loss (7). Current critical loads for N deposition are primarily based on N addition experiments, which are valuable for identifying cause-effect relationships but have some limitations. Many experiments are located in regions with high ambient N deposition (often above the established critical load) (8) and use elevated N loads with few treatments over relatively short time periods. As a result, they are poorly suited to identifying the initial impacts of N deposition, vulnerable to treatment artifacts, and predisposed to short-term responses. Given these limits, there is a need to test experimentally derived critical load values with field survey data. Field surveys also have limitations, but the strengths of each approach complement the drawbacks of the other. Properly constructed and analyzed, survey data along pollution gradients provide a means of testing the efficacy of experimentally determined critical loads in the real world. If the experimentally derived critical load value adequately protects

plant communities, we would expect to find no evidence of harmful effects in sites receiving N deposition below this level.

We focus on European acid grasslands: a widespread seminatural habitat in which gradient studies (9-12), time series analvses (13), and manipulation experiments (14) all show N deposition to be a primary driver of change in vegetation composition and diversity. The critical load for this habitat is currently set at 10-15 kg N ha<sup> $-1^{\prime}$ </sup> y<sup>-1</sup>, with three indicators of critical load exceedance: an increase in graminoids, a decrease in total species richness, and a decline of typical species (the indicator that we focus on here) (2). We apply Threshold Indicator Taxon Analysis (TITAN) to identify the points along the N deposition gradient at which individual plant species change in abundance (15) and compare these values with the critical load. TITAN identifies for each species the point along the N deposition gradient at which any systematic difference in cover is maximized (the change point), whether the difference in cover on either side of this change point is statistically significant, and if so, the abruptness of the difference. We use strict criteria (99%) for both "purity" (the proportion of change-point response directions among bootstrap replicates that agree with the observed response) and "reliability" [the proportion of bootstrap change points that are significant at the chosen probability level (in our case, 0.05)]. This conservative approach provides a higher level of confidence in the reliability of change points, but at a lower level of confidence that all species that change are identified. We also applied TITAN to examine relationships between species abundance and climate to account for any multicollinearity between N deposition and climate.

#### Results

All species showing high-significance change points have lower cover in sites with greater N deposition. Change points are identified from the lowest N deposition at which it is possible to identify any change  $(7 \text{ kg N } ha^{-1} \text{ y}^{-1})$  (Fig. 1). The species change points predominately are at the lower end of the N deposition gradient: one-third occur below the lower boundary of the currently established critical load for this habitat, and a similar number is within the critical load range. Independent evidence of sensitivity to N deposition exists for many of these species, such as *Hypericum pulchrum* (11), *Plantago lanceolata* (16, 17), *Hylocomium splendens* (11, 18, 19), *Achillea millefolium* (20), and *Succisa pratensis* (21, 22). Most of the species that decline in cover are forbs, and this finding agrees with studies showing this functional group to be particularly sensitive to N deposition (12). Above 25 kg N ha<sup>-1</sup> y<sup>-1</sup>, there are very few species change

Data deposition: The BEGIN dataset reported in this paper is archived in Ecological Archives, http://www.esapubs.org/archive/ecol/E092/128/metadata.htm.

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Author contributions: R.J.P. designed research; R.J.P., N.B.D., C.J.S., D.J.G., and B.P. performed research; R.J.P. analyzed data; and R.J.P., N.B.D., C.J.S., and D.J.G. wrote the paper.

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Fig. 1. Species change points (purity > 99%, P < 0.05 in >99% bootstraps) showing 5% and 95% bootstrap percentiles; symbols are sized in proportion to z score. \*Species that also show a change point at the equivalent position on the precipitation gradient.

points, suggesting that sensitive species have already been impacted and that changes in remaining species are comparatively minor. Change points strongly converge at an N deposition of 14.2 kg N ha<sup>-1</sup> y<sup>-1</sup> (Fig. 2), indicating a community-level ecological threshold (15). These results suggest that the impacts of N deposition on sensitive species typical of acid grasslands begin at very low deposition levels and are highly nonlinear, with most change occurring below the upper limit of the critical load.

Our key results showing individual species change points below the current critical load and a community-level threshold around 14.2 kg N ha<sup>-1</sup> y<sup>-1</sup> seem quite robust. Excluding all species showing significant change points at corresponding positions on the precipitation gradient, all data from any one sampling region, taxa with more than 75% of occurrences in a sampling region, or the most extreme three instead of five of the candidate change points (shifting the minimum deposition to 6 kg N ha<sup>-1</sup> y<sup>-1</sup>) displaces the community threshold by no more than 1.5 kg N ha<sup>-1</sup> y<sup>-1</sup>, and in all cases, species respond from the lowest levels of N deposition detectable. We, therefore, have confidence in the general robustness of our results to potential confounding factors such as regional biogeography and climate. Relaxing our criteria for purity and reliability to 95% instead of 99% identifies almost twice as many species, but does not alter the proportion of approximately 60% of species change points occurring at or below the range of the critical load.

#### Discussion

Based on this detailed analysis of a large targeted survey, we cannot confirm the existence of a level of N pollution at which impacts on plants are not detectable. Individual species decline in abundance from the lowest level of N deposition at which any change could be identified by our analysis (6–7 kg N ha<sup>-1</sup> y<sup>-1</sup>). We are not aware of any long-term acid grassland field experiment with a total treatment (including ambient) of less than 10 kg

N ha<sup>-1</sup> y<sup>-1</sup>. Because gradient studies cannot identify a deposition level at which there is no decline of typical species, and N addition experiments have limited ability to identify impacts at low levels of pollution or at a fine scale, we conclude that a true critical load for acid grasslands, if it exists, is too low to detect with existing data and methods. However, we do find a convergence of many species change points at an N deposition of 14.2 kg N ha<sup>-1</sup> y<sup>-1</sup> (Fig. 2), indicating a community-level ecological threshold (15). This threshold coincides with the upper boundary of the critical load, suggesting that, rather than the onset of change, the experimentally observed changes on which the critical load is based represent the point at which the community shifts to a more pollution-tolerant assemblage (23).

Our analysis identifies many more changes in the community below the critical load than above it. Thus, a long-term incremental increase in N deposition would have a greater impact in a site with a current loading of 10 kg N ha<sup>-1</sup> y<sup>-1</sup> than a site with a current loading of 30 kg N ha<sup>-1</sup> y<sup>-1</sup>. This finding highlights the importance of protecting currently unpolluted areas from new pollution sources: we cannot rule out ecological impacts from even relatively small increases in reactive N deposition in these areas. It also suggests that less overall ecological damage may result from new sources of reactive N in current high-N deposition regions, where a shift to a more pollution-tolerant community may have already occurred, rather than regions that currently receive low levels of pollution and are sensitive to even small increases in N deposition. Effective pollution control policy should focus on avoiding all new pollution sources in currently unpolluted regions.

Critical loads have been highly successful in reversing the acidification of European ecosystems, and we support the continuation and expansion of effects-based pollution policy. In addition, one practical application of critical loads is to rank the relative sensitivity of different habitats for making decisions on ECOLOGY

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**Fig. 2.** Community change for species reduced in abundance [sum (z–)] showing critical load, inferred community threshold (dotted line at 14.2 kg N ha<sup>-1</sup> y<sup>-1</sup>), and 5–95% bootstrap percentile range.

which may be most affected by new emissions sources. Our results do not challenge the value of critical loads for identifying pollution levels associated with undesirable biogeochemical change (such as leaching of reactive N to downstream ecosystems) or assessing relative harm to ecological communities. However, by introducing a quantitative way of evaluating individual species change, our study suggests that, for this community, the only way to have no harm is to have no pollution. If the results from this study are found more widely, the central no-harm concept of critical loads may have to be replaced with a sliding scale of harm, in which informed decisions must be made on which elements in an environment should be protected and which elements may have to be compromised.

- 1. Nilsson J, Grennfelt P (1988) Critical Loads for Sulphur and Nitrogen (UNECE/Nordic Council of Ministers, Skokloster, Sweden).
- 2. UNECE (2010) Empirical Critical Loads and Dose-Response Relationships (UNECE, Geneva).
- Burns D, Blett T, Haeuber R, Pardo L (2008) Critical loads as a policy tool for protecting ecosystems from the effects of air pollutants. Front Ecol Environ 6(3):156–159.
- Liu X, et al. (2011) Nitrogen deposition and its ecological impact in China: An overview. Environ Pollut 159(10):2251–2264.
- Ouimet R, Arp P, Watmough S, Aherne J, DeMerchant I (2006) Determination and mapping critical loads of acidity and exceedances for upland forest soils in eastern Canada. Water Air Soil Pollut 172(1-4):57–66.
- Phoenix G, et al. (2006) Atmospheric nitrogen deposition in world biodiversity hotspots: The need for a greater global perspective in assessing N deposition impacts. *Glob Change Biol* 12(3):470–476.
- Sala O, et al. (2000) Global biodiversity scenarios for the year 2100. Science 287(5459): 1770–1774.
- RoTAP (2012) Review of Transboundary Air Pollution: Acidification, Eutrophication, Ground Level Ozone and Heavy Metals in the UK (Centre for Ecology and Hydrology, Edinburgh).
- Stevens C, et al. (2010) Nitrogen deposition threatens species richness of grasslands across Europe. Environ Pollut 158(9):2940–2945.
- Stevens C, et al. (2010) Changes in species composition of European acid grasslands observed along a gradient of nitrogen. J Veg Sci 22(2):207–215.

#### Methods

One hundred fifty-three unimproved and undisturbed acid grasslands (*Violion caninae* association) were surveyed across the Atlantic fringe of northwest Europe (United Kingdom, Ireland, France, Belgium, The Netherlands, Germany, Norway, Sweden, and Denmark), with cover of all species estimated in five  $2 \times 2$ -m quadrats (9, 10). In total, 155 species were identified from sites that fulfilled our criteria: a typical  $2 \times 2$ -m quadrat contained 15–25 species in the less-polluted sites and 7–12 species in the more-polluted sites (9). For each site, estimates of N deposition were produced using the EMEP (European Monitoring and Evaluation Programme)-based IDEM (Integrated Deposition mage is 2.4–43.4 kg N ha<sup>-1</sup> y<sup>-1</sup> (mean = 18.6 kg N ha<sup>-1</sup> y<sup>-1</sup>); most of this gradient is evenly sampled, with the exception of a gap between 2.4 and 5.1 kg N ha<sup>-1</sup> y<sup>-1</sup>.

Underlying the TITAN approach is the Indicator Value (IndVal) (24) method for the identification of taxa that typify groups of an a priori sample classification. A taxon with a high IndVal score will have a high concentration of abundances and high fidelity to a single group. A taxon with a maximal IndVal score of 100% would be found in all samples of a group and only in that group. In TITAN, IndVal scores are calculated for all species for all possible change points along the environmental gradient, with permutation tests to assess the uncertainty in these scores. Permuted IndVal scores are standardized as z scores and summed for positive [sum(z+)] and negative [sum(z-)] responses for each possible change point. Sum(z) peaks highlight values of the environmental variable around which many taxa exhibit strong directional changes in abundance representing community thresholds. Uncertainty is assessed by bootstrapping; quantiles of the bootstrapped maxima provide a guide to the abruptness of the response (15). Change points will be identified with both abrupt (threshold-like) and more gradual species responses, but the latter will have broader confidence intervals. For each species, TITAN also returns measures of purity (proportion of bootstrap replicates matching group assignment in the original data) and reliability (proportion of bootstrap replicates with maximum IndVal reaching a specified P value). TITAN has been shown to be effective at identifying known species and community thresholds in real and simulated data (15).

We applied TITAN to the mean Domin (25) score of the five quadrats for each species. TITAN was implemented in R (26) with 250 IndVal permutations and 500 bootstrap replicates. We excluded both rare species found in five or fewer sites and the five lowest and five highest candidate change points, because the partitioned sample size is considered too small to confidently identify differences (15). Because N deposition is correlated with mean annual precipitation (but not temperature) across these sites, we also applied TITAN to precipitation data.

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- Maskell L, Smart S, Bullock J, Thompson K, Stevens C (2010) Nitrogen deposition causes widespread loss of species richness in British habitats. *Glob Change Biol* 16(2): 671–679.
- Stevens C, Dise N, Gowing D, Mountford J (2006) Loss of forb diversity in relation to nitrogen deposition in the UK: Regional trends and potential controls. *Glob Change Biol* 12(10):1823–1833.
- Duprè C, et al. (2010) Changes in species richness and composition in European acidic grasslands over the past 70 years: The contribution of cumulative atmospheric nitrogen deposition. *Glob Change Biol* 16(1):344–357.
- 14. Sutton M, et al. (2011) European Nitrogen Assessment (Cambridge Univ Press, Cambridge, United Kingdom).
- Baker M, King R (2010) A new method for detecting and interpreting biodiversity and ecological community thresholds. *Methods Ecol Evol* 1(1):25–37.
- Mountford J, Lakhani K, Kirkham F (1993) Experimental assessment of the effects of nitrogen addition under hay-cutting and aftermath grazing on the vegetation of meadows on a Somerset peat moor. J Appl Ecol 30(2):321–332.
- Kirkham F, Mountford J, Wilkins R (1996) The effects of nitrogen, potassium and phosphorus addition on the vegetation of a Somerset peat moor under cutting management. J Appl Ecol 33(5):1013–1029.
- Dirkse G, Martakis G (1992) Effects of fertilizer on bryophytes in Swedish experiments on forest fertilization. *Biol Conserv* 59(2-3):155–161.
- Salemaa M, Mäkipää R, Oksanen J (2008) Differences in the growth response of three bryophyte species to nitrogen. Environ Pollut 152(1):82–91.

- Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* 451(7179):712–715.
- 21. Pauli D, Peintinger M, Schmid B (2002) Nutrient enrichment in calcareous fens: Effects on plant species and community structure. *Basic Appl Ecol* 3(3):255–266.
- Vergeer P, Rengelink R, Ouborg N, Roelofs J (2003) Effects of population size and genetic variation on the response of *Succisa pratensis* to eutrophication and acidification. J Ecol 91(4):600–609.
- 23. Folke C, et al. (2004) Regime shifts, resilience and biodiversity in ecosystem management. Annu Rev Ecol Evol Syst 35:557–581.
- Dufrêne M, Legendre P (1997) Species assemblages and indicator species: The need for a flexible asymmetrical approach. Ecol Monogr 67(3):345–366.
- 25. Bannister P (1966) The use of subjective estimates of cover-abundance as the basis for ordination. *J Ecol* 54(3):665–674.
- 26. R Development Core Team (1993) The R Project for Statistical Computing (R Project, Vienna, Austria).

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## Nitrogen deposition threatens species richness of grasslands across Europe

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Atmospheric nitrogen deposition is reducing biodiversity in grasslands across Europe.

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#### ABSTRACT

Evidence from an international survey in the Atlantic biogeographic region of Europe indicates that chronic nitrogen deposition is reducing plant species richness in acid grasslands. Across the deposition gradient in this region  $(2-44 \text{ kg N ha}^{-1} \text{ yr}^{-1})$  species richness showed a curvilinear response, with greatest reductions in species richness when deposition increased from low levels. This has important implications for conservation policies, suggesting that to protect the most sensitive grasslands resources should be focussed where deposition is currently low. Soil pH is also an important driver of species richness reductions. The results of this survey suggest that the impacts of nitrogen deposition can be observed over a large geographical range.

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#### 1. Introduction

In recent years the global threat posed by atmospheric nitrogen (N) deposition has become clear (Sala et al., 2000; Galloway et al., 2008; Bobbink et al., 2010), but to date, impacts of N deposition on the biodiversity and ecosystem function in semi-natural environments have only been demonstrated at local and national scales (Stevens et al., 2004; Jones et al., 2004; Smart et al., 2005; Maskell et al., 2010). The deposition of reactive N has more than doubled

over the last one hundred years as a result of agricultural intensification and increased burning of fossil fuels by traffic and industry (Galloway et al., 2004; Fowler et al., 2005). Atmospheric deposition of reactive N has the potential to enrich the N content of soils, resulting in increased plant growth and hence competition for light (Bobbink et al., 1998; Hautier et al., 2009) and other resources, and to acidify soils reducing the number of species that can tolerate these conditions and coexist (Schuster and Diekmann, 2003). Globally, the deposition of reactive N is predicted to increase in the future due to the expanding global population leading to increased demand for food and increased use of fossil fuels (Tilman et al., 2002; Dentener et al., 2006). The potential loss of biodiversity as a result of N deposition has important implications for both environmental and agricultural policy.

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In 2004, Stevens et al. identified a linear decline in plant species richness of acid grasslands in Great Britain associated with long-term chronic N deposition. This has since been demonstrated in other habitats in Great Britain such as heathlands, calcareous grasslands and dune grasslands (Maskell et al., 2010; Jones et al., 2004). However, until now there has been little research showing a) what happens at levels of deposition lower than those found in Great Britain b) how important N deposition is as a driver of species richness and c) whether the losses of biodiversity reported by Stevens et al. (2004) are occurring on a larger, international scale.

To address these research needs, we surveyed 153 semi-natural acid grasslands belonging to the *Violion caninae* alliance (Schwickerath, 1944) on a transect across the Atlantic biogeographic zone of Europe with total atmospheric N deposition ranging from 2.4 to 43.5 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Fig. 1), covering much of the range of deposition found in the industrialised world. *V. caninae* grasslands are widespread across western Europe, but changes in land use have decreased their cover in some regions (Ellenberg, 1996). They are economically valuable providing a number of ecosystem services including extensive sheep and cattle grazing in some regions, and are important for biodiversity supporting a range of plants, invertebrates and mammals. These grasslands are dominated by species such as *Agrostis capillaris, Festuca ovina* and *rubra, Potentialla erecta* and *Galium saxatile*, typically with a dense sward where species are intimately mixed.

Grasslands were surveyed in Atlantic regions of Great Britain, Isle of Man, Ireland, France, Germany, Belgium, the Netherlands, Denmark, Sweden and Norway (Fig. 1). All of the grasslands were surveyed between 2002 and 2007 using a consistent methodology; all were unfertilised and many were in areas protected for nature conservation.

#### 2. Materials and methods

#### 2.1. Field methodology

Between 2002 and 2007 153 acid grasslands belonging to the *V. caninae* alliance (Schwickerath, 1944) were surveyed within the Atlantic biogeographic zone of Europe. All grasslands were surveyed between May and September. The survey consisted of: nine grasslands in Belgium, three grasslands in Denmark, twenty-five grasslands in France, twelve grasslands in Germany, eleven grasslands in Ireland, Northern Ireland and the Isle of Man, seven grasslands in the Netherlands, nine grasslands in Norway, four grasslands in Sweden and seventy-seven grasslands in Great Britain. The large number of sites surveyed in Great Britain derives from the intensive national survey of the earlier work and the fact that *V. caninae* grasslands cover a much larger area than in other countries in the study (Stevens et al., 2004).

The grasslands were selected to cover the range of atmospheric N deposition in Europe and to give a good range of sites at different latitudes and longitudes for different deposition values. Grasslands surveyed were not agriculturally improved or in the vicinity of a point source of N and were managed by grazing or cutting. Within each site, five randomly located 4 m<sup>2</sup> quadrats were surveyed, avoiding areas of vegetation belonging to a different community (e.g. areas of shrub) or were strongly affected by animals, tracks and paths, or were in the rain shadows of trees or hedges. Within each quadrat all vascular plants and bryophytes were identified to species level and percent cover was estimated by eye. A description of the site was made including latitude, longitude, aspect, slope, extent of grassland, soil depth and surrounding vegetation communities.

Soil samples were collected from each quadrat. Topsoil samples (0–10 cm below the litter layer) were taken from two opposing corners of the quadrat using a trowel and bulked to give one sample per quadrat. Subsoil samples (20–30 cm deep or as deep as possible in shallow soil) were taken from the centre of the quadrat using a 5 cm diameter soil auger. All soil samples were kept cool during transit and air dried on return to the laboratory.

For each site, N and sulphur deposition data were modelled using the EMEPbased IDEM model (Pieterse et al., 2007) or national deposition models depending on which were available in each of the countries surveyed. Full details are given below. Meteorological data were obtained from the European Space Agency Monitoring Agriculture with Remote Sensing (MARS) unit (Monitoring Agricultural Resources, 2009); ten year averages were calculated for each site for mean annual potential transpiration from crop canopy, mean minimum daily temperature, mean maximum daily temperature and mean annual rainfall. Radiation index was calculated based on aspect, slope and latitude according to Oke (1987).

#### 2.2. Laboratory methodology

All analyses were conducted on air-dried soils due to the large number and geographical spread of sites being surveyed (MAFF, 1986). Soil pH was determined using a pH probe in a 1:5 soil and deionised water mixture.

Nitrate, ammonium, calcium and aluminium concentrations were determined using two different methods. For samples collected in 2002 and 2003 from Great Britain, soils were extracted with 1 M KCl and analysed using an ion chromatograph. Soil samples collected in 2007 were shaken with 0.4 M NaCl and analysed using an auto-analyser. All extracts were determined using a 1:10 soil and extractant mixture. Aluminium and calcium concentrations were determined using the methodology of the later survey showing that results of the two extraction methodologies were comparable. Phosphorus availability was determined using a standard Olsen extraction and colourometric determination (MAFF, 1986). Total C and N content of the soil was determined using a CN elemental analyser by combustion and gas detection.

#### 2.3. Deposition models

For all of the sites visited, the best available deposition model was used for estimating the deposition of nitrogen and sulphur (S), resulting in some variation in the models used. National models were used for Germany (Gauger et al., 2002), the Netherlands (Van Jaarsveld, 1995, 2004; Asman and van Jaarsveld, 2002) and Great Britain (NEGTAP, 2001). For all other countries the EMEP-based IDEM (Pieterse et al., 2007) models were used. The different model use similar approaches to model reduced and oxidised deposition. Comparison between the models showed that these models provided the best estimates of deposition across the region. For all of the models, deposition was calculated as a three-year average to provide a more robust estimate of longer-term nitrogen inputs.

#### 2.4. Statistical analysis

For all analyses mean values from the five samples per site were used. Simple regression and forward and backward multiple regressions were conducted using SPSS v17. All variables were checked for normality and corrected if necessary (Table 1) and strongly inter-correlated independent variables (r > 0.5) were removed from the models. The variables to be retained were selected based on ecological relevance. Soil ammonia concentration and plant available P concentration were highly skewed and correction did not result in a normal distribution. In these cases the model was run with and without these variables included. In each case, results did not differ between model runs so they were excluded from the analysis. The regression tree was analysed in R according to the method set out in Crawley (2007) and variance partitioning using stepwise multiple regression were analysed in Minitab.

#### 3. Results

Examining the relationship between atmospheric N deposition and species richness shows a significant negative linear relationship (linear regression  $r^2 = 0.36$ , p < 0.001). The distribution of sampling sites differs between countries and Great Britain contributes a large proportion of the overall data set. However, when data for Great Britain is removed from the regression model, the relationship remains significant ( $r^2 = 0.28$ , p < 0.001). Analysis of covariance shows that the intercept of the regression line for mainland European species richness differs significantly from the regression line for the Great Britain (p < 0.001), but gradients of the two regression lines for the relationship between N deposition and species richness are not significantly different.

The relationship between N deposition and species richness is better fitted with a negative exponential curve giving an  $r^2$  of 0.40 (p < 0.0001, Fig. 1). This shows a potentially greater loss of species richness when deposition increases from a low background level than a high level.

Most of the decline in species richness is accounted for by a reduction in forb species richness, with grass richness and bryophyte richness showing weaker but still significant negative relationships with N deposition (forbs:  $r^2 = 0.31$ , p < 0.001



**Fig. 1.** Map of acid grasslands surveyed in the Atlantic Biogeographic region of Europe and graph showing total plant species richness (mean number of species in five 2 by 2 m quadrats per site) against total inorganic N deposition for each of the countries surveyed: Belgium (red), Denmark (yellow), France (dark blue), Great Britain (green), Germany (brown), Ireland, Northern Ireland and Isle of Man (pink), Netherlands (purple), Norway (turquoise) and Sweden (orange). The curvilinear relationship between N deposition and species richness is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

(exponential relationship) grass:  $r^2 = 0.27$ , p < 0.001 (linear relationship), bryophyte:  $r^2 = 0.05$ , p < 0.005 (linear relationship); Fig. 2).

Multiple regression (forward and backward stepwise regression) was used to identify additional drivers of species richness in this data set. Correlated variables were removed and corrections for non-normality applied, leaving a set of sixteen variables for analysis (Table 1). Multiple regression showed that total inorganic N deposition, topsoil pH, radiation index and extractable soil nitrate concentration (log transformed for normality) explained a total of 55% of the variation in species richness (Eq. (1)):

$$-0.243(\text{N deposition}) - 3.978(\text{Radiation index}) - 0.304(\ln \text{NO}_3^-)$$
(1)

Topsoil pH showed the strongest linear correlation with species richness, with an  $r^2$  of 0.38 (p < 0.001) (Fig. 3a). As topsoil pH is influenced by site physical characteristics and N deposition, this correlation may be related to acidification of the soils, but the approach does not distinguish between the sources of acidification

#### Table 1

Variables recorded in this study. Variables entered into the regression models are shown in bold. Some variables had to be excluded due to correlation with other variables or data distributions that were too strongly skewed for analysis.

Variable	Range
Dependent variable	
Species richness (mean number of species per five 4 m <sup>2</sup> quadrats)	6.4-33.2
Deposition variables	
Total inorganic N deposition	2.4-43.5
(kg N ha <sup>-1</sup> yr <sup>-1</sup> )	
Total inorganic S deposition	2.2-19.6
(kg N ha ' yr ')	
Geographical and physical variables	
Longitude	-9.951-13.25
Latitude	43.303-60.697
Altitude (m)	4–812 (Ln)
Inclination (°)	0-60
Aspect (°)	0-350
Vegetation height (cm)	1.5-40
Radiation index	-0.43 - 0.99
Mean maximum daily temperature (°C)	6.8-18.8
Mean minimum daily temperature (°C)	0.6-10.2
Mean annual rainfall (mm)	498-1971
Mean annual potential transpiration from canopy (mm)	487-834
Soil variables	
Topsoil pH	3.7-5.7
Subsoil pH	3.3-6.1
Extractable aluminium concentration (mg kg <sup>-1</sup> dry soil)	2.3–1319 (Ln)
Extractable ammonium concentration (mg $kg^{-1}$ dry soil)	0-305
Extractable nitrate concentration (mg kg $^{-1}$ dry soil)	0–172 (Ln)
Olsen P (mg kg <sup>-1</sup> dry soil)	0-86
C (%)	0.03-40.63
N (%)	0.09-22.89
C:N ratio (%/%)	8.9-30.5
Ca:Al ratio (g/g)	0.01-20.49
Management variables	
Management type	Cutting/grazing
Management intensity (estimated from standing	High, medium
crop biomass)	and low

Variables that did not show a normal distribution were log (Ln) - transformed.





which include sulphur deposition. Indeed, there is a significant relationship between soil pH and N deposition ( $r^2 = 0.20$ , p < 0.001) (Fig. 3b) although as a result of the variability of soil types in this study and the large amount of variation in soil pH that is independent of N deposition, it was not necessary to remove this variable from the analysis. There was also a significant correlation between soil pH and S deposition ( $r^2 = 0.18$ , p < 0.001).

A regression tree confirmed the primary importance of N deposition as a driver of species richness. The division in the regression shows that at high deposition (greater than 20.3 kg N ha<sup>-1</sup> yr<sup>-1</sup>), topsoil pH is the next most significant variable, followed by soil nitrate concentration, but at deposition less than 20.3 kg N ha<sup>-1</sup> yr<sup>-1</sup>, extractable aluminium concentration is the next most significant.

#### 3.1. Discussion and conclusions

The results of this large-scale survey suggest that the impacts of N deposition can be observed over a large geographical range and are not restricted to Great Britain, as initially demonstrated by Stevens et al. (2006). Analysis of covariance shows that the gradients of the two regression lines for the relationship between N deposition and species richness are not significantly different, implying that the species richness of grasslands in the two regions are equally sensitive to N deposition.

The results of this investigation are consistent with those found by Dupre et al. (2010) who conducted a temporal analysis of changes in species richness in the same grassland type. They found that during the last 70 years, species richness in Great Britain, Germany and the Netherlands have all declined significantly in relation to estimated cumulative N deposition. These results provide further evidence for declines in species richness related to chronic atmospheric N deposition and support the use of a space for time substitution for detecting the effects of N deposition.

The curvilinear relationship found in this investigation implies that small increases in N deposition will have a larger impact when background deposition levels are low or moderate than when initial deposition levels are higher (above 20 kg N  $ha^{-1}$  yr<sup>-1</sup>, reflecting the point for splitting the data identified in the regression



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**Fig. 2.** a) Forb richness (curvilinear relationship) b) grass richness (linear relationship) and c) bryophyte richness (linear relationship) (mean number of species in five 2 by 2 m quadrats per site) against total inorganic N deposition (kg N ha<sup>-1</sup> yr<sup>-1</sup>) for 153 acid grasslands surveyed in the Atlantic Biogeographic region of Europe.



**Fig. 3.** a) Topsoil pH against plant species richness (mean number of species in five 2 by 2 m quadrats per site), b) topsoil pH against total inorganic N deposition (kg N ha<sup>-1</sup> yr<sup>-1</sup>) for 153 acid grasslands surveyed in the Atlantic Biogeographic region of Europe.

tree (Fig. 4)). These results support the experimental findings of Clark and Tilman (2008), who demonstrated the potential for species loss in prairie grasslands as a result of chronic low-level deposition. Below 20 kg N ha<sup>-1</sup> yr<sup>-1</sup>, linear regression indicates that a deposition rate of 2.3 kg N ha<sup>-1</sup> yr<sup>-1</sup> reduces species richness by one species per 4 m<sup>2</sup> quadrat. Above 20, 3.5 kg N ha<sup>-1</sup> vr<sup>-1</sup> is tolerated before species richness is reduced by the same amount. Such a relationship indicates that at high deposition, many of the species sensitive to N deposition may have already declined leaving mainly the less nitrogen-sensitive species. These findings have important implications for conservation and restoration suggesting that to protect the most sensitive grasslands, resources should be focussed on protecting areas that are as vet relatively undamaged by N deposition, since the potential for species loss in these areas is greater (Emmett, 2007). There may also be important repercussions for the regulation of point source emissions of pollutants. Current legislation aims to maintain deposition below the critical load for N deposition (currently 10–20 kg N  $ha^{-1}$  yr<sup>-1</sup> for acid grassland communities (Bobbink et al., 2003)), but the results of this study indicate that increasing deposition by very small amounts where background levels are low will result in reductions in species richness, even if the total deposition to the site (diffuse source plus point source) remains below the critical load. It is also important to note that even where the critical load is exceeded by background deposition, the addition of further N from a point source may still result in a reduction of species richness, even at high levels of deposition.

The majority of the decline in species richness is accounted for by a loss of forbs. This decline in forb species richness is the same trend as identified in earlier acid grassland surveys in Great Britain (Stevens et al., 2004, 2006, 2009) and is consistent with experimental N additions in other grassland types (e.g. Bobbink, 1991; Mountford et al., 1993; Wedin and Tilman, 1996) and historical studies (Dupre et al., 2010). The wedge shaped distribution observed for forbs and bryophytes indicates that at low deposition richness can be both high and low, but at high deposition high richness is not observed. A loss of species richness within the grassland sward potentially has wide implications for biodiversity further up the food chain (Throop and Lerdau, 2004; Weiss, 1999) as well as an impact on ecosystem functioning and ecosystem service provision.

The results of this study demonstrate the importance of soil pH as a driver of species richness. Of the variables examined, topsoil pH showed the strongest linear correlation with species richness, with an  $r^2$  of 0.38 (p < 0.001) (Fig. 3a). As soil pH is reduced the forms and availability of nutrients and potentially toxic metals are affected, reducing the pool of species able to survive (Tyler, 2003). S deposition also shows a significant negative relationship with topsoil pH ( $r^2 = 0.18$ , p < 0.001) showing that both N and S deposition (or their legacy) remain important drivers of soil pH.

Variance partitioning allows assessment of the relative contributions of different groups of variables to the total variation in species richness. This analysis showed that both a site's geographical and physical characteristics (Table 1), and its management type and intensity, exclusively explain very little of the variation in species richness ( $r^2 = 0.004$  and 0.003, respectively). These small numbers reflect the tightly defined plant community type and the similarities in management practices across the transect. Soil variables exclusively explain 13.9% of the variation in species richness and deposition variables explain 6.9% of the variation in species richness, indicating that N deposition modifies the response of the plant community to site characteristics. However, the potential for N deposition to influence soil variables means that these two sets could be considered together. The sum of the variation in species richness explained exclusively and variation explained by N deposition and soil in combination is 53% of the variation in species richness.

The regression tree (Fig. 4) sheds further light on the interaction between independent variables. In the regression tree, the first division is with N deposition, reflecting the importance of this variable as a driver of species richness with pH and aluminium as the next most significant variables. Aluminium may be a more important driver of species richness at low deposition because here, species intolerant of aluminium toxicity have yet to be eliminated by acidification. The acid substrate in these habitats means that availability of aluminium in the soil can change greatly with small changes in soil pH. Below this level of the regression tree, other variables become important. pH is clearly a very important driver of species richness in these grasslands and soil acidification



**Fig. 4.** Regression tree showing relationships between species richness and dependent variables (Table 1) using dichotomous partitioning criteria. Variables in the regression tree are: total inorganic N deposition (kg N ha<sup>-1</sup> yr<sup>-1</sup>) (N deposition), topsoil pH (pH), In soil KCI extractable aluminium concentration (mg kg<sup>-1</sup>) (In Al) and In soil KCI extractable nitrate concentration (mg kg<sup>-1</sup>) (ln NO<sub>3</sub>).

is likely to be playing an important role in the reduction of species richness. This was also found in analysis of temporal trends in species richness in relation to N deposition (Dupre et al., 2010).

#### 4. Conclusion

The results of this large-scale survey indicate that chronic nitrogen deposition is reducing plant species richness in acid grasslands. Across the deposition gradient in the Atlantic biogeographic region of Europe species richness showed a curvilinear relationship with N deposition, with greatest differences in species richness where deposition increased from low levels. Given the large range over which we observe reduced species richness associated with high N deposition, the similar relationships that have been observed in other habitats (Maskell et al., 2010) and the results of experimental N additions in a wide range of habitats (e.g. Power et al., 1998; Morecroft et al., 1994; Jones et al., 2004; Pilkington et al., 2005) it is reasonable to assume that N deposition represents a global threat to the biodiversity of semi-natural ecosystems.

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#### References

- Asman, W.A.H., van Jaarsveld, J.A., 2002. A variable-resolution transport model applied for NHx in Europe. Atmospheric Environment 26A, 445–464.
- Bobbink, R., Ashmore, M., Braum, S., Fluckinger, W., Van den Wyngaert, I.J.J., 2003. Empirical nitrogen critical loads for natural and semi-natural ecosystems: 2002 update. In: Achermann, B, Bobbink, R (Eds.), Empirical Critical Loads for Nitrogen, Environmental Documentation No. 164 Air. Swiss Agency for Environment, Forest and Landscape SAEFL, Berne.
- Bobbink, R., Hornung, M., Roelofs, J.G.M., 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. Journal of Ecology 86, 717–738.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J. W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L., De Vries, W., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications 20, 30–59.
- Bobbink, R., 1991. Effects of nutrient enrichment in Dutch chalk grassland. Journal of Applied Ecology 28, 28–41.
- Clark, C.M., Tilman, D., 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451, 712–715.
- Crawley, M.J., 2007. The R Book. John Wiley and Sons, Chichester.
- Dentener, F., Stevenson, D., Ellingsen, K., van Noije, T., Schultz, M., Amann, M., Atherton, C., Bell, N., Bergmann, D., Bey, I., Bouwman, L., Butler, T., Cofala, J., Collins, B., Drevet, J., Doherty, R., Eickhout, B., Eskes, H., Fiore, A., Gauss, M., Hauglustaine, D., Horowitz, L., Isaksen, I.S.A., Josse, B., Lawrence, M., Krol, M., Lamarque, J.F., Montanaro, V., Muller, J.F., Peuch, V.H., Pitari, G., Pyle, J., Rast, S., Rodriguez, J., Sanderson, M., Savage, N.H., Shindell, D., Strahan, S., Szopa, S., Sudo, K., Van Dingenen, R., Wild, O., Zeng, G., 2006. The global atmospheric environment for the next generation. Environmental Science & Technology 40, 3586–3594.
- Dupre, C., Stevens, C.J., Ranke, T., Bleeker, A., Peppler-Lisbach, C., Gowing, D.J.G., Dise, N.B., Dorland, E., Bobbink, R., Diekmann, M., 2010. Changes in species richness and composition in European acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. Global Change Biology 16, 344–357.
- Ellenberg, H., 1996. Vegetation Mitteleuropas mit den Alpen, fifth ed. Eugen Ulmer, Stuttgart.
- Emmett, B.A., 2007. Nitrogen saturation of terrestrial ecosystems: some recent findings and their implications for our conceptual framework. Water Air and Soil Pollution Focus 7, 99–109.
- Fowler, D., Muller, J.B.A., Hayman, G., Vincent, K.J., 2005. Changes in the atmospheric deposition of acidifying compounds in the UK between 1986 and 2001. Environmental Pollution 137, 15–25.

- Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S. P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vorosmarty, C.J., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70, 153–226.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions and potential solutions. Science 320, 889–892.
- Gauger, T., Anshelm, F., Schuster, H., Erisman, J.W., Vermeulen, A.T., Draaijers, G.P.J., Bleeker, A., Nagel, H.-D., 2002. Mapping of ecosystems specific long-term trends in deposition loads and concentrations of air pollutants in Germany and their comparison with critical loads and critical levels. In: Institut Fur Navigation. University of Stuttgart Germany.
- Hautier, Y., Niklaus, P.A., Hector, A., 2009. Competition for light causes plant biodiversity loss after eutrophication. Science 324, 636–638.
- Jones, M.L.M., Wallace, H.L., Norris, D., Brittain, S.A., Haria, S., Jones, R.E., Rhind, P.M., Reynolds, B.R., Emmett, B.A., 2004. Changes in vegetation and soil characteristics in coastal sand dunes along a gradient of atmospheric nitrogen deposition. Plant Biology 6, 598–605.
- MAFF, 1986. The Analysis of Agricultural Materials, third ed.. Her Majesty's Stationary Office, London.
- Maskell, L.C., Smart, S.M., Bullock, J.M., Thompson, K., Stevens, C.J., 2010. Nitrogen Deposition causes widespread species loss in British Habitats. Global Change Biology 16, 671–679.
- Monitoring Agricultural Resources (MARS), 2009. European Commission Joint Research Centre URL. http://mars.jrc.it/mars/About-us/The-MARS-Unit.
- Morecroft, M.D., Sellers, E.K., Lee, J.A., 1994. An experimental investigation into the effects of atmospheric deposition on two semi-natural grasslands. Journal of Ecology 82, 475–483.
- Mountford, J.O., Lakhani, K.H., Kirkham, F.W., 1993. Experimental assessment of the effects of nitrogen addition under hay-cutting and aftermath grazing on the vegetation of meadows on a Somerset peat moor. Journal of Applied Ecology 30, 321–332.
- NEGTAP, 2001. Transboundary Air Pollution: acidification, Eutrophication and Ground-level Ozone in the UK. CEH, Edinburgh.
- Oke, T.R., 1987. Boundary Layer Climates. second ed., Methuen, New York.
- Pieterse, G., Bleeker, A., Vermeulen, A.T., Wu, Y., Erisman, J.W., 2007. High resolution modelling of atmosphere-canopy exchange of acidifying and eutrophying components and carbon dioxide for European forests. Tellus 59B, 412–424.
- Pilkington, M.G., Caporn, S.J.M., Carroll, J.A., Cresswell, N., Lee, J.A., Emmett, B.A., Johnson, D., 2005. Effects of increased deposition of atmospheric nitrogen on an upland Calluna moor: N and P transformation. Environmental Pollution 135, 469–480.
- Power, S.A., Ashmore, M.R., Cousins, D.A., 1998. Impacts and fate of experimentally enhanced nitrogen deposition on a British lowland heath. Environmental Pollution 102, 27–34.
- Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Biodiversity – Global biodiversity scenarios for the year 2100. Science 287, 1770–1774.
- Schuster, B., Diekmann, M., 2003. Changes in species density along the soil pH gradient – evidence from German plant communities. Folia Geobotanica 38, 367–379.
- Schwickerath, M., 1944. Das Hohe Venn und seine Randgebiete. Pflanzensoziologie 6, 1–278.
- Smart, S.M., Bunce, R.G.H., Marrs, R.H., LeDuc, M., Firbank, L.G., Maskell, L.C., Scott, W.A., Thompson, K., Walker, K.J., 2005. Large-scale changes in the abundance of common higher plant species across Britain between 1978, 1990 and 1998 as a consequence of human activity: tests of hypothesised changes in trait representation. Biological Conservation 124, 355–371.
- Stevens, C.J., Dise, N.B., Mountford, J.O., Gowing, D.J., 2004. Impact of nitrogen deposition on the species richness of grasslands. Science 303, 1876–1879.
- Stevens, C.J., Dise, N.B., Gowing, D.J., Mountford, J.O., 2006. Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls. Global Change Biology 12, 1823–1833.
- Stevens, C.J., Maskell, L.C., Smart, S.M., Caporn, S.J.M., Dise, N.B., Gowing, D.J., 2009. Identifying indicators of atmospheric nitrogen deposition impacts in acid grasslands. Biological Conservation 142, 2069–2075.
- Throop, H.L., Lerdau, M.T., 2004. Effects of nitrogen deposition on insect herbivory: implications for community and ecosystem processes. Ecosystems 7, 109–133.
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature 418.
- Tyler, G., 2003. Some ecophysiological and historical approaches to species richness and calcicole/calcifuge behaviour – contribution to a debate. Folia Geobotanica 38, 419–428.
- Van Jaarsveld, J.A., 1995. Modelling the long-term atmospheric behaviour of pollutants on various spatial scales. PhD thesis, University of Utrecht, The Netherlands.
- Van Jaarsveld, J.A., 2004. The Operation Priority Substances Model. Report No. 500045001/2004. National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.
- Wedin, D., Tilman, D., 1996. Influence of nitrogen loading and species composition on the carbon balance of grasslands. Science 274, 1720–1723.
- Weiss, S.B., 1999. Cars, cows, and checkerspot butterflies: nitrogen deposition and management of nutrient-poor grassland for a threatened species. Conservation Biology 13, 1476–1486.

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# The impact of nitrogen deposition on acid grasslands in the Atlantic region of Europe

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#### ABSTRACT

A survey of 153 acid grasslands from the Atlantic biogeographic region of Europe indicates that chronic nitrogen deposition is changing plant species composition and soil and plant-tissue chemistry. Across the deposition gradient  $(2-44 \text{ kg N ha}^{-1} \text{ yr}^{-1})$  grass richness as a proportion of total species richness increased whereas forb richness decreased. Soil C:N ratio increased, but soil extractable nitrate and ammonium concentrations did not show any relationship with nitrogen deposition. The above-ground tissue nitrogen contents of three plant species were examined: *Agrostis capillaris* (grass), *Galium saxatile* (forb) and *Rhytidiadelphus squarrosus* (bryophyte). The tissue nitrogen content of neither vascular plant species showed any relationship with nitrogen deposition. None of the species showed strong relationships between *R. squarrosus* nitrogen content and nitrogen deposition. None of the species showed strong relationships between above-ground tissue N:P or C:N and nitrogen deposition, indicating that they are not good indicators of deposition rate.

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#### 1. Introduction

Atmospheric nitrogen (N) deposition is a global environmental problem. Nitrogen oxides are predominantly emitted from the burning of fossil fuels. Between 1980 and 2003 most of Europe (excluding Portugal, Spain and Greece) saw a reduction in oxidised

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N emissions of between 20 and 50%. This downward trend is generally reflected in wet deposition (in rainfall) but not in dry deposition (as a gas or fine particulate) (Fagerli and Aas, 2008). For ammonia emissions and deposition the pattern is much more mixed, with some European countries showing small decreases and others increase. 90% of ammonia emissions are from agricultural sources (Erisman et al., 2008) and consequently can vary greatly within a small area. Nevertheless, total deposition of N remains high in many parts of Europe and critical loads (thresholds above which there is risk of damage to sensitive components of the ecosystem) are already exceeded in many countries (Galloway et al., 2008), representing a threat to biodiversity and ecosystem services

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(Mace et al., 2005). Indeed, N deposition is forecast to be, together with land use and climate change, in the top three drivers of change in global biodiversity by the year 2100 (Sala et al., 2000).

Nitrogen deposition has a number of different potential impacts on semi-natural ecosystems. At high air concentrations  $NH_3$  and  $NH_4^+$  are phytotoxic, causing leaf damage and growth reduction (e.g. Pearson and Stewart, 1993; Sheppard and Leith, 2002). Concentrations of ammonia high enough to cause direct toxicity are quite rare in Europe and generally only occur in the immediate vicinity of point sources. However, at lower levels, the effects of N deposition on vegetation can still be observed, including soil-mediated effects of eutrophication and acidification, and increased susceptibility to secondary stress (Bobbink et al., 2010).

In many semi-natural terrestrial ecosystems N is the limiting nutrient, so the addition of N has the potential to increase primary productivity. This can lead to increased plant competition for other resources, such as light and phosphorus, shifting the plant community towards one dominated by more competitive species (e.g. Bobbink et al., 1998; Clark et al., 2007; Hautier et al., 2009). Grasses are generally considered to be the more productive component of grasslands and are frequently thought to increase in response to eutrophication, shading out stress-tolerant components of the sward (e.g. Hautier et al., 2009). This results in changes in species composition and the balance of functional groups. The increase in plant litter from the enhanced productivity of vegetation can increase N turnover. More decomposable species are often favoured and tissue quality increases leading to an increase in N mineralisation rates (Aerts and Chapin, 2000). Other effects of N deposition include an increased susceptibility to insect herbivory, such as the increased frequency and intensity of attacks on Calluna vulgaris from the heather beetle (Lochmaea suturalis) (e.g. Brunsting and Heil, 1985). Increased attacks from insect herbivores are thought to be related to elevated concentrations of N in plant tissues and decreased concentrations of carbon (C) -based defensive compounds (Throop and Lerdau, 2004). Indeed, many studies have reported changes in plant-tissue chemistry related to N deposition (e.g. Pitcairn et al., 1998; Gidman et al., 2006). N deposition also leads to an increased incidence of drought and frost stress (e.g. Carroll et al., 1999), which, due to species differences in susceptibility, can alter plant species composition.

Deposition of acids in precipitation, oxidation of dry-deposited compounds, loss of basic cations through ion exchange, plant uptake, and nitrification of ammonium, result in a reduction of soil pH either directly or indirectly related to N deposition (Bobbink et al., 2010). Increased soil acidity can have a number of potential consequences including increased solubility of potentially toxic metals, changes to nutrient cycling, reducing or changing the populations of soil microbes and fauna, and changing plant species composition and richness (Johnston et al., 1986; Falkengren-Grerup, 1995; Stevens et al., 2004, 2009a). The potential for N deposition to impact plant species composition, soil chemistry and plant-tissue chemistry has been clearly demonstrated in numerous N addition experiments in a range of habitats (e.g. Phoenix et al., 2003; Pilkington et al., 2005; Berger et al., 2009). However, there have been far fewer investigations on whether these changes are actually occurring at a regional scale. There have been several studies in Great Britain demonstrating changes in plant species richness and composition (e.g. Stevens et al., 2004; Maskell et al., 2010) and soil chemistry (e.g. Stevens et al., 2006; Stevens et al., 2009a) across an N deposition gradient but, until now, none that investigate correlations between N deposition and soil chemistry and plant-tissue chemistry in acid grasslands at a European scale.

Here we report data from a study that gathered data on species composition, soil chemistry and plant-tissue chemistry from 153 acid grasslands in 13 different countries within the Atlantic biogeographic zone of Europe. This was used to test the hypotheses that 1) the proportion of species richness accounted for by forbs will decrease whilst the proportion of species richness accounted for by grasses will increase; 2) Al:Ca ratio and soil nitrogen (available nitrate, ammonium and total N) will increase and C:N ratio will decrease with increasing N deposition and 3) the nitrogen concentration of plant tissues will increase with increasing N deposition. Richness of functional groups as a proportion of total species richness was used to assess relative changes in composition and to determine whether the proportion of richness made up by the functional groups is changing rather than changes observed being a function of changing richness. The results of this study will demonstrate impacts of ambient levels of N deposition over a largescale spatial gradient providing evidence for European policy and, in particular, the development of critical loads for nitrogen.

#### 2. Methods

153 acid grasslands belonging to the Violion caninae association (Schwickerath, 1944) were surveyed within the Atlantic biogeographic zone of Europe (Fig. 1). This community is typically dominated by grasses including Agrostis capillaris and Festuca ovina or rubra with forbs such as Galium saxatile and Potentilla erecta and bryophytes including Rhytidiadelphus squarrosus occurring at a high constancy. Soils are variable in texture and of acidic pH (approximately pH 3.5-6). This community is found throughout the Atlantic biogeographic region of Europe. The grasslands were selected to cover the range of atmospheric N deposition in Europe (N deposition at sites ranged from 2 to 44 kg N ha<sup>-1</sup> yr<sup>-1</sup>). None of the grasslands surveyed were fertilised and all were managed by grazing or cutting. Areas within the grassland that belonged to other plant communities or were strongly affected by animals, tracks and paths, or were in the rain shadows of trees or hedges were excluded from the survey. Surveys were conducted between May and September in 2002/3 and 2007. At each site, five randomly located  $2 \times 2$  m quadrats were surveyed within a 1 ha area. Within each quadrat all vascular plants and bryophytes were identified to a species level. A description of the site was made including latitude, longitude, aspect, slope, patch size of the habitat from which the sample was taken, soil depth, management intensity (estimated based on vegetation height and number of grazing animals on a scale of one to three from intensive to extensive) and dominant species in adjacent vegetation communities.

Soil samples were collected from each quadrat. Topsoil samples were taken at a depth of 0-10 cm below the litter layer. Samples were taken from two opposing corners of the quadrat using a trowel and bulked to give one sample per quadrat. Subsoil samples were taken at a depth of 20-30 cm deep or, where soils were shallower than 20 cm, as deep as possible. Subsoil samples were taken from the



**Fig. 1.** Map of the 153 acid grasslands surveyed in the Atlantic biogeographic zone of Europe.

centre of the quadrat using a 5 cm diameter soil auger. All soil samples were bagged and kept cool during transit.

Plant-tissue samples were collected from within the vicinity of the quadrats. Approximately two grams (dry weight) of above-ground material were collected for three species: *Agrostis capillaris* L. (bent grass), *Galium saxatile* L. (lady's bedstraw) and the moss *Rhytidiadelphus squarrosus* (Hedw.) Warnst at all sites where the species occurred. These species were selected as they are both common and abundant throughout the geographical area investigated in this project. Only living material was collected. Samples were washed in deionised water.

In the laboratory, soil samples were air dried, roots and stones removed and remaining soil ground to <2 mm prior to analysis using a pestle and mortar. For total carbon (C) and N analysis, soils were ground to a fine powder. Plant samples were oven dried for three days at 55 °C and then ground to <2 mm.

Nitrate, ammonium, dissolved calcium (Ca) and aluminium (Al) concentrations were analysed using two different methods. Samples collected in 2002 and 2003 were leached with 1 M KCl and the resulting nitrate and ammonium analysed using an ion chromatograph. Other samples were shaken with 0.4 M NaCl and analysed using an auto-analyser. For all samples metal concentrations were determined using an ICP-MS. A comparison between the two methodologies was made to ensure they were compatible. Total C and N content of the soil and plant material was analysed using a CN element analyser. Plant-tissue phosphorus (P) concentration was determined using a dry ashing extraction method (Chapman and Pratt, 1985; Ryan et al., 2001) followed by a Barton colour complex (MAFF, 1986). Absorbance was determined using a colorimeter at a wavelength of 410 nm. Soil pH was determined using a pH probe in a 1:5 slurry of soil and deionised water.

Meteorological data for all the sites were obtained from the European Space Agency Monitoring Agriculture with Remote Sensing (MARS) unit (MARS, 2009). Ten year averages (1996–2006) were calculated for each site for mean annual potential transpiration, mean minimum daily temperature, mean maximum daily temperature and mean annual rainfall. Radiation index (based on latitude, aspect and slope) was calculated according to Oke (1987). The European gradient gives a greater range of N deposition compared to the UK alone but also covers a broader range of climatic conditions including sites that were wetter, drier, warmer and cooler than found in the UK alone.

For each site, total N, reduced N, oxidised N and sulphur (S) deposition data were modelled using the best available deposition model. National models were used for Germany (Gauger et al., 2002), the Netherlands (Van Jaarsveld, 2004; Asman and van Jaarsveld, 2002; Van Jaarsveld, 1995) and the United Kingdom (NEGTAP, 2001; Smith et al., 2000). For all other countries the EMEP-based IDEM model (Pieterse et al., 2007) was used. For all of the models, deposition was calculated as a three-year average (2000–2003) to provide a robust estimate of longer-term N inputs. Acid deposition was calculated from the molar equivalents of reactive N and S (NO<sub>3</sub> + NH<sub>4</sub><sup>+</sup> + SO<sub>4</sub><sup>2-</sup>, mmol<sub>c</sub> m<sup>-2</sup> y<sup>-1</sup>).

Linear regression and linear mixed-effects models were conducted using R (R Core Development Team, 2007; package nlme). All variables were checked for normality and corrected if necessary and strongly inter-correlated independent variables (r > 0.6) were removed from the models. Linear mixed-effects models (dependent variables topsoil pH, Al, nitrate and ammonium concentrations, total soil C, total soil N, soil C:N and plant-tissue N concentration for Agrostis capillaris, Galium saxatile and Rhytidiadelphus squarrosus) used the following predictors: latitude, longitude, altitude, aspect, inclination, mean maximum daily temperature, mean minimum daily temperature, mean annual rainfall, radiation index, vegetation height, management intensity, total N deposition and S deposition, and country as a random variable. For plant-tissue variables soil topsoil pH, Al, nitrate and ammonium concentrations, total C, total N, C:N were included as predictors. Minimum adequate models were selected manually by deleting variables until only significant variables remained. For plant composition data, the five replicate quadrats at each site were added together to give a list of species per site. Within functional group richness for grasses, forbs and bryophytes was calculated as a proportion of total species richness. Species richness for each functional group is presented in Stevens et al. (2010a).

#### 3. Results

#### 3.1. Nitrogen deposition

Total inorganic N deposition ranged from 2 to 44 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Oxidised N deposition ranged from 1.2 to 18.3 kg N ha<sup>-1</sup> yr<sup>-1</sup> and reduced deposition ranged from 1.2 to 28.7. Oxidised and reduced deposition was highly correlated with total N deposition (oxidised: r = 0.85; reduced: r = 0.96) and each other (r = 0.69).

#### 3.2. Functional group composition

Results for total species richness are presented in Stevens et al. (2010). Species richness of grasses (mean of five  $2 \times 2$  m quadrats)

ranged from 2.2 to 8.4 species. Grass richness as a proportion of total species richness shows a significant positive relationship with N deposition ( $r^2 = 0.21$ , p < 0.001) (Fig. 2a). Breaking this down into reduced and oxidised N shows that both have very similar relationships with grass richness (reduced N:  $r^2 = 0.18$ , p < 0.001, oxidised N:  $r^2 = 0.16$ , p < 0.001). Species richness of forbs (mean of five  $2 \times 2$  m quadrats) ranged from 0.2 to 14.4 species. Forb richness as a proportion of total species richness shows a significant negative relationship with N deposition ( $r^2 = 0.13$ , p < 0.001) (Fig. 2b). As with grass richness, both reduced and oxidised N have very similar negative relationships with forb richness (reduced N:  $r^2 = 0.12$ , p < 0.001, oxidised N:  $r^2 = 0.10$ , p < 0.001). Species richness of bryophytes (mean of five  $2 \times 2$  m quadrats) ranged from 0 to 8 species. Bryophyte richness as a proportion of total species richness shows a weak but significant positive relationship with N deposition ( $r^2 = 0.06$ , p < 0.001) (Fig. 2c). Reduced and oxidised N also shows similar positive results when analysed against the proportion of bryophytes (reduced N:  $r^2 = 0.06$ , p < 0.01, oxidised N:  $r^2 = 0.02$ , p < 0.05).

#### 3.3. Soil chemistry

Both topsoil and subsoil pH show a significant negative relationship with total inorganic N deposition (topsoil:  $r^2 = 0.20$ , p < 0.001, subsoil N  $r^2 = 0.20$ , p < 0.001). Both topsoil and subsoil pH show stronger negative relationships with oxidised N deposition than reduced N deposition (Topsoil: reduced N  $r^2 = 0.14$ , p < 0.001, oxidised N  $r^2 = 0.25$ , p < 0.001; subsoil:reduced N  $r^2 = 0.14$ , p < 0.001, oxidised N  $r^2 = 0.25$ , p < 0.001; subsoil:reduced N  $r^2 = 0.13$ , p < 0.001, oxidised N  $r^2 = 0.24$ , p < 0.001). However, the close correlation between oxidised and reduced deposition mean that this should be interpreted with care. When total acid deposition is used instead of total inorganic N deposition, the relationship is slightly improved (topsoil  $r^2 = 0.25$ , p < 0.001, subsoil  $r^2 = 0.22$ , p < 0.001). The minimum adequate linear mixed-effects model for topsoil pH showed significant effects of N deposition (parameter value -0.149, p < 0.01), latitude (parameter value 0.412, p < 0.001) and inclination (parameter value -0.006, p < 0.01).

Aluminium concentration (ln transformed for normality) in the topsoil is negatively correlated with topsoil pH ( $r^2 = 0.35$ , p < 0.001). There is a weak but significant positive relationship between aluminium concentration and N deposition ( $r^2 = 0.07$ , p < 0.001). The positive relationship between Al concentration and acid deposition is slightly stronger than with N deposition ( $r^2 = 0.11$ , p < 0.001). The minimum adequate linear mixed-effects model showed significant effects of N deposition (parameter value 0.01, p < 0.05), altitude (parameter value 0.14, p < 0.001) and rainfall (parameter value 0.0003, p < 0.05). Aluminium: calcium ratio (Al:Ca) (ln transformed for normality) shows a weak but significant positive relationship with N deposition ( $r^2 = 0.05$ , p < 0.01). There is no improvement in the relationship by using acid deposition rather than N deposition (data not shown).

Topsoil nitrate concentration (ln transformed for normality) shows no significant relationship with N deposition (p = 0.92). The minimum adequate linear mixed-effects model showed a significant relationship with latitude (parameter value 0.339, p < 0.001), altitude (parameter value 0.447, p < 0.05), inclination (parameter value -0.046, p < 0.01) and rainfall (parameter value -0.001, p < 0.05).

Topsoil ammonium concentration (ln transformed for normality) shows a weak but significant positive relationship with N deposition ( $r^2 = 0.03$ , p < 0.05) but this relationship is mainly driven by a few high points. The minimum adequate linear mixed-effects model showed significant effects of N deposition (parameter value 0.043, p < 0.001), altitude (parameter value 0.287, p < 0.001) and rainfall (parameter value 0.001, p < 0.05).

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0 10 20 30 40 50 Total inorganic N deposition (kg N ha<sup>-1</sup> yr<sup>-1</sup>)

Fig. 2. Within functional group richness as a proportion of species richness for a) grasses, b) forbs and c) bryophytes against total inorganic N deposition for 153 acid grasslands in the Atlantic biogeographic regions of Europe. Symbols show the country from which data were collected: □ Belgium, ○ Denmark, ■ France, + Germany, ◇ Ireland and Isle of Man, ● Netherlands, × Norway, ◆ Sweden, and ▲ United Kingdom.

Total N content of the soil shows a weak but significant negative relationship with N deposition ( $r^2 = 0.04$ , p < 0.05) but, again, this relationship is mainly driven by high results from a few sites. This is confirmed by N deposition not being included in the minimum adequate linear mixed-effects model, significant effects were identified for longitude (parameter value -0.023, p < 0.001), latitude (parameter value 0.035, p < 0.001) and altitude (parameter value 0.110, p < 0.001). There is no significant relationship between soil C content and N deposition (p = 0.06). The minimum adequate linear mixed-effects model significant effects of longitude (parameter value -0.310, p < 0.001), latitude (parameter value 0.645, p < 0.001), altitude (parameter value -0.155, p < 0.05). However, C:N ratio shows

a significant positive relationship with N deposition ( $r^2 = 0.15$ , p < 0.001) (Fig. 3a). There is a clear significant negative relationship between C:N ratio and pH ( $r^2 = 0.29$ , p < 0.001) (Fig. 3b).

#### 3.4. Plant-tissue chemistry

*A. capillaris* was collected from a total of 148 sites. There is no significant relationship between N deposition and *A. capillaris* tissue N content (p = 0.87) (Fig. 4a), C:N ratio (p = 0.66) or N:P ratio (p = 0.29). For tissue N content the minimum adequate linear mixed-effects model showed significant effects of latitude (parameter value -0.482, p < 0.001), soil pH (parameter value -1.608, p < 0.05), S deposition (parameter value 0.172, p < 0.05), management intensity (parameter



Fig. 3. Topsoil C:N ratio against a) total inorganic N deposition and b) pH for 153 acid grasslands in the Atlantic biogeographic regions of Europe. Symbols for countries are as in Fig. 2.

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Fig. 4. Above-ground tissue N content for a) A. capillaris, b) G. saxatile and c) R. Squarrosus against total inorganic N deposition for 153 acid grasslands in the Atlantic biogeographic regions of Europe. Symbols for countries are as in Fig. 2.

value 1.159, p < 0.01) and soil nitrate concentration (parameter value 0.413, p < 0.01). *G. saxatile* was collected from 115 sites, being most commonly absent from sites in France. There is no significant relationship between N deposition and G. saxatile tissue N content (p = 0.42) (Fig. 4b) or C:N ratio (p = 0.32). N:P ratio has a weak but significant positive relationship with N deposition ( $r^2 = 0.04, p < 0.05$ ). R. squarrosus was collected from a 148 sites. For tissue N content the minimum adequate linear mixed-effects model showed significant effects of soil carbon (parameter value -0.167, p < 0.001), soil ammonium concentration (parameter value 0.726, p < 0.001) and soil nitrate concentration (parameter value 1.267, p < 0.001). There is a weak but significant positive relationship between N deposition and *R. squarrosus* tissue N content ( $r^2 = 0.10$ , p < 0.001) (Fig. 4c). There is also a weak but significant negative relationship between N deposition and *R. squarrosus* C:N ( $r^2 = 0.08$ , p < 0.001) but not N:P (p = 0.20). For tissue N content the minimum adequate linear mixed-effects model showed significant effects of N deposition (parameter value 0.086, p < 0.001), longitude (parameter value 0.167, p < 0.001), and management intensity (parameter value 0.584, p < 0.05).

#### 4. Discussion

#### 4.1. Functional group composition

There are clear relationships in the plant species composition data that show that the proportion of grass richness in the sward increases with increasing N deposition, whereas the proportion of forb richness decreases steeply. Species richness of forbs showed a significant reduction with increasing N deposition (Stevens et al., 2010a). A similar trend was observed in the earlier UK survey (Stevens et al., 2004) and other surveys (e.g. Stevens et al., 2009b; Duprè et al., 2010) as well as in N addition experiments (e.g. Mountford et al., 1993; Wilson et al., 1995). Although N deposition impacts are usually attributed primarily to eutrophication and the alteration of competitive interactions (e.g. Hautier et al., 2009), evidence from the UK now suggests that these grasslands may be responding primarily to acidification and its impacts on soil chemistry (Stevens et al., 2010b). In this acid grassland community competition for light may not be as important as in some other communities (e.g. prairie grasslands, Wedin and Tilman, 1993) in determining the response to N deposition. Although there is likely to be some effect of eutrophication, the continual removal of plant material through grazing and/or cutting may prevent competition for light and nutrients from being a strong determinant of community composition. An increase in the proportion of grasses in this community may simply reflect that many of the grasses found are very generalist species (e.g. Agrostis capillaris, Festuca ovina) or acid specialists (e.g. Deschampsia flexuosa, Molinia caerulea) that benefit from a reduction in forb cover. The strong relationship between forb richness and N deposition identified in the survey (Stevens et al., 2010a) indicates that this is the strongest driver of the changes in the proportional changes in richness.

The slight increase in bryophytes as a proportion of species number is a surprising trend, although this relationship is very weak and may be a consequence of the decline in forb richness. However, if the increase in bryophytes as a proportion of total species richness is a genuine trend, this would support the theory that competition for light is less important because in a short sward where competition for light was intense, we would expect an impoverished bryophyte flora (Peintinger and Bergamini, 2006) and that bryophytes would form a small proportion of total richness. Total species richness of bryophytes declines slightly with increasing N deposition but this is also not a strong trend (Stevens et al., 2010a).

For all of the functional groups, the proportion of richness shows weaker relationships with the oxidised and reduced components of N deposition considered when they are separately. However, in all cases the relationships between oxidised N deposition or reduced N deposition and the proportion of species richness are similar. This may be because they are correlated or because changes in species composition are slow reflecting chronic deposition over many years. The effects of recent changes in the ratio of reduced to oxidised N in deposition may not yet be apparent in the vegetation community. Although oxidised N and reduced N tend to have different sources (oxidised from burning fossil fuels and reduced from agricultural emissions) they are correlated within this data set. This makes it difficult to separate their effects through statistical means.

#### 4.2. Soil chemistry

Stevens et al. (2004, 2006) reported a clear relationship between N deposition and soil pH and consequent mobilisation of metals within the soil (Stevens et al., 2009a) in the UK sites and, although the relationship is still apparent in this larger European data set, it is not as strong as in the UK alone ( $r^2 = 0.39$ , Stevens et al., 2006, 2010a). The relationship between N deposition and soil pH found here supports evidence from other, smaller-scale surveys (e.g. Skiba et al., 1989) and long-term monitoring studies (e.g. Blake et al., 1999) that have demonstrated evidence for widespread soil acidification related to N deposition or acid deposition in weakly buffered soils.

It is possible that the wide range of soil types and underlying geology encompassed in the mainland European sites, together with a greater range in grassland-management techniques meant that trends in soil pH and aluminium concentration are not as clear in the large-scale European transect compared to the UK survey. Some of this variation is removed by looking at the Al:Ca ratio in the topsoil, but this only shows a weak relationship with N deposition. The availability of reactive aluminium in the soil is clearly related to pH despite variations in soil type. This is consistent with the known solubility of aluminium from experimental acidification of soils (Ulrich, 1991; Tyler and Olsson, 2001).

The slightly stronger relationship between soil pH or metal concentrations and total acid deposition (rather than N deposition alone) indicates that soil acidification in Europe is still related to either current or previous S deposition. However, because N and S deposition are somewhat correlated it is difficult to determine their relative contributions. This is also true when considering the relative contributions of the oxidised and reduced components of N deposition and soil pH has implications for the species composition of the grasslands, which are reflected in the importance of soil pH as a driver of species richness (Stevens et al., 2010a). Reduction in pH and consequent mobilisation of metals could be changing the species composition from species typical of intermediate pH to acid-resistant species, especially considering that this habitat is

already at the lower end of pH tolerance for most of the species typical of grasslands.

Given the variation in soil types and site conditions it is not surprising to see a lack of clear relationships between N deposition and soil nitrate and ammonium concentrations. In addition to soil variability, both nitrate and ammonium are readily utilised by plants and microbial communities, and nitrate is relatively mobile in the soil. Mixed-effects models showed that for both nitrate and ammonium concentration, climatic variables and altitude are important variables. Given that microbial processing of N is sensitive to climatic and seasonal variation and the direct impact these have on the amount of readily available N, this relationship is not surprising (Morecroft et al., 1992). A significant positive relationship between N deposition and soil ammonium concentration was observed for the UK alone ( $r^2 = 0.34$ ; Stevens et al., 2006) adding further support to the hypothesis that increased variability in the soils, climate and site conditions in this larger study prevent trends being readily identified.

Although there is a relationship between total soil N and N deposition this is weak, and once one outlying point was removed the relationship was no longer significant. Given that leaching losses of N from these grasslands are likely to be quite low (Phoenix et al., 2003) it is possible that yet again soil variability, and particularly variation in soil organic matter content, is masking any changes in soil total N. The soil N results from the European survey are in agreement with results from the UK alone ( $r^2 = 0.08$ , Stevens et al., 2006) although the relationship is stronger in the European survey. Interestingly, C:N ratio increases with increasing N deposition (Fig. 3). This means that rather than N increasing relative to the amount of C as might be expected through the addition of N, C is actually increasing relative to N. Increased soil C in response to N deposition has been reported in several studies in different habitats (e.g. Knorr et al., 2005; de Vries et al., 2009). Increases in soil C relative to soil N are possibly related to increased productivity leading to increased litter and/or changes in the decomposition rate of litter. Measurement of standing biomass, soil organic matter dynamics and litter decomposition would confirm whether this is the case in the acid grassland system investigated in this study. All of the grasslands in this survey were managed for agriculture either by cutting or grazing. This continual removal of plant material make it less likely that changes in productivity will impact the ecosystem. Another possible explanation is reduced soil respiration leading to C accumulation in the soil as has been observed in forest ecosystems (e.g. Mo et al., 2007; Bowden et al., 2004; Burton et al., 2004). The clear relationship with soil pH suggests that the effect is related to acidification impacting on soil processes.

#### 4.3. Plant-tissue chemistry

Many experimental studies have demonstrated relationships between N addition and plant-tissue N content (e.g. Carroll et al., 2003; Gordon et al., 2001; Leith et al., 1999,) but these are not reflected in this gradient study. It is possible that short-term N additions in experimental applications are not fully representative of chronic N deposition leading to a different response from plants or background variation in N availability may be masking this trend across natural gradients. It is clear from the plant-tissue chemistry results that responses are species-specific which may also account for some of the different results observed. For A. capillaris there were no significant relationships between N deposition and any of the tissuenutrient content measurements (N, C:N and N:P). R. squarrosus showed slightly different results, with a significant relationships observed between tissue N content and C:N ratio with N deposition, but not for N:P ratio. In the UK alone the relationship between N deposition and tissue N was not observed (Stevens et al., 2006).

Results are generally in good agreement with Stevens et al. (2006). *R. squarrosus* showed slightly different results, with a significant relationships observed between tissue N content and C:N ratio with N deposition but this relationship is very weak and is unlikely to be ecologically relevant. In the UK alone the relationship between N deposition and tissue N was not observed (Stevens et al., 2006). This suggests that although this was the strongest of the relationships between tissue chemistry and N deposition recorded in this study, it is not necessarily suitable for application as an indicator of N deposition at a national scale. This conclusion is supported by an intensive investigation of experimental N additions to a similar grassland community who also found no relationship between N deposition and *R. squarrosus* tissue N content (Arroniz-Crespo et al., 2008).

There are a number of potential reasons that strong relationships between tissue-nutrient concentrations and N deposition are not observed in this study. In this gradient study, there are many different variables with the potential to affect tissue N content, including climatic and management variations. The mixed-effects models show that some of these variables are significantly related to tissue N content. In a controlled experimental situation these may be less apparent. The lack of relationship between N deposition and plant-nutrient concentrations may also relate to the microbial processing of N in the soil resulting in different amounts and forms of N being available to plants compared to that which is deposited. The microbial processing of N varies on a small spatial and temporal scale and is affected by a range of factors including temperature, soil moisture, soil pH and soil carbon content as well as N input (e.g. Riaz et al., 2008). Furthermore, the plants investigated in this study may be using the additional N deposited from the atmosphere for increased growth, as supported by optimal partitioning theory, which suggests that plants equalize nutrient exchange ratios in order to maximize the efficiency of resource use (Bloom et al., 1985). The collection of productivity data would confirm whether this is the case. An alternative possibility is that these plants may be showing a very plastic response to N deposition, with tissue nutrients changing in response to very short-term changes in N deposition levels (Dise and Gundersen, 2004). If this is the case, small-scale spatial and temporal variations in deposition, which are not accounted for in using a larger scale modelled N deposition data, may have a role to play in the variability of the results observed. Seasonal variation in resource allocation may also play a role in the lack of relationships between tissue-nutrient concentrations and N deposition. Relationships between tissue N content and N deposition may not be apparent in above-ground tissues year-round and there may be seasonal variability in tissue N concentration which has not been eliminated in this study. An alternative indicator to tissue-nutrient concentrations may be the use of metabolic fingerprints or enzymes such as phosphomonoesterase which have generated some interesting results from initial studies (Arroniz-Crespo et al., 2008; Gidman et al., 2006; Hogan et al., 2010).

#### 5. Conclusion

Species composition of acid grasslands in Europe shows clear relationships with N deposition. The proportion of species richness that is made up of forb species is negatively associated with N deposition whilst the proportion of grasses increases.

Soil chemistry results show no relationships between N deposition and soil N (extractable nitrate, ammonium and total N), but relationships between N deposition and soil pH and Al concentration can be observed. Soil C:N shows a positive relationship with N deposition. This result has important implications for carbon storage but further research is needed to determine the mechanism causing this increase. The results of this survey demonstrate that tissue N concentrations of the three species investigated are not suitable for use as indicators of N deposition. Some species may be more suitable, but before they are recommended for use as an indicator of N deposition, their response to chronic N deposition should be investigated outside of controlled experimental situations.

These results clearly show a correlation between atmospheric N deposition and species composition and soil chemistry of European grasslands at ambient levels of N deposition suggesting that deposition levels need to be reduced to protect these grasslands. The results show that changes occur at all levels of deposition found within the region including impacts on vegetation community and soil below the critical load for N deposition for this habitat. This would suggest that achieving no damage to sensitive acid grasslands from N deposition is not a realistic goal and policy makers should instead aim to minimise impacts on acid grasslands. The results also show that even at high deposition impacts continue to occur suggesting that further N additions to areas where the critical load is currently exceeded could cause further damage.

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#### References

- Aerts, R., Chapin, F.S., 2000. The mineral nutrition of wild plants revisited: a reevaluation of processes and patterns. Advances in Ecological Research 30, 1–67.
- Arroniz-Crespo, M., Leake, J.R., Horton, P., Phoenix, G.K., 2008. Bryophyte physiological responses to, and recovery from, long-term nitrogen deposition and phosphorus fertilisation in acidic grassland. New Phytologist 180, 864–874.
- Asman, W.A.H., van Jaarsveld, J.A., 2002. A variable-resolution transport model applied for NH<sub>x</sub> in Europe. Atmospheric Environment 26A, 445-464.
- Berger, T.W., Inselsbacher, E., Mutsch, F., Pfeffer, M., 2009. Nutrient cycling and soil leaching in eighteen pure and mixed stands of beech (*Fagus sylvatica*) and spruce (*Picea abies*). Forest Ecology and Management 258, 2578–2592.
- Blake, L., Goulding, K.W.T., Mott, C.J.B., Johnston, A.E., 1999. Changes in soil chemistry accompanying acidification over more than 100 years under woodland and grass at Rothamsted Experimental Station, UK. European Journal of Soil Science 50, 401–412.
- Bloom, A.J., Chapin, F.S., Mooney, H.A., 1985. Resource limitation in plants an economic analogy. Annual Review of Ecology and Systematics 16, 363–392.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J.W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L., De Vries, W., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications 20, 30–59.
- Bobbink, R., Hornung, M., Roelofs, J.G.M., 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. Journal of Ecology 86, 717–738.
- Bowden, R.D., Davidson, E., Savage, K., Arabia, C., Steudler, P., 2004. Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. Forest Ecology and Management 196, 43–56.
- Brunsting, A.M.H., Heil, G.W., 1985. The role of nutrients in the interactions between a herbivorous beetle and some competing plant species in heathlands. Oikos 44, 23–26.
- Burton, A.J., Pregitzer, K.S., Crawford, J.N., Zogg, G.P., Zak, D.R., 2004. Simulated chronic NO<sub>3</sub>– deposition reduces soil respiration in northern hardwood forests. Global Change Biology 10, 1080–1091.
- Carroll, J.A., Caporn, S.J.M., Cawley, L., Read, D.J., Lee, J.A., 1999. The effect of increased atmospheric nitrogen on *Calluna vulgaris* in upland Britain. New Phytologist 141, 423–431.
- Carroll, J.A., Caporn, S.J.M., Johnson, D., Morecroft, M.D., Lee, J.A., 2003. The interactions between plant growth, vegetation structure and soil processes in seminatural acidic and calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen deposition. Environmental Pollution 121, 363–376.
- Chapman, H.D., Pratt, P.F., 1985. Methods for Analysis of Soils, Plants and Waters. Agricultural Sciences Publications, California.

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- Clark, C.M., Cleland, E.E., Collins, S.L., Fargione, J.E., Gough, L., Gross, K.L., Pennings, S.C., Suding, K.N., Grace, J.B., 2007. Environmental and plant community determinants of species loss following nitrogen enrichment. Ecology Letters 10, 596–607.
- Duprè, C., Stevens, C.J., Ranke, T., Bleeker, A., Peppler-Lisbach, C., Gowing, D.J.G., Dise, N.B., Dorland, E., Bobbink, R., Diekmann, M., 2010. Changes in species richness and composition in European acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. Global Change Biology 16, 344–357.
- de Vries, W., Solberg, S., Dobbertin, M., Sterba, H., Laubhann, D., van Oijen, M., Evans, C., Gundersen, P., Kros, J., Wamelink, G.W.W., Reinds, G.J., Sutton, M.A., 2009. The impact of nitrogen deposition on carbon sequestration by European forests and heathlands. Forest Ecology and Management 258, 1814–1823.
- Dise, N.B., Gundersen, P., 2004. Forest ecosystem responses to atmospheric pollution: linking the empirical with the experimental. Water, Air and Soil Pollution: Focus 4, 207–220.
- Erisman, J.W., Bleeker, A., Hensen, A., Vermeulen, A., 2008. Agricultural air quality in Europe and the future perspectives. Atmospheric Environment 42, 3209–3217.
   Fagerli, H., Aas, W., 2008. Trends of nitrogen in air and precipitation: model results
- Fagerli, H., Aas, W., 2008. Trends of nitrogen in air and precipitation: model results and observations at EMEP sites in Europe, 1980–2003. Environmental Pollution 154. 448–461.
- Falkengren-Grerup, U., 1995. Long-term changes in flora and vegetation in deciduous forests of southern Sweden. Ecological Bulletins 44, 215–226.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions and potential solutions. Science 320, 889–892.
- Gauger, T., Anshelm, F., Schuster, H., Erisman, J.W., Vermeulen, A.T., Draaijers, G.P.J., Bleeker, A., Nagel, H.-D., 2002. Mapping of Ecosystems Specific Long-term Trends in Deposition Loads and Concentrations of Air Pollutants in Germany and Their Comparison with Critical Loads and Critical Levels. Institut fur Navigation, University of Stuttgart, Germany.
- Gidman, E.A., Stevens, C.J., Goodacre, R., Broadhurst, D., Emmett, B., Gwynn-Jones, D., 2006. Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls. Global Change Biology 12, 1823–1833.
- Gordon, C., Wynn, J.M., Woodin, S.J., 2001. Impacts of increased nitrogen supply on high Arctic heath: the importance of bryophytes and phosphorus availability. New Phytologist 149, 461–471.
- Hautier, Y., Niklaus, P.A., Hector, A., 2009. Competition for light causes plant biodiversity loss after eutrophication. Science 324, 636–638.
- Hogan, E.J., Minnullina, G., Smith, R.I., Crittenden, P.D., 2010. Effects of nitrogen enrichment on phosphatase activity and N/P relationships in Cladonia portentosa. New Phytologist 186, 911–925.
   Johnston, A.E., Goulding, K.W.T., Poulton, P.R., 1986. Soil acidification during more
- Johnston, A.E., Goulding, K.W.T., Poulton, P.R., 1986. Soil acidification during more than 100 years under permanent grassland and woodland at Rothamstead. Soil Use and Management 2, 3–10.
- Knorr, M., Frey, S.D., Curtis, P.S., 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86, 3252–3257.
- Leith, I.D., Hicks, W.K., Fowler, D., Woodin, S.J., 1999. Differential responses of UK upland plants to nitrogen deposition. New Phytologist 141, 277–289. Mace, G., Masundire, H., Baille, J. (Eds.), 2005. Millenium Ecosystem Assessment.
- Mace, G., Masundire, H., Baille, J. (Eds.), 2005. Millenium Ecosystem Assessment. Current States and Trends, vol. 1. Island Press, Washington.
- MAFF, 1986. The Analysis of Agricultural Materials, third ed. Her Majesty's Stationary Office, London.
- Maskell, L.C., Smart, S.M., Bullock, J.M., Thompson, K., Stevens, C.J., 2010. Nitrogen deposition causes widespread species loss in British habitats. Global Change Biology 16, 671–679.
- Mo, J.M., Brown, S., Xue, J.H., Fang, Y.T., Li, Z., Li, D.J., Dong, S.F., 2007. Response of nutrient dynamics of decomposing pine (*Pinus massoniana*) needles to simulated N deposition in a disturbed and a rehabilitated forest in tropical China. Ecological Research 22, 649–658.
- Monitoring Agricultural Resources (MARS), European Commission Joint Research Centre. http://mars.jrc.it/mars/About-us/The-MARS-Unit (accessed November 2009).
- Morecroft, M.D., Marrs, R.H., Woodward, F.I., 1992. Altitudinal and seasonal trends in soil mineralization rate in the Scottish Highlands. Journal of Ecology 80, 49–56.
- Mountford, J.O., Lakhani, K.H., Kirkham, F.W., 1993. Experimental assessment of the effects of nitrogen addition under hay-cutting and aftermath grazing on the vegetation of meadows on a Somerset peat moor. Journal of Applied Ecology 30, 321–332.
- NEGTAP, 2001. Transboundary Air Pollution: Acidification, Eutrophication and Ground-level Ozone in the UK. CEH, Edinburgh.
- Oke, T.R., 1987. Boundary Layer Climates, second ed.. Methuen, New York.
- Pearson, J., Stewart, G.R., 1993. Tansley Review No.56. The deposition of atmospheric ammonia and its effects on plants. New Phytologist 125, 283–305.

- Peintinger, M., Bergamini, A., 2006. Community structure and diversity of bryophytes and vascular plants in abandoned fen meadows. Plant Ecology 185, 1–17.
- Phoenix, G.K., Booth, R.E., Leake, J.R., Read, D.J., Grime, P., Lee, J.A., 2003. Effects of enhanced nitrogen deposition and phosphorus limitation on nitrogen budgets of semi-natural grasslands. Global Change Biology 9, 1309–1321.
- of semi-natural grasslands. Global Change Biology 9, 1309–1321. Pieterse, G., Bleeker, A., Vermeulen, A.T., Wu, Y., Erisman, J.W., 2007. High resolution modelling of atmosphere–canopy exchange of acidifying and eutrophying components and carbon dioxide for European forests. Tellus 59B, 412–424.
- Pilkington, M.G., Caporn, S.J.M., Carroll, J.A., Cresswell, N., Lee, J.A., Emmett, B.A., Johnson, D., 2005. Effects of increased deposition of atmospheric nitrogen on an upland *Calluna* moor: N and P transformation. Environmental Pollution 135, 469–480.
- Pitcairn, C.E.R., Leith, I.D., Sheppard, L.J., Sutton, M.A., Fowler, D., Munro, R.C., Tang, S., Wilson, D., 1998. The relationship between nitrogen deposition, species composition and foliar nitrogen concentrations in woodland flora in the vicinity of livestock farms. Environmental Pollution 102, 41–48.
- R Development Core Team, 2007. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Riaz, M., Mian, I.A., Cresser, M.S., 2008. Extent and causes of 3D spatial variations in
- Riaz, M., Mian, I.A., Cresser, M.S., 2008. Extent and causes of 3D spatial variations in potential N mineralization and the risk of ammonium and nitrate leaching from an N-impacted permanent grassland near York, UK. Environmental Pollution 156, 1075–1082.
- Ryan, J., Estefan, G., Rashid, A., 2001. Soil and Plant Analysis Laboratory Manual, second ed. ICARDA, Aleppo, Syria.
- Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Biodiversity – global biodiversity scenarios for the year 2100. Science 287, 1770–1774.
- Schwickerath, M., 1944. Das Hohe Venn und seine Randgebiete. Pflanzensoziologie 6, 1–278.
- Sheppard, L.J., Leith, I.D., 2002. Effects of NH<sub>3</sub> fumigation on the frost hardiness of *Calluna* – does N deposition increase winter damage by frost? Phyton-annales Rei Botanicae 42, 183–190.
- Skiba, U., Cresser, M.S., Derwent, R.G., Futty, D.W., 1989. Peat acidification in Scotland. Nature 337, 68–70.
- Smith, R.I., Fowler, D., Sutton, M.A., Flechard, C., Coyle, M., 2000. Regional estimation of pollutant gas dry deposition in the UK: model description, sensitivity analyses and outputs. Atmospheric Environment 34, 3757–3777.
- Stevens, C.J., Dise, N.B., Mountford, J.O., Gowing, D.J., 2004. Impact of nitrogen deposition on the species richness of grasslands. Science 303, 1876–1879.
- Stevens, C.J., Dise, N.B., Gowing, D.J., Mountford, J.O., 2006. Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls. Global Change Biology 12, 1823–1833.
- Stevens, C.J., Dise, N.B., Gowing, D.J., 2009a. Regional trends in soil acidification and metal mobilisation related to acid deposition. Environmental Pollution 157, 313–319.
- Stevens, C.J., Maskell, L.C., Smart, S.M., Caporn, S.J.M., Dise, N.B., Gowing, D.J., 2009b. Identifying indicators of atmospheric nitrogen deposition impacts in acid grasslands. Biological Conservation 142, 2069–2075.
- Gentuying indicators of atmospheric introgen deposition impacts in acting grasslands. Biological Conservation 142, 2069–2075.
  Stevens, C.J., Duprè, C., Dorland, E., Gaudnik, C., Gowing, D.J.G., Bleeker, A., Diekmann, M., Alard, D., Bobbink, R., Fowler, D., Corcket, E., Mountford, J.O., Vandvik, V., Aarrestad, P.A., Muller, S., Dise, N.B., 2010a. Nitrogen deposition threatens species richness of grasslands across Europe. Environmental Pollution 158, 2940–2945.
- Stevens, C.J., Thompson, K., Grime, J.P., Long, C.J., Gowing, D.J.G., 2010b. Contribution of acidification and eutrophication to declines in species richness of calcifuge grasslands along a gradient of atmospheric nitrogen deposition. Functional Ecology 24, 478–484.
- Throop, H.L., Lerdau, M.T., 2004. Effects of nitrogen deposition on insect herbivory: implications for community and ecosystem processes. Ecosystems 7, 109–133.
- Tyler, G., Olsson, T., 2001. Concentrations of 60 elements in the soil solution as related to soil acidity. European Journal of Soil Science 52, 151–165.
- Ulrich, B., 1991. An ecosystem approach to soil acidification. In: Ulrich, B., Sumner, M.E. (Eds.), Soil Acidity. Springer-Verlag, Berlin, pp. 28–79.Van Jaarsveld, J.A., 1995. Modelling the Long-term Atmospheric Behaviour of
- Vali Jaarsveid, J.A., 1995. Modelling the Long-term Annospheric Benaviour of Pollutants on Various Spatial Scales. University of Utrecht, The Netherlands. Van Jazerveld J.A. 2004. The Operation Priority Systemace Model National Insti-
- Van Jaarsveld, J.A., 2004. The Operation Priority Substances Model. National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands. Wedin, D., Tilman, D., 1993. Competition among grasses along a nitrogen gradient:
- initial conditions and mechanisms of competition. Ecological Monographs 63, 199–229.
- Wilson, E.J., Wells, T.C.E., Sparks, T.H., 1995. Are calcareous grasslands in the UK under threat from nitrogen deposition? - an experimental determination of a critical load. Journal of Ecology 83, 823–832.

# **Data Papers**

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# Grassland species composition and biogeochemistry in 153 sites along environmental gradients in Europe

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Abstract. This data set consists of vascular plant and bryophyte species composition and plant and soil biogeochemical data from 153 acid grasslands located in the Atlantic biogeographic region of Europe. Data were collected between 2002 and 2007. The grasslands all belong to the Violion caninae association and were managed by grazing or cutting but had not received fertilizer inputs. These data provide plant composition from five randomly located  $2 \times 2$  m quadrats at each site with all vascular plants and bryophytes identified to species level with cover estimates for each species. Topsoil and subsoil were collected in each quadrat, and data are provided for pH, metal concentrations, nitrate and ammonium concentrations, total carbon and N, and Olsen extractable phosphorus. Aboveground plant tissues were collected for three species (Rhytidiadelphus squarrosus, Galium saxatile, and Agrostis capillaris), and data are provided for percentage N, carbon, and phosphorus. These data have already been used in a number of research papers focusing on the impacts of atmospheric N deposition on grassland plant community and biogeochemistry. The unique data set presented here provides the opportunity to test theories about the effect of environmental variation on plant communities, biogeochemistry, and plant-soil interactions, as well as spatial ecology and biogeography.

Key words: Acid grasslands; Atlantic region of Europe; plant tissue chemistry; soil chemistry; species composition.

The complete data sets corresponding to abstracts published in the Data Papers section of the journal are published electronically in Ecological Archives at (http://esapubs.org/archive). (The accession number for each Data Paper is given directly beneath the title.)

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# Changes in species composition of European acid grasslands observed along a gradient of nitrogen deposition

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#### Keywords

Acid grassland; Climate; Nitrogen deposition; Ordination; Soil biogeochemistry; Variation partitioning; *Violion caninae* 

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#### Abstract

**Question:** Which environmental variables affect floristic species composition of acid grasslands in the Atlantic biogeographic region of Europe along a gradient of atmospheric N deposition?

**Location:** Transect across the Atlantic biogeographic region of Europe including Ireland, Great Britain, Isle of Man, France, Belgium, The Netherlands, Germany, Norway, Denmark and Sweden.

**Materials and Methods:** In 153 acid grasslands we assessed plant and bryophyte species composition, soil chemistry (pH, base cations, metals, nitrate and ammonium concentrations, total C and N, and Olsen plant available phosphorus), climatic variables, N deposition and S deposition. Ordination and variation partitioning were used to determine the relative importance of different drivers on the species composition of the studied grasslands.

**Results:** Climate, soil and deposition variables explained 24% of the total variation in species composition. Variance partitioning showed that soil variables explained the most variation in the data set and that climate and geographic variables accounted for slightly less variation. Deposition variables (N and S deposition) explained 9.8% of the variation in the ordination. Species positively associated with N deposition included *Holcus mollis* and *Leontodon hispidus*. Species negatively associated with N deposition included *Agrostis curtisii, Leontodon autumnalis, Campanula rotundifolia* and *Hylocomium splendens*.

**Conclusion:** Although secondary to climate gradients and soil biogeochemistry, and not as strong as for species richness, the impact of N and S deposition on species composition can be detected in acid grasslands, influencing community composition both directly and indirectly, presumably through soil-mediated effects.

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#### Introduction

The global nitrogen (N) cycle has been transformed by human activities. The global creation of reactive N increased by a factor of ten, from 15 to  $156 \text{ Tg N yr}^{-1}$ between 1860 and 1995 and by a further 31 to 187 Tg N  $yr^{-1}$  between 1995 and 2005 (Galloway et al. 2008). With continued growth of the world population and increasing demand for food, pressures on the global N cycle are set to increase. Excess reactive N in the atmosphere is deposited to terrestrial and aquatic ecosystems as wet and dry deposition. Atmospheric deposition of reactive N is considered a global threat to biodiversity (Sala et al 2000; Phoenix et al. 2006). Levels of N deposition in Western Europe are among the highest in the world (Galloway et al. 2008), and although there have been small declines in deposition in some regions in recent years (Fagerli & Aas 2008), deposition of N remains high in many areas and critical loads are exceeded in many parts of Europe (Galloway et al. 2008). Sulphur (S) deposition has also increased steadily through the twentieth century, peaking in the 1980s. Between 1880 and 1991 cumulative deposition of S reached  $6000 \text{ kg S ha}^{-1}$  in high emission areas (Mylona 2002). Since then S deposition has fallen considerably as a result of political initiatives in Europe. The 1985 Helsinki Protocol on the Reduction of Sulphur Emissions or their Transboundary Fluxes and the 1994 Oslo Protocol on Further Reduction of Sulphur Emissions have achieved a 60% reduction in S emissions in Europe (1980-1997) (EMEP 1999).

N deposition has the potential to impact on grassland plant community composition in a number of different ways, resulting in changes in tissue nutrient stoichiometry and metabolism (e.g. Pitcairn et al. 1998; Gidman et al. 2006; Arroniz-Crespo et al. 2008), changes in species composition (e.g. Mountford et al. 1993; Stevens et al. 2009b) and changes in species richness (e.g. Stevens et al. 2004; Clark & Tilman 2008; Duprè et al. 2010). There are several ways in which N deposition can bring about these changes.

Because N is the limiting nutrient in many terrestrial ecosystems, the addition of N can increase primary productivity resulting in increased competition for light and other resources. This can lead to an increased dominance of competitive species that are better able to take advantage of the increased nutrients (Bobbink et al. 1998; Hautier et al. 2009). N also has the potential to acidify soils, through the deposition of nitric acid in precipitation, oxidation of dry-deposited compounds and an increase in plant uptake and N transformations in the soil. The resultant reductions in soil pH can reduce the available species pool and result in changes in species composition (Schuster & Diekmann 2003; Tyler 2003). N deposition can also result in increased susceptibility to insect herbivory (Brunsting & Heil 1985), increased incidence of drought and frost stress (Caporn et al. 2000; Sheppard & Leith 2002) and, at high air concentrations of nitrite, nitrate and ammonium, can cause leaf damage and growth reduction (Pearson & Stewart 1993), although concentrations this high are generally only found in the immediate vicinity of point sources.

The addition of N to semi-natural vegetation typically results in an increase in competitive species (Wedin & Tilman 1993; Wilson et al. 1995) or a reduction in acid intolerant species (Stevens et al. 2010b). Results from previous studies on acid grasslands have shown that species richness declined in relation to N deposition over both spatial gradients (Stevens et al. 2004; Maskell et al. 2010) and through time (Duprè et al. 2010). Changes in species richness and composition in acid grasslands in the UK have been associated with higher KCl-extractable ammonium in the soil, lower pH (Stevens et al. 2006) and higher aluminium and other metal availabilities in soils (Stevens et al. 2009a).

Changes in species composition in relation to N deposition have previously been examined at local and national scales in a range of habitats (e.g. Smart et al. 2003; Bennie et al. 2006) as well as in experimental manipulations (e.g. Mountford et al. 1993; Carroll et al. 2003). In this investigation, we use a survey of 153 acid grasslands belonging to the Violion caninae alliance (Schwickerath 1944) in ten countries within the Atlantic biogeographic region of Europe to investigate variation in species composition and underlying explanatory variables. We examine the variation in species composition in a clearly defined community type along a long N deposition gradient (total atmospheric N deposition ranging from 2.4 to  $43.5 \text{ kg} \cdot \text{N} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ ), and aim to quantify the amount of variation in species composition attributed to different explanatory variables and specifically to deposition variables.

#### Methods

One hundred and fifty-three *Violion caninae* grasslands were surveyed between 2002 and 2007 within the Atlantic biogeographic zone of Europe (Fig. 1). The acidic grasslands visited were selected in a stratified manner to cover the range of atmospheric N deposition in Europe. Grasslands in the vicinity of point sources of nitrogen (e.g. large pig or poultry farms) were avoided. All of the grasslands were managed by grazing or cutting and none were fertilized. To ensure consistent community selection across the geographic gradient, a list of indicator or dominant species of the community was drawn up that had to be found on a site before the survey was carried



Fig. 1. Distribution of the 153 Violion caninae grasslands surveyed in the Altantic biogeographic region of Europe.

out. Despite the large geographical range over which the community was surveyed, there were no marked differences in the community between countries, as shown by the relatively short DCA gradient (Fig. 2). At each site, five randomly located  $2 \text{ m} \times 2 \text{ m}$  quadrats were surveyed within a 1-ha area. Within each quadrat, all vascular plants and bryophytes were identified to species level and their cover estimated using the Domin scale (see Rich et al. 2005). Areas within the grassland that belonged to other plant communities (according to the dominant or indicator species), those strongly affected by animals, tracks and paths, or in the rain shadows of trees or hedges were excluded from the survey. A description of the site was made and data collected on latitude, longitude, aspect, slope, extent of grassland, soil depth (to bedrock) and surrounding vegetation.

Soil samples were collected from each quadrat. Topsoil samples were taken at a depth of 0–10 cm below the litter layer. Samples were taken from two opposing corners of the quadrat, bulked to make one sample per quadrat and kept cool during transit.

In the laboratory, soil samples were air dried and ground to < 2 mm prior to analysis. For total carbon (C) and N analysis, soils were ground to a fine powder. Soil pH was determined using a pH probe in a 1:5 slurry of soil:deionized water (Thomas 1996). Nitrate, ammonium and metal concentrations were analysed using two



**Fig. 2.** DCA ordination of sites surveyed, coded according to country. The gradient lengths for axes 1 and 2 are 2.73 and 2.27, respectively; eigen values are 0.236 and 0.190.

different methods. Sixty-eight samples from the UK collected in 2002 and 2003 were leached with 1 M KCl (MAFF 1986) and the resulting nitrate and ammonium analysed using ion chromatography. Other samples were shaken with 0.4 M NaCl and analysed using an autoanalyser. For all samples, metal concentrations were determined using an ICP-MS. A comparison between the two methodologies demonstrated that results were comparable (not shown). Total C and N content of the soil and plant material was analysed using a CN element analyser. Plant available phosphorus was calculated in an Olsen extraction (MAFF 1986). All samples were analysed within 3 months of collection. Full details of soil analysis are given in Stevens et al. (2010a).

Meteorological data for all sites were obtained from the European Space Agency Monitoring Agriculture with Remote Sensing (MARS) unit (MARS 2009); 10-year averages (1996–2006) were calculated for each site for mean annual potential evapotranspiration, mean minimum daily temperature, mean maximum daily temperature and mean annual rainfall. Radiation index was calculated based on latitude, aspect and slope (Oke 1987).

For each site, total N, reduced N, oxidized N and sulphur (S) deposition data were modelled using the best available deposition model. National models were used for Germany (Gauger et al. 2002), the Netherlands (Van Jaarsveld 1995; Asman & van Jaarsveld 2002; Van Jaarsveld 2004) and the United Kingdom (Smith et al. 2000; NEGTAP 2001). For all other countries, the European Monitoring and Evaluation Programme (EMEP)-based Integrated Deposition Model (IDEM) (Pieterse et al. 2007) was used. Comparisons between models revealed that results were very similar for many areas where both models were available. The exceptions were areas with very variable altitude; for these areas, national models, which have a smaller resolution than the EMEP model, were used. For all of the models, deposition was calculated as a 3-year average (2000-2003).

For the five quadrats at each site, both mean Domin scores (groupings of percentage cover) and constancy values (frequency in the five quadrats) were tested and gave very similar results, so constancy scores were selected for the final analysis. Major gradients were explored using indirect gradient analysis with detrended correspondence analysis (DCA) in CANOCO 4.5 (Biometris, Wageningen, The Netherlands). Correlation coefficients between 19 environmental variables (latitude, longitude, radiation index, inclination, management type, mean daily maximum temperature, soil pH, soil aluminium, calcium, magnesium and manganese concentrations, nitrate concentration, ammonium concentration, Olsen phosphorus concentration, total C and N content, C:N, total atmospheric N and S deposition) and site scores of DCA axes were calculated. A log-transformation was applied to some variables to achieve normality. For further analysis, highly intercorrelated variables

(r > 0.6) were removed (altitude, radiation index, transpiration, mean daily minimum temperature, rainfall, subsoil pH, iron concentration, nitrate concentration, ammonium concentration and Olsen extractable phosphate). Latitude and temperature although highly correlated were both retained due to their potential importance as drivers of species composition on such a large geographical scale. A correlation matrix is provided in Appendix S1. To reduce the number of environmental variables, those variables that were significantly correlated with the DCA axes were selected using Minitab 15 (Minitab Inc., 2007, USA). Divalent base cations (calcium, magnesium) and manganese were added together to further reduce the number of variables (Kleinebecker et al. 2008). Sulphur deposition and soil N were retained in the analyses, as they were variables of particular interest to this investigation, although they were correlated with some other variables. These environmental variables were used in a canonical correspondence analysis (CCA) with forward selection and rare species down-weighted. Variables that did not show a significant relationship in the forward selection were removed. Variance partitioning was conducted by running a series of partial CCAs using three groups of variables: deposition, soil and climate and geographic variables (Table 1) to determine the relative contributions of each group to the overall variance (Borcard et al. 1992). CCA was performed using CANOCO 4.5 (Biometris, Wageningen, The Netherlands).

#### Results

A total of 398 species were found in the 153 sites. The species recorded most frequently in the data set were *Agrostis capillaris* L. (150 sites), *Luzula campestris* (L.) DC. (128 sites), *Rhytidiadelphus squarrosus* (Hedw.) Warnst (124 sites), *Potentilla erecta* (L.) Räuschel (116 sites) and *Galium saxatile* L. (113 sites). Grassland swards were typically grass-dominated, with variable amounts of forb and bryophyte cover. DCA (Fig. 2) showed good overlap between the sites surveyed in different countries, but a latitudinal gradient is apparent on axis 1. The DCA ordination analyses showed relatively short gradient lengths considering the large geographical variance in the grasslands surveyed. The gradient length of axis 1

Table 1.	Grouping	of variables	used in	variance	partitioning
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Group	Variables
Deposition Soil	Total inorganic N deposition and S deposition Topsoil pH, exchangeable aluminium concentration, exchangeable base cation
Climate and geographic location	concentration, % C, %N and C:N ratio Latitude, longitude and mean daily maximum temperature

Table 2.	Correlation	coefficients	between DCA	axis scores	and environment	ntal variables	. Significant	correlation	coefficients are	marked as:	*P <	0.05,
**P < 0.0	D1, ***P < 0	0.001.										

Environmental Variable	Axis 1	Axis 2	Axis 3
Latitude	- 0.649***	0.070	0.073
Longitude	0.294***	- 0.255***	0.627***
Altitude (m asl)	- 0.360***	0.155	- 0.108
Log inclination (°)	- 0.398***	0.165*	- 0.252**
Radiation index	0.328***	- 0.042	0.118
Mean monthly maximum temperature ( °C)	0.663***	- 0.131	- 0.078
Mean monthly minimum temperature ( °C)	0.468***	- 0.080	- 0.290***
Mean annual rainfall (mm)	- 0.029	0.095	- 0.258**
Mean annual evapotranspiration (mm)	- 0.604***	- 0.019	- 0.078
Topsoil pH	0.629***	0.308***	-0.190*
Log aluminium concentration (mg kg $^{-1}$ dry soil)	$-0.446^{***}$	- 0.139	- 0.102
Log base cation concentration (mg kg $^{-1}$ dry soil)	- 0.351***	0.474***	- 0.381***
Log C (%)	$-0.458^{***}$	0.151	0.151
Log N (%)	- 0.203*	-0.114	- 0.113
C:N	- 0.301	- 0.447***	0.166*
KCl extractable nitrate concentration (mg kg <sup><math>-1</math></sup> · dry soil)	- 0.368***	0.397***	0.179*
KCl extractable ammonium concentration (mg kg $^{-1}$ · dry soil)	- 0.196*	- 0.129	- 0.011
Olsen extractable P concentration (mg kg $^{-1}$ dry soil)	0.013	0.073	0.122
Management type (grazing or mowing)	0.551***	- 0.196*	- 0.018
Total inorganic N deposition (kg N $\cdot$ ha <sup>-1</sup> $\cdot$ yr <sup>-1</sup> )	- 0.301***	- 0.293***	0.464***
Total inorganic S deposition (kg $S \cdot ha^{-1} \cdot yr^{-1}$ )	- 0.355***	- 0.104***	0.006

was 2.73 and that of axis 2 was 2.27. The total inertia in the DCA was 3.006. The sample scores of axis 1 of the DCA analysis were significantly correlated with a number of variables. Significant correlations with an *r* value greater than 0.4 were observed for latitude, management type, mean daily maximum temperature, topsoil pH, aluminium concentration and C content. For axis 2 of the DCA analysis, sample scores were significantly and strongly correlated with base cation concentration and soil C:N ratio. Sample scores on axis 3 were significantly correlated with total N deposition and longitude (Table 2).

After excluding highly inter-correlated variables we used 11 variables (Table 1) in the CCA. These variables explained 24% of the total variation in the species composition. Variance partitioning of the explained variation showed that soil variables (topsoil pH, log aluminium concentration, log C content, log N content, C:N ratio) were the group that explained the most variation in the data set, accounting for 38.0% of the constrained total inertia. Climate and geographic variables (latitude, longitude and mean daily maximum temperature) accounted for 30.8% of the variation in the constrained total inertia. A further 13.3% of the variation was accounted for by a combination of these variables. Deposition variables (N and S deposition) alone explained 9.8% of the variation in the constrained total inertia, with a further 6.2% overlap in explanatory power between deposition and soil variables. The remaining 1.9% of the variation was



**Fig. 3.** Amount of variation in species composition described by CCA analysis that is explained by three groups of explanatory variables: deposition (N deposition and S deposition), soil (topsoil pH, aluminium concentration, base cation concentration, C content, N content and C:N ratio) and climate and geographic location (latitude, longitude and mean daily maximum temperature). Areas of circles in the Venn diagram show approximately the percentage of variation explained relative to the total variation explained by the full CCA model (24%).

explained by overlap between the three variable groups (Fig. 3).

CCA was also used to identify species positively and negatively associated with N deposition. For this constrained ordination, N and S deposition were used as environmental variables and all other variables were used as co-variables. Figure 4 shows only those species that occurred in more than 10% of sites. Species most strongly



**Fig. 4.** CCA ordination diagram (axes 1 and 2) for all species with N and S deposition as environmental variables and climate and soil variables used as co-variables. Rare species are down-weighted. Species plotted occurred in more than 10% of sites and species positively or negatively associated with N deposition (assessed by their positions in the ordination diagram) are named.

positively associated with N deposition in the ordination diagram were *Holcus mollis* L., *Leontodon hispidus* L., *Festuca ovina sensu lato* L., *Nardus stricta* L., *Cerastium fontanum* Baumg. and *Juncus effusus* L. Species that were rarer within the data set but showed a particularly strong association with high N deposition were *Senecio jacobaea* L. and *Cynosurus cristatus* L. Species most strongly negatively associated with N deposition were *Agrostis curtisii* Kerguélen, *Viola riviniana* Reichenb., *Leontodon autumnalis* L., *Campanula rotundifolia* L. and *Hylocomium splendens* (Hedw.) Br. Eur. Species that were rarer within the data set but showed a particularly strong association with low N deposition were *Vaccinium vitis-idaea* L. and *Hypericum pulchrum* L.

#### Discussion

Climate and geographic variables explain almost a third of species composition variation in our study. Further influence of climate may have been missed, as we did not consider the hydrology and water-holding capacity of each soil at each site. Given the large spatial gradient over which this study has been conducted, the importance of climate in influencing species composition is also of no surprise. The variability in climatic factors across the gradient is large, with mean daily minimum temperatures ranging from -0.6 °C to 10.2 °C and mean daily max-

imum temperatures ranging from 6.8 °C to 18.8 °C. Rainfall also varies considerably across the gradient, from  $498 \text{ mm yr}^{-1}$  to  $1971 \text{ mm yr}^{-1}$ .

Atmospheric deposition alone explains 9.8% of the variation in species composition in our data set. As shown in Fig. 3, there is a strong influence of soil on the species composition found along the gradient of atmospheric deposition used in this study. We need to consider, however, that N and S deposition have the potential to acidify soils, which presents problems in disentangling their impacts on the vegetation community. Soil acidification and consequent mobilization of metals and reduction in base cation availability have been observed in this grassland community and related to N deposition (Stevens et al. 2009a, 2010b), and changes in soil C:N have also been related to N deposition (Stevens et al. in press). As the proportion of variation that is jointly explained by deposition and soil is small, it is likely that the influence of deposition on soils is not fully accounted for in the overlap found here. This may be partly due to the large variability in the soil textures and types encountered in this survey, leading to differences in how the deposited N is processed in the soil. As a consequence of the influence of N and S deposition on soil chemistry, the variation explained by deposition and the variation explained by soil cannot be considered entirely independent. N and S deposition were considered together in our analysis since they are highly correlated (r=0.45) in our data set, which presents problems in disentangling their degree of influence on the community composition.

The results for species composition found in this study contrast with results obtained for species richness (Stevens et al. 2010a). For species richness, geographic and physical variables (location, climate and site characteristics) explained very little of the variation (<1%), whereas here climate and geographical variables explain almost one-third of the variation. Species richness was reduced by atmospheric deposition, most likely due to the loss of rare species in the different regions. As a result, in this study the compositional shift is not as evident, given that the more dominant species remain the same. The vast majority of the species found in this survey occurred across the whole of the spatial extent of the survey, but there were some notable exceptions, such as Agrostis curtisii, which replaces A. capillaris as the dominant grass in some sites in the west of France and the southwest of England. The restricted distribution of A. curtisii is probably related to climatic and edaphic factors (Ivimey-Cook 1959). There were a number of other species which, although not showing strongly restricted distribution in our study area, were only found in this community in some geographical areas or were at a much higher abundance in some areas (e.g. Arnica montana L.).

Species that were most strongly associated with low N deposition tend to be forbs that are poor competitors and are not tolerant of highly acidic soils. Viola riviniana is described in Grime et al. (2007) as intermediate between stress-tolerator and C-S-R strategist but, perhaps more importantly, it is rarely found in the most acid soils. This may also be true of Campanula rotundifolia, also intermediate between stress-tolerator and C-S-R, but again, rarely found on strongly acid soils (Grime et al. 2007). C. rotundifolia is also a poor competitor with vigorous grasses (Sinker et al. 1991) so may not be competing well with grass species that are encouraged by high N deposition. Leontodon autumnalis is a species typical of intermediate fertility but is also found commonly on weakly acid soils rather than highly acid soils (Ellenberg et al. 1991; Hill et al. 1999). The moss Hylocomium splendens has been shown to decrease with N additions in several forest experiments. Doses of  $30 \text{ kg} \cdot \text{N} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$  caused a strong decline of *H*. splendens abundance in Sweden (Dirkse & Martakis 1992), and a decline was also identified in coniferous forests in southern Germany over a 20-40 year period. The latter was attributed to sensitivity to acidification (Rodenkirchen 1992). Duprè et al. (2010) also identified a decline in H. splendens in the UK from analysis of historic quadrat data collected between 1960-1975 and from 1975-2003. The limited distribution of Agrostis curtisii means that the strong association with low N deposition for this species should be interpreted with some caution; however, it is a species very typical of infertile habitats (Ellenberg N score of 1 in Hill et al. 1999).

Species most strongly associated with high N deposition were Holcus mollis, Festuca ovina, Nardus stricta, Cerastium fontanum, Leontodon hispidus and Juncus effusus. None of these species are typical of fertile habitats but, given that the vegetation community in which we were working is characterized by extremely poor soils, this is what would be expected. Both H. mollis and J. effusus tend towards being competitive species and are both tolerant of very acid soils, while C. fontanum is a more ruderal species (Grime et al. 2007). An increase in graminoid species is often associated with increased N deposition (Stevens et al. 2009b; Duprè et al. 2010) and H. mollis increased in relative frequency in Germany and the UK between 1939–1975 and 1975–2007. The association of L. hispidus with high deposition is more surprising as this species is not typical of highly acidic or nutrient-rich habitats (Ellenberg et al. 1991; Hill et al. 1999) and requires further investigation.

It is clear from this analysis that N deposition has the potential to influence vegetation community composition in acid grasslands, both directly and indirectly through soil-mediated effects. Although secondary to climate gradients and soil biogeochemistry, the impact of N and S deposition on species composition can be detected, even at a large spatial scale. These results have important implications for conservation management, and suggest that in order to maintain acid grasslands in good condition we need to reduce N deposition or manage grasslands in a way that mitigates its effects.

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#### References

- Arroniz-Crespo, M., Leake, J.R., Horton, P. & Phoenix, G.K. 2008. Bryophyte physiological responses to, and recovery from, long-term nitrogen deposition and phosphorus fertilisation in acidic grassland. *New Phytologist* 180: 864–874.
- Asman, W.A.H. & van Jaarsveld, J.A. 2002. A variableresolution transport model applied for NHx in Europe. *Atmospheric Environment* 26A: 445–464.
- Bennie, J., Hill, M.O., Baxter, R. & Huntley, B. 2006. Influence of slope and aspect on long-term vegetation change in British chalk grasslands. *Journal of Ecology* 94: 355–368.
- Bobbink, R., Hornung, M. & Roelofs, J.G.M. 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology* 86: 717–738.
- Borcard, D., Legendre, P. & Drapeau, P. 1992. Partialling out the spatial component of ecological variation. *Ecology* 73: 1045–1055.
- Brunsting, A.M.H. & Heil, G.W. 1985. The role of nutrients in the interactions between a herbivorous beetle and some competing plant species in heathlands. *Oikos* 44: 23–26.
- Caporn, S.J.M., Ashenden, T.W. & Lee, J.A. 2000. The effect of exposure to NO<sub>2</sub> and SO<sub>2</sub> on frost hardiness in *Calluna* vulgaris. Environmental and Experimental Botany 43: 111–119.
- Carroll, J.A., Caporn, S.J.M., Johnson, D., Morecroft, M.D. & Lee, J.A. 2003. The interactions between plant growth, vegetation structure and soil processes in semi-natural acidic and calcareous grasslands receiving long-term inputs of simulated pollutant nitrogen deposition. *Environmental Pollution* 121: 363–376.
- Clark, C.M. & Tilman, D. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* 451: 712–715.

- Dirkse, G.M. & Martakis, G.F.P. 1992. Effects of fertilizer on bryophytes in Swedish experiments on forest fertilization. *Biological Conservation* 59: 155–161.
- Duprè, C., Stevens, C.J., Ranke, T., Bleeker, A., Peppler-Lisbach, C., Gowing, D.J.G., Dise, N.B., Dorland, E., Bobbink, R. & Diekmann, M. 2010. Changes in species richness and composition in European acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. *Global Change Biology* 16: 344–357.
- Ellenberg, H., Weber, H.E., Dull, R., Wirth, V., Werner, W. & Paulissen, D. 1991. Zeigerwerte von pflanzen in Mitteleuropa. *Scripta Geobotanica* 18: 1–248.
- EMEP 1999. Transboundary acid deposition in Europe. EMEP emission data. Status report 1999 of the European Monitoring and Evaluation Programme. EMEP/MSC-W
- Fagerli, H. & Aas, W. 2008. Trends of nitrogen in air and precipitation: model results and observations at EMEP sites in Europe, 1980–2003. *Environmental Pollution* 154: 448–461.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P. & Sutton, M.A. 2008. Transformation of the nitrogen cycle: recent trends, questions and potential solutions. *Science* 320: 889–892.
- Gauger, T., Anshelm, F., Schuster, H., Erisman, J.W., Vermeulen, A.T., Draaijers, G.P.J., Bleeker, A. & Nagel, H.-D. 2002. Mapping of ecosystems specific long-term trends in deposition loads and concentrations of air pollutants in Germany and their comparison with Critical Loads and Critical Levels. Institut für Navigation, University of Stuttgart, Stuttgart, DE.
- Gidman, E.A., Stevens, C.J., Goodacre, R., Broadhurst, D., Emmett, B. & Gwynn-Jones, D. 2006. Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls. *Global Change Biology* 12: 1823–1833.
- Grime, J.P., Hodgeson, J.G. & Hunt, R. 2007. *Comparative plant ecology: a functional approach to common British species*. Unwin Hyman, London, UK.
- Hautier, Y., Niklaus, P.A. & Hector, A. 2009. Competition for light causes plant biodiversity loss after eutrophication. *Science* 324: 636–638.
- Hill, M.O., Mountford, J.O., Roy, D.B. & Bunce, R.G.H. 1999. Ellenberg's indicator values for British plants. ECOFACT Volume 2. Technical Annex. DETR, Rotherham, UK.
- Ivimey-Cook, R.B. 1959. Biological flora of the British Isles: *Agrostis setacea. Journal of Ecology* 47: 691–706.
- Kleinebecker, T., Holzel, N. & Vogel, A. 2008. South Patagonian ombrotrophic bog vegetation reflects biogeochemical gradients at the landscape level. *Journal of Vegetation Science* 19: 151–160.
- MAFF 1986. *The analysis of agricultural materials*. Her Majesty's Stationery Office, London, UK.
- MARS 2009. "European Commission Joint Research Centre". http://www.mars.jrc.it.

- Maskell, L.C., Smart, S.M., Bullock, J.M., Thompson, K. & Stevens, C.J. 2010. Nitrogen deposition causes widespread species loss in British habitats. *Global Change Biology* 16: 671–679.
- Mountford, J.O., Lakhani, K.H. & Kirkham, F.W. 1993. Experimental assessment of the effects of nitrogen addition under hay-cutting and aftermath grazing on the vegetation of meadows on a Somerset peat moor. *Journal of Applied Ecology* 30: 321–332.
- Mylona, S. 2002. Sulphur dioxide emissions in Europe 1880–1991 and their effect on sulphur concentrations and depositions. *Tellus* 48: 662–689.
- NEGTAP 2001. *Transboundary air pollution: acidification, eutrophication and ground-level ozone in the UK*. Centre for Ecology and Hydrology, Edinburgh, UK.
- Oke, T.R. 1987. *Boundary layer climates*. Methuen, New York, NY, US.
- Pearson, J. & Stewart, G.R. 1993. Tansley Review No.56. The deposition of atmospheric ammonia and its effects on plants. *New Phytologist* 125: 283–305.
- Phoenix, G.K., Hicks, W.K., Cinderby, S., Kuylenstierna, J.C.L., Stock, W.D., Dentener, F.J., Giller, K.E., Austin, A.T., Lefroy, R.D., Gimeno, B.S., Ashmore, M.R. & Ineson, P. 2006. Atmospheric nitrogen deposition in world biodiversity hotspots: the need for a greater global perspective in assessing N deposition impacts. *Global Change Biology* 12: 470–476.
- Pieterse, G., Bleeker, A., Vermeulen, A.T., Wu, Y. & Erisman, J.W. 2007. High resolution modelling of atmosphere –canopy exchange of acidifying and eutrophying components and carbon dioxide for European forests. *Tellus* 59B: 412–424.
- Pitcairn, C.E.R., Leith, I.D., Sheppard, L.J., Sutton, M.A., Fowler, D., Munro, R.C., Tang, S. & Wilson, D. 1998. The relationship between nitrogen deposition, species composition and foliar nitrogen concentrations in woodland flora in the vicinity of livestock farms. *Environmental Pollution* 102: 41–48.
- Rich, T., Redbane, M., Fasham, M., McMeechan, F. & Dobson, D. 2005. Ground and shrub vegetation. In: Hill, D., Fasham, M., Tucker, G., Shewry, M. & Shaw, P. (eds.) *Handbook of biodiversity methods: survey, evaluation and monitoring.* pp. 201–222. Cambridge University Press, Cambridge, UK.
- Rodenkirchen, H. 1992. Effects of acidic precipitation, fertilization and liming on the ground vegetation in coniferous forests of Southern Sweden. *Water, Air and Soil Pollution* 61: 279–294.
- Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M. & Wall, D.H. 2000. Biodiversity global biodiversity scenarios for the year 2100. *Science* 287: 1770–1774.

- Schuster, B. & Diekmann, M. 2003. Changes in species density along the soil pH gradient – evidence from German plant communities. *Folia Geobotanica* 38: 367–379.
- Schwickerath, M. 1944. Das Hohe Venn und seine Randgebiete. *Pflanzensoziologie* 6: 1–278.
- Sheppard, L.J. & Leith, I.D. 2002. Effects of NH<sub>3</sub> fumigation on the frost hardiness of *Calluna* – does N deposition increase winter damage by frost? *Phyton-annales rei botanicae* 42: 183–190.
- Sinker, C.A., Packham, J.R., Trueman, I.C., Oswald, P.H., Perring, F.H. & Prestwood, W.V. 1991. *Ecological flora of the Shropshire region*. Shropshire Wildlife Trust, Shrewsbury, UK.
- Smart, S.M., Robertson, J.C., Shiels, E.J. & Van de Poll, H.M. 2003. Locating eutrophication effects across British vegetation between 1990 and 1998. *Global Change Biology* 9: 1763–1774.
- Smith, R.I., Fowler, D., Sutton, M.A., Flechard, C. & Coyle, M. 2000. Regional estimation of pollutant gas dry deposition in the UK: model description, sensitivity analyses and outputs. *Atmospheric Environment* 34: 3757–3777.
- Stevens, C.J., Dise, N.B., Mountford, J.O. & Gowing, D.J. 2004. Impact of nitrogen deposition on the species richness of grasslands. *Science* 303: 1876–1879.
- Stevens, C.J., Dise, N.B., Gowing, D.J. & Mountford, J.O. 2006. Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls. *Global Change Biology* 12: 1823–1833.
- Stevens, C.J., Dise, N.B. & Gowing, D.J. 2009a. Regional trends in soil acidification and metal mobilisation related to acid deposition. *Environmental Pollution* 157: 313–319.
- Stevens, C.J., Maskell, L.C., Smart, S.M., Caporn, S.J.M., Dise, N.B. & Gowing, D.J. 2009b. Identifying indicators of atmospheric nitrogen deposition impacts in acid grasslands. *Biological Conservation* 142: 2069–2075.
- Stevens, C.J., Duprè, C., Dorland, E., Gaudnik, C., Gowing, D.J.G., Bleeker, A., Diekmann, M., Alard, D., Bobbink, R., Fowler, D., Corcket, E., Mountford, J.O., Vandvik, V., Aarrestad, P.A., Muller, S. & Dise, N.B. 2010a. Nitrogen deposition threatens species richness of grasslands across Europe. *Environmental Pollution* 158: 2940–2945.
- Stevens, C.J., Thompson, K., Grime, J.P., Long, C.J. & Gowing, D.J.G. 2010b. Contribution of acidification and eutrophication to declines in species richness of calcifuge grasslands along a gradient of atmospheric nitrogen deposition. *Functional Ecology* 24: 478–484.
- Stevens, C.J., Duprè, C., Dorland, E., Gaudnik, C., Gowing, D.J.G., Bleeker, A., Diekmann, M., Alard, D., Bobbink, R., Fowler, D., Corcket, E., Mountford, J.O., Vandvik, V., Aarrestad, P.A., Muller, S. & Dise, N.B. In press. The impact of nitrogen deposition on acid grasslands in the

Atlantic region of Europe. *Environmental Pollution* doi.10:1016/j.envpol.2010.11.026.

- Thomas, G.W. 1996. Soil pH and soil acidity. In: Sparks, D.L. (ed.) *Chemical methods*. pp. 475–490. Soil Science Society of America, Madison, WI, US.
- Tyler, G. 2003. Some ecophysiological and historical approaches to species richness and calcicole/calcifuge behaviour contribution to a debate. *Folia Geobotanica* 38: 419–428.
- Van Jaarsveld, J.A. 1995. *Modelling the long-term atmospheric behaviour of pollutants on various spatial scales*. University of Utrecht, Utrecht, NL.
- Van Jaarsveld, J.A. 2004. The operation priority substances model. National Institute for Public Health and the Environment (RIVM), Bilthoven, NL.
- Wedin, D. & Tilman, D. 1993. Competition among grasses along a nitrogen gradient: initial conditions and mechanisms of competition. *Ecological Monographs* 63: 199–219.
- Wilson, E.J., Wells, T.C.E. & Sparks, T.H. 1995. Are calcareous grasslands in the UK under threat from nitrogen deposition? – an experimental determination of a critical load. *Journal of Ecology* 83: 823–832.

#### Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Correlation matrix (abbreviations used in table shown in parentheses) for longitude, latitude, altitude, radiation index (radiation), log inclination (inclination), management type (manage), mean annual evapotranspiration (evopot), mean monthly maximum temperature (max temp), mean monthly minimum temperature (min temp), mean annual rainfall (rainfall), topsoil pH (pH), log soil aluminium concentration (Al), log soil nitrate concentration (NO3), log soil ammonium concentration (NH4), soil Olsen extractable phosphorus concentration (Olsen P), soil C content (carbon), soil N content (nitrogen), soil C:N ratio (C:N), total inorganic N deposition (N dep) and total inorganic S deposition (S dep), log soil base cation concentration (cation). Statistically significant correlations (P < 0.05) are shown in red. Units for all measurements are given in Table 2.

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PROFILE

# Addressing the Impact of Atmospheric Nitrogen Deposition on Western European Grasslands

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Abstract There is a growing evidence base demonstrating that atmospheric nitrogen deposition presents a threat to biodiversity and ecosystem function in acid grasslands in Western Europe. Here, we report the findings of a workshop held for European policy makers to assess the perceived importance of reactive nitrogen deposition for grassland conservation, identify areas for policy development in Europe and assess the potential for managing and mitigating the impacts of nitrogen deposition. The importance of nitrogen as a pollutant is already recognized in European legislation, but there is little emphasis in policy on the evaluation of changes in biodiversity due to nitrogen. We assess the potential value of using typical species, as defined in the European Union Habitats Directive, for determining the impact of nitrogen deposition on acid grasslands. Although some species could potentially be

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Department of Air Quality & Climate Change, Energy Research Centre of the Netherlands, PO Box 1, 1755 ZG Petten, The Netherlands used as indicators of nitrogen deposition, many of the typical species do not respond strongly to nitrogen deposition and are unlikely to be useful for identifying impact on an individual site. We also discuss potential mitigation measures and novel ways in which emissions from agriculture could be reduced.

**Keywords** Acid grasslands · Biodiversity · Convention on long-range transboundary air pollution (CLRTAP) · Nitrogen deposition · Species-rich *Nardus* grassland

#### Introduction

The natural global nitrogen (N) cycle has been transformed by human activities as a consequence of agricultural

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intensification and fossil fuel combustion. Between 1860 and 1995 the global creation of reactive N (all forms of N that are biologically or photochemically active) increased from 15 to 156 Tg N year<sup>-1</sup>. Between 1995 and 2005, it increased by a further 31-187 Tg N year<sup>-1</sup> (Galloway and others 2008). With continued growth of the world population and increasing demand for food, pressures on the global N cycle are set to increase (Tilman 1999).

Nitrogen oxides are mainly produced by fossil fuel combustion in transport, power generation and industrial processes whereas 90% of ammonia emissions come from agricultural sources (mainly fertilizers and animal manure) (Erisman and others 2008). Excess reactive N in the atmosphere is deposited to terrestrial and aquatic ecosystems as wet or dry deposition. Wet deposition occurs when soluble N compounds are dissolved in rain and cloud drops; dry deposition consists of gases and particles that are deposited directly to surfaces. In Europe as a whole, deposition of N showed a slight decline between 1980 and 2003 (Fagerli and Aas 2008), but in many areas, levels of deposition remain above those that are known to have an impact on semi-natural ecosystems. In other parts of the world, such as developing nations and growing economies, N deposition is increasing (Galloway and others 2008). These changes are in contrast to sulfur (S) for which emissions have been reduced by between 90 and 70% and deposition has declined rapidly since the 1970s (Fowler and others 2007).

Atmospheric N deposition can potentially have a wide range of effects on semi-natural ecosystems including direct toxicity, increased sensitivity to secondary stress, acidification and eutrophication (Bobbink and others 2010). Acidification can be caused directly by acid deposition (N and S), but also indirectly through leaching of basic cations, soil microbial processes and plant uptake. Increased soil acidity can result in an increased solubility of metals and a reduced availability of nutrients (Tyler and Olsson 2001). In Europe, plant species diversity tends to decline with increasing soil acidity and so species composition and richness can be impacted (Johnston and others 1986; Falkengren-Grerup 1995; Stevens and others 2004). Because N is the limiting nutrient in many semi-natural terrestrial ecosystems, the addition of N also has the potential to increase primary productivity. N deposition may be less of a threat where phosphorus is the limiting nutrient. For plant communities the consequence of this increase in productivity can be a shift towards domination by species with a high competitive ability under high resource availability (e.g., Bobbink and others 1998; Hautier and others 2009).

In acid grasslands (found on soils with a pH of around 5 or below regardless of N deposition status), the main effects on plant communities are a loss of species richness (Duprè and others 2010; Maskell and others 2010; Stevens and others 2010a), especially forbs, an increase in the prevalence of competitive species (e.g., Wilson and others 1995), and an increase in the prevalence of acid tolerant species (e.g., Stevens and others 2010b) resulting in a species composition which is not typical of this community. These changes are a cause for concern because they represent a loss of biodiversity across large areas of Western Europe. Changes in plant tissue chemistry have also been observed on the gradient of deposition (Gidman and others 2006; Stevens and others 2011b).

Sala and others (2000) identified the five most important determinants of changes in biodiversity on a global scale: changes in land-use, increasing atmospheric  $CO_2$  concentration, increasing N deposition and acid rain, climate change, and biotic exchanges (introduction of exotic species). They used a series of scenarios of predicted future change to identify the relative effects of these drivers on biodiversity by the year 2100. For global biodiversity, land-use change was considered the greatest threat followed by climate change and N deposition. The relative importance of these drivers differed between habitats with N deposition being among the top three drivers for eight out of ten terrestrial biomes and the most important in northern temperate forests.

The Millennium Ecosystem Assessment also identified N and sulfur deposition together with fertilization as amongst the most important threats to biodiversity (Mace and others 2005). It concludes that N inputs are a threat to biodiversity at the biome and species level.

Both of these global studies are concerned with the assessment of threat to ecosystems at a global level, whereas this paper is primarily concerned with a single vegetation type (acid grassland) in Western Europe.

Semi-natural grasslands are an important component of European agriculture, supporting extensive grazing and providing hay. They also support a wide range of plant, invertebrate and bird species. Acid grasslands are found throughout Europe in both upland and lowland areas. They were formerly widespread but, in some areas of Europe, have been heavily impacted by land-use change and agricultural abandonment (Ellenberg 1996). The habitat we focus on is identified in the Conservation of Natural Habitats and of Wild Fauna and Flora Directive (92/43/EEC) (the "Habitats Directive") and is a subgroup of the Natura 2000 habitat "species-rich *Nardus* grassland".

Focusing on grassland systems in Western Europe, particularly acid grasslands, this paper aims to assess the perceived threat of N deposition to biodiversity by policy makers, identify relevant policy drivers, assess the potential for EU Habitats Directive 'typical species' to be used as indicators of N deposition and identify management options for mitigating the effects of N deposition. We also identify outstanding policy-related questions that scientists and policy makers need to address to effectively protect biodiversity. To do this we report on the results of a workshop held for policy makers concerned with N deposition in Western Europe.

# Perceived Importance of N Deposition as a Threat to Biodiversity

In order to assess the perceived threat of N deposition on biodiversity we asked participants at a workshop for policy makers and others concerned with environmental policy (with a focus on national or European conservation and biodiversity policy) to give their opinions. Workshop participants came from nine countries across Western Europe (Denmark, France, Germany, Ireland, Netherlands, Norway, Sweden, Switzerland, United Kingdom). The workshop presented results of the European Science Foundation project 'BEGIN-Biodiversity of European Grasslands-Impacts of Nitrogen Deposition' and was held in October 2009 in Barsac, Gironde, France. The workshop addressed the following topics: identification of drivers of grassland habitat change, the use of indicators in assessing impacts of N deposition on grasslands and mitigation of impacts through responsive management. In order to assess the perceived importance of N deposition on grasslands we asked stakeholders at the workshop to assess the relative importance of ten drivers of biodiversity loss in grasslands ranking them from the most important (score 10) to the least important (score 1). Threats to the biodiversity of acid grasslands may differ at the Western European scale from those that we see at a global scale, so the possible drivers that delegates considered were threats specific to grassland biodiversity over the next 20 years. The candidate drivers presented for consideration by the delegates were: atmospheric N deposition, atmospheric sulfur deposition, intensification of agriculture (including addition of inorganic fertilizers), abandonment of management, fragmentation of habitats, climate change, overgrazing, invasion by exotic species, recreation and tourism pressures and soil compaction. There was also an option to add further drivers.

Abandonment of management was the category that was most commonly rated as the greatest threat to biodiversity (45% of delegates), followed by intensification of agriculture and atmospheric N deposition. These were rated as the top three priorities for many of the delegates as can be seen by examining the average scores (Fig. 1). Afforestation and building development were both identified as a threat by one delegate each.

The high score allocated to N deposition reflects the perceived threat that N deposition presents to biodiversity.



Fig. 1 Average scores allocated by 18 delegates from Western Europe to identify the most important drivers of grassland biodiversity over the next 20 years. A high score was allocated to the greatest threats

It also reflects the awareness policy makers have of the problems that N deposition presents. However, that these delegates participated in the workshop already indicated that they were concerned about this issue and the impacts it may be having in their countries. The results also reflect the transboundary and widespread nature of the threat presented by N deposition (Fagerli and Aas 2008). Differences in allocation of scores reflect personal opinion, but also national priorities and policy. In a country such as the Netherlands, where N deposition is well publicized and levels of deposition are high but declining as a result of successful introduction of abatement measures (Netherlands Environmental Assessment Agency 2005), we saw a higher ranking than in a country like Ireland, where N deposition is relatively low. In other parts of the world the perceived importance of N deposition as a driver of species change will vary depending on awareness and the perceived importance of other issues. For example, in Eastern Europe N deposition is high (Dentener and others 2006) and is thought to be impacting vegetation community composition (Hejcman and others 2009) but issues associated with land use change and abandonment are more likely to be considered of high priority (Sikor 2003).

#### Nitrogen Deposition in European Policy

The transboundary nature of N deposition and other air pollutants means that European legislation and international conventions are very important in dealing N emission and its impact on biodiversity. Key policy related to air pollution includes the Convention on long-range transboundary air pollution (CLRTAP), National Emission Ceilings Directive (2001/81/EC), Large Combustion Plant Directive (2000/80/EC), Ambient Air Quality Assessment and Management Directive (96/62/EC) and Integrated Pollution Prevention and Control Directive (91/61/EC and 2008/1/EC). However, although all the above directives are concerned with reducing air pollution and setting limits for N emissions, concentrations or deposition rates, many of these pieces of legislation do not directly consider the impact of the pollutants on semi-natural habitats. The CLRTAP is the main exception to this generalization, considering impacts on human health and environment. Indeed, of the seven International Co-operative Programmes (ICPs) reporting to the Working Group on Effects for the CLRTAP, five consider air pollution impacts on biodiversity.

The 1999 Gothenburg Protocol to abate acidification, eutrophication and ground-level ozone sets out an obligation to reduce emissions of sulfur, N and volatile organic compounds and ambient concentrations of ozone below specified levels. Parties are required to monitor emissions, ambient concentrations and deposition and collect information on the effects of these pollutants on human health, terrestrial and aquatic ecosystems and materials. The Gothenburg Protocol also sets out critical loads and levels for air pollutants. A critical load is defined as "a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur, according to present knowledge" (ICP Modelling and Mapping 2004; Nilsson and Grennfelt 1988). Empirical critical loads for N deposition are set for a range of habitats (Achermann and Bobbink 2003) and are based on expert knowledge and research. They consider the impacts of N on indicators of biodiversity, such as an increase in the dominance of N-favored species, decreases in diversity and changes in soil chemistry. Empirical critical loads are regularly revised to take account of the most up-to-date scientific knowledge. Exceedance of these critical loads can be mapped and provide a tool for determining the potential damage of N deposition.

Biodiversity policy is also important for assessing the impact of N deposition. The UN Convention on Biological Diversity (CBD) and the Ramsar Convention both have the potential to protect habitats from the deleterious effects of N deposition (see Bleeker and others 2011) and critical-load exceedance for N deposition is used as one of the indicators in 'Streamlining European 2010 Biodiversity Indicators' (EEA 2007). Relevant European legislation includes the Directive on the Conservation of Wild Birds (2009/147/ES) and on the Habitats Directive. The Habitats Directive requires member states to take measures to ensure habitats and wild species are in a favorable conservation status.

#### **Conservation Management Tools and Options**

Detecting N Deposition Impacts Using "Typical Species" as Indicators

In order to fulfill the requirements of the Habitats Directive, member states are required to undertake surveillance of the conservation status of the natural habitats and species and to produce a report every six years. A number of methods are used to define 'favorable status' for each habitat, including habitat range and area, and presence of typical species. Typical species are those which can be considered good indicators of favorable habitat quality, are sensitive to changes in the condition of the habitat and are detectable by non-destructive means (European Commission 2006). Typical species are defined by each member state and are species considered typical of the habitat. If these species were suitable as indicators of N deposition impact they would be very useful for conservation managers.

We undertook an analysis of acid grassland survey data from Western Europe to determine whether Habitats Directive typical species are suitable for use as indicators of N deposition. Typical species were taken from the Habitats Directive Article 17 database (EEA 2008). The most appropriate habitat classification under the Habitats Directive for the grasslands surveyed is 'species-rich *Nardus* grassland' (habitat code 6230). Only four countries submitted lists of typical species to the European Environment Agency for species-rich *Nardus* grassland in the Atlantic region: Netherlands, Ireland, France and Germany. This gave a total of 55 typical species with some species reported for more than one country (Table 1).

The data used in this analysis are from 153 species-rich Nardus grasslands in ten countries (Belgium, Denmark, France, Germany, Great Britain, Ireland, Isle of Man, Netherlands, Norway and Sweden) (Stevens and others 2010a). The grasslands surveyed were selected to cover the range of atmospheric N deposition in Western Europe and to give a good range of sites at different latitudes and longitudes for different deposition values. The grasslands surveyed all belonged to the association Violion caninae grassland. The definition of species-rich Nardus grassland is slightly broader than the definition of the association Violion caninae grassland (Schwickerath 1944), but Violion caninae can be considered a sub-type of the speciesrich Nardus grassland (Galvánek and Janák 2008; Krahulec 1985). Canonical correspondence analysis (CANOCO 4.5; ter Braak and Smilauer 2002) was used to show the distribution of species in relation to N deposition. In this ordination, N and sulfur (S) deposition were used as variables in the analysis whilst soil pH, aluminum concentration, base cation concentration, nitrogen content, carbon content, C:N ratio, latitude, longitude and mean daily **Table 1** Typical species for dry acid grassland (habitat code 6230) inthe Atlantic region for countries that submitted data to the HabitatsDirective Article 17 database (EEA 2008)

Typical species	Country
Achillea millefolium	IE
Agrostis capillaris	IE
Agrostis curtisii	FR
Antennaria dioica	DE
Anthoxanthum odoratum	IE
Arnica montana	DE
Avenula lodunensis	FR
Botrychium lunaria	DE
Carex arenaria	FR
Carex ericetorum	NL
Carex pallescens	DE
Carex panicea	DE
Carex pilulifera	DE, IE
Chamaespartium sagittale	DE
Dactylorhiza viridis	NL
Danthonia decumbens	FR, IE
Dianthus deltoides	FR
Euphrasia stricta	DE
Festuca filiformis (sub-species of F. ovina)	DE, FR
Festuca ovina	IE
Galium saxatile	DE, FR, IE, NL
Gentiana pneumonanthe	DE
Hypericum maculatum	DE, IE
Hypochaeris radicata	DE
Jasione montana	FR
Juncus squarrosus	DE, IE
Lathyrus linifolius (synonym of L. montanus)	DE
Lathyrus montanus	IE
Luzula campestris	DE, FR
Luzula multiflora	IE
Meum athamanticum	DE
Narcissus bulbocodium	FR
Nardus stricta	DE, FR, IE, NL
Pedicularis sylvatica	DE, FR, IE, NL
Platanthera bifolia	DE, NL
Polygala serpyllifolia	DE, FR, IE, NL
Polygala vulgaris	DE, IE
Potentilla erecta	DE, FR
Pseudarrhenatherum longifolium	FR
Pseudorchis albida	IE
Rhytidiadelphus squarrosus	IE
Rumex acetosella	FR
Sedum anglicum	FR
Serapias lingua	FR
Spiranthes spiralis	NL
Stachys officinalis	NL
Succisa pratensis	IE

Typical species	Country		
Thymus pulegioides	FR		
Veronica officinalis	DE		
Viola canina	DE, FR, IE		
Viola lactea	FR		
Viola riviniana	IE		

Species in bold occurred within more than 5% of the acid grasslands surveyed

maximum temperature were used as co-variables. There were no strong geographical trends in the data (Stevens and others 2011a). For all of the sites, N deposition was modeled using the best available deposition model. National models were used for Germany (Gauger and others 2002), the Netherlands (Asman and van Jaarsveld 2002; Van Jaarsveld 1995, 2004) and Great Britain (NEGTAP 2001; Smith and others 2000). For all other countries, the EMEP-based IDEM model (Pieterse and others 2007) was used.

Many typical species did not occur in sufficient numbers (more than 5% of sites) in our database to be included in this ordination analysis. There are two potential reasons for this. The first is that if a species is particularly common on a regional level, but not at a broader level, they may make an ideal typical species for a country, but our dataset may not have sufficient sites within that region for it to appear in our dataset at more than 5% of all sites. The second potential reason that a typical species may not be found in our dataset is that we have not covered the full range of types of species-rich *Nardus* grassland as described in Natura 2000. Nevertheless, these data can give us an indication of how suitable a species may be for assessing the impact of N deposition.

Figure 2 shows the position of the 55 selected Article 17 typical species within an ordination diagram of a canonical correspondence analysis created using data gathered in the above-mentioned European survey. Species to the right of the ordination diagram at the top of the arrow are more commonly found at high N deposition (based on their occurrence and cover) within this dataset, those in the center are neutral with regards to N deposition and those to the left are more commonly found at lower N deposition. It is those species on the left that are likely to be most suitable as indicators of low nitrogen deposition impact. As can be seen from Fig. 2, typical species are scattered across the ordination diagram, so using typical species lists from the Netherlands, Ireland, France and Germany, this group of species is not suitable as an indicator of N deposition impact in this habitat. Analysis of each of these countries individually and comparison with national lists showed a similar scatter of typical species with respect to pollutant



**Fig. 2** Ordination diagram of a canonical correspondence analysis showing species from 153 *Violion caninae* grasslands in the Atlantic biogeographic region of Europe. *Filled circles* show Habitats Directive typical species for species-rich *Nardus* grassland and *empty circles* show other species. The *empty circles* have not been individually labelled for clarity; a full discussion of species associated with high and low levels of N deposition is given in Stevens and others (2011a). The *arrows* represent increasing total nitrogen deposition rate (N) and increasing total sulfur deposition rate (S). Eigen values for axis 1 and 2 are 0.097 and 0.276 respectively. The total inertia of the analysis was 3.79

deposition (data not shown). There are some individual species that may be suitable, most notably *Agrostis curtisii* (typical species in France), *Stachys officinalis* (typical species in the Netherlands) and *Polygala serpyllifolia* (typical species in Ireland and Germany). All of these species are relatively common across Western Europe (although *Agrostis curtisii* has a restricted distribution in the western warm oceanic regions) and so merit further investigation of their potential as indicators of N deposition in this particular region.

The presence or absence and abundance of most typical species are not suitable indicators of N deposition at a Western European scale, indeed, many of the species selected as typical species are too common to be useful indicators even at a national scale. Given the widespread nature of this result in Western Europe it is likely that this will be true throughout the whole of Europe. Alternative species may provide more suitable indicators although other measures such as Ellenberg N scores, species richness or grass:forb ratio may be more useful, particularly if temporal changes in these measures could be assessed (Duprè and others 2010; Stevens and others 2009).

#### Mitigation of Acidification and Eutrophication

Detecting the potential impacts of atmospheric N deposition is only the initial stage of dealing with the problem. Once affected sites are identified, appropriate management should be put in place to mitigate the effects of deposition. There are a number of different options for the management of grasslands to control the impact of N deposition. These fall into two categories, measures to mitigate acidification and measures to mitigate eutrophication.

The main method of mitigating soil acidification is liming. Liming has a long history of use in agricultural sciences, indeed the Park Grass Experiment at Rothamsted Experimental Station in Hertfordshire, England commenced experimental lime addition in 1903 (Silvertown and others 1994). Liming has been widely used to combat acidification from atmospheric pollutants and nutrient addition in many habitats, including grasslands (e.g., Blake and others 1999; De Graaf and others 1998). Lime (usually calcium carbonate) reduces soil acidity by exchange of calcium or magnesium ions with hydrogen ions on soil particles resulting in a higher soil pH. In a heathlandcatchment liming experiment, Dorland and others (2005b) found liming resulted in higher soil pH, higher concentrations of base cations and a reduced Al:Ca ratio. Despite this, there was only a small positive response by vegetation, but changes in species composition were observed including the cessation of vigorous growth of some competitive species and an increase in rarer species. The addition of too much lime can damage plants and the effects if liming can vary depending on the site history, soil type, amount of N in the soil and the plant species present. In areas fed by base-rich groundwater, raising water-tables in the soil has also been used to combat acidification (Roelofs and others 1996).

Where species composition is changing as a result of eutrophication and consequent increases in productivity, measures can be taken to reduce competition for light and to remove nitrogen from the system. These could include an increase in cutting frequency or grazing intensity. These methods are commonly used in the restoration of grassland from former agricultural land (Walker and others 2003) and can change species composition, reducing the cover of productive grasses promoted by the addition of N. Additional winter grazing to disturb the grassland turf is also an option (Jones and Hayes 1999). The removal of biomass by cutting and taking off the hay also removes N from the system with the potential to reduce nutrient status of the soil and reduce productivity in the long-term. However, the rate of nutrient removal by this method is usually low (Hejcman and others 2010), and so it may take some time for a significant change to be detected (Olff and Bakker 1991). Burning also provides a means of biomass removal and is traditionally used as a management tool in some habitats (e.g., heathlands) but it is not commonly used in grasslands and may not be well accepted by the general public, because it produces greenhouse gases, particulate

pollution and other air pollutants, leaves large areas looking unsightly and generates safety concerns. Another method that has been tested experimentally for the removal of reactive N from soils is the addition of carbon (C). C addition in the form of sucrose, sawdust, starch or cellulose increases the C:N ratio of soil and can induce microbial communities to immobilize N in the soil, thus making it inaccessible to plants (e.g., Eschen and others 2007; Török and others 2000).

Turf stripping or cutting is the most dramatic method for the removal of nutrients, but it also removes acidified surface soil, and so can address both acidification and eutrophication problems. Turf stripping has been used extensively in some countries, especially the Netherlands, for the restoration of heathland (De Graaf and others 1998; Dorland and others 2005a). Topsoil removal is an alternative method that has been used for the restoration of grasslands (e.g., Buisson and others 2006) but it presents similar problems to turf stripping. Although turf stripping has undergone experimental trials in grasslands (e.g., Jansen and Roelofs 1996; Pywell and others 2002), it is an expensive form of management and in addition to removing nutrients and acidified soil, it also removes the soil seed bank and organic matter, as well as reducing the water holding capacity of the soil (van den Berg and others 2003a). The removal of the soil seed bank means that if the local species pool is already depleted, appropriate species may not be able to re-colonize and may need to be reintroduced (Dorland and others 2004; van den Berg and others 2003b). Dispersal into large cleared areas is usually not sufficient for rapid colonization by target species, and would require large source populations nearby. For perennial species that do not produce large annual seed crops, unassisted dispersal is likely to be quite limited (e.g., Soons and others 2005). To facilitate re-colonization of cleared areas, hay from target communities can be spread out to supply seeds and improve micro-environmental conditions. This has been successful in some situations (Poschlod and Biewer 2005; Coiffait-Gombault and others 2010), but is limited by the supply of hay from nearby source sites.

Many of these measures are unsuitable for application at a landscape scale and it would only be appropriate to apply them to sites of conservation importance or other targeted areas. Currently it falls to landowners to mitigate against the effects of N deposition. When conservation organizations are responsible for land management, such mitigation may be possible albeit expensive, but in many cases the land is owned by private individuals, so appropriate management needs to be promoted through agri-environment schemes. Mitigation measures to reduce the effect of N deposition are not currently incorporated into these schemes in many parts of Europe, but some of the measures described above are feasible at different scales. Farmers need incentives to encourage appropriate management. Payment based on results of management, such as an increase in species richness or reduction in eutrophic species, could increase motivation and help farmers to value their land as a conservation resource but may reduce participation in the scheme if farmers are concerned about the probability of success.

#### **Emission Management Options**

Managing the effects of N deposition is frequently expensive, impractical and in some cases can change the landscape and the ecosystem dramatically. The only truly effective and sustainable method to reduce the impact of N is through the reduction of N emissions.

There are a number of potential methods that could be used to reduce emissions of N. Emissions of N oxides from industrial sources are already controlled within Europe to a large extent through the legislation outlined above. However, this is not always the case world-wide. Options for the control of reduced N emissions from agriculture have poor uptake and oxidized N emissions from traffic and transport are mainly mitigated through efforts to reduce  $CO_2$  emissions.

In principle, the Habitats Directive provides protection for designated areas of conservation importance. Under the Directive, projects cannot be approved if they are assessed to have an adverse effect on a Special Area of Conservation (SAC) or Special Protection Area (SPA). However, this decision can only be made if an appropriate review and assessment is undertaken which, for many agricultural activities resulting in increased nitrogen deposition to nearby sites, is not the case. Legislative control of N emissions from agriculture, and indeed measuring and monitoring of emissions, provides many challenges due to the diffuse nature of emissions and the cost of emission reduction technology. Existing options for reducing N emissions from agriculture include direct injection of fertilizers and slurries, suitable storage of animal waste, utilizing technologies to minimize fertilizer use and filtering air before it leaves animal housing areas but all of these options have the potential to incur additional cost which may make them unattractive to farmers. Sutton and others (2011) suggest that improving the nitrogen use efficiency of crops through improving the genetic potential of crop varieties, increasing the genetic potential of animals to increase productivity, improving animal feed quality to increase feed conversion efficiency and increasing the efficiency of use of animal manures are key priorities to reduce agricultural N losses in Europe.

Workshop delegates suggested a number of possible options for reducing emissions. Diffuse nitrate pollution to water is currently controlled in Europe through the Nitrates Directive (91/676/EEC,) which requires Member States to identify areas where groundwater nitrate concentrations exceed 50 mg  $l^{-1}$  or are at risk of doing so. These areas are designated as Nitrate Vulnerable Zones (NVZs) and Member States must establish 'Action Programmes' in order to reduce and prevent further nitrate contamination. A similar approach could be taken to N emissions to the atmosphere.

Slurry spreading is an agricultural activity, which is currently managed for the protection of water quality, but could additionally be managed for air quality. For example, regulations in the UK (The Nitrate Pollution Prevention Regulations 2008) currently prohibit application of farmyard manure to grassland in NVZs between the mid-September and mid-January, when biological uptake is lowest and runoff is often high. If slurry spreading were also not permitted during the hottest months of the year, this would minimize ammonia volatilization.

Reduced meat production and the potential to educate the public to reduce meat consumption is an effective way to reduce ammonia emissions. World-wide meat consumption increased dramatically between 1961 and 1994, and although the rate of growth in *per capita* meat consumption has now slowed in the developed world, this is because it had already reached a very high level (Rosegrant and others 1999). Using incentives and education to reduce meat consumption could have environmental and health benefits. Initiatives such as 'meat free Mondays' (http://www. supportmfm.org/), meat free days in schools (such as seen in Ghent, Belgium), and government recommendations for reduced consumption of meat (such as seen in Sweden) are becoming increasingly popular and could reduce N emissions. Attendees at the workshop signed the 'Barsac declaration' (http://www.nine-esf.org/barsac-declaration) to reduce meat consumption encourage the availability of reduced meat portions.

Another suggestion proposed at the workshop was promoting grass-fed animals over housed ones, which generate higher ammonia emissions. The latter option has benefits for consumer health (e.g., Daley and others 2010), animal welfare and the management of the grasslands, since biomass is removed by grazing, but means that emissions cannot be managed by filtering N compounds from the air. "Green meat" (meat produced on high value grasslands) provides a further benefit of ensuring that grasslands of high value for nature conservation remain agriculturally productive and economically viable. A further option is to take measures to reduce population growth.

#### **Policy Questions for Research**

Results of study and personal observations have convinced policy makers and scientists from a range of countries and backgrounds at the workshop that N deposition is having an impact on species-rich acid grasslands in the Atlantic biogeographic region of Europe. However, there remain some important questions that require attention from scientists and policy makers in order for progress to be made toward providing greater protection for sensitive habitats.

#### What Changes are of Conservation Concern?

The evidence, briefly summarized above, shows a wide range of impacts on vegetation and soils, but there is a need to determine which of these changes are of conservation concern. For example, it may be that while changes in chemistry of plant tissues on a gradient of N deposition have the potential to provide an early warning of plant stress (Gidman and others 2006), it is likely to be of less concern in terms of the assessment and management of sites of conservation importance than changes in community structure and function. A reduction in plant species richness or a change in community composition may be of much greater concern, because it represents a loss of biodiversity. Of the changes that are considered of conservation concern in a particular habitat, it is necessary to determine which of these changes are the most important and which should trigger the need for action.

How Much Change is Necessary for it to be Considered Significant?

Once important changes have been identified, the magnitude of these changes needs to be considered. For example, Stevens and others (2004), in a gradient survey of acid grasslands in Great Britain, reported an average reduction in species richness of one species for every additional 2.5 kg N ha<sup>-1</sup> year<sup>-1</sup> and suggested that this pattern was the result of long-term elevated N deposition. Given a response curve such as this, it is up to scientists and policy makers to determine 'acceptable' changes in community composition or reductions in species abundance or biodiversity before taking action. The dependence of ecosystem services on species diversity is a rapidly advancing research front (e.g., Engelhardt and Ritchie 2001) and will inform the extent to which species loss can be tolerated.

Addressing these questions will require close collaboration between scientists and policy makers and will provide a future direction for nitrogen-deposition research.

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### References

- Achermann B, Bobbink R (2003) Empirical critical loads for nitrogen. In: Proceedings of the expert workshop, Berne, 11–13 November 2002. Swiss Department for the Environment, Forests and Landscape, Berne
- Asman WAH, van Jaarsveld JA (2002) A variable-resolution transport model applied for NHx in Europe. Atmospheric Environment 26A:445–464
- Blake L, Goulding KWT, Mott CJB, Johnston AE (1999) Changes in soil chemistry accompanying acidification over more than 100 years under woodland and grass at Rothamstead Experimental Station, UK. European Journal of Soil Science 50: 401–412
- Bleeker A, Hicks WK, Dentener F, Galloway J, Erisman JW (2011) N deposition as a threat to the world's protected areas under the convention on biological diversity. Environmental Pollution 159:2280–2288
- Bobbink R, Hornung M, Roelofs JGM (1998) The effects of air-borne nitrogen pollutants on species diversity in natural and seminatural European vegetation. Journal of Ecology 86:717–738
- Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M, Cinderby S, Davidson E, Dentener F, Emmett B, Erisman JW, Fenn M, Gilliam F, Nordin A, Pardo L, De Vries W (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications 20:30–59
- Buisson E, Holl KD, Anderson S, Corcket E, Hayes GF, Torre F, Peteers A, Dutoit T (2006) Effect of seed source, topsoil removal, and plant neighbour removal on restoring California coastal prairies. Restoration Ecology 14:569–577
- Coiffait-Gombault C, Buisson E, Dutoit T (2010) Hay transfer promotes establishment of mediterranean steppe vegetation on soil disturbed by pipeline construction. Restoration Ecology 19:214–222
- Daley CA, Abbott A, Doyle PS, Nader GA, Larson S (2010) A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutrition Journal 9:1–12
- De Graaf MCC, Verbeek PJM, Bobbink R, Roelofs JGM (1998) Restoration of species-rich dry heaths. The importance of appropriate soil conditions. Acta Botanica Neerlandica 47: 98–111
- Dentener F, Drevet J, Lamarque JF, Bey I, Eickout B, Fiore AM, Hauglustaine D, Horowitz LW, Krol M, Kulshrestha UC, Lawrence M, Galy-Lacaux C, Rast S, Shindell D, Stevenson D, Van Noije T, Atherton C, Bell N, Bergman D, Butler T, Cofala J, Collins B, Doherty R, Ellingsen K, Galloway J, Gauss M, Montanaro V, Müller JF, Pitari G, Rodriguez J, Sanderson M, Solmon F, Strahan S, Schultz M, Sudo K, Szopa S, Wild O (2006) Nitrogen and sulfur deposition on regional and global scales: a multimodel evaluation. Global Biogeochemical Cycles 20:1–21
- Dorland E, Van den Berg LJL, Van den Berg AJ, Vermeer M, Roelofs JGM, Bobbink R (2004) The effects of sod cutting and additional liming on potential net nitrification in heathland soils. Plant and Soil 265:267–277
- Dorland E, Hart MAC, Vermeer ML, Bobbink R (2005a) Assessing the success of wet heath restoration by combined sod cutting and liming. Applied Vegetation Science 8:209–311
- Dorland E, van den Berg LJL, Brouwer E, Roelofs JGM, Bobbink R (2005b) Catchment liming to restore degraded, acidified heathlands and moorland pools. Restoration Ecology 13:302–311
- Duprè C, Stevens CJ, Ranke T, Bleeker A, Peppler-Lisbach C, Gowing DJG, Dise NB, Dorland E, Bobbink R, Diekmann M (2010) Changes in species richness and composition in European

acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. Global Change Biology 16:344–357

- EEA (2007) Halting the loss of biodiversity by 2010: proposal for a first set of indicators to monitor progress in Europe. European Environment Agency, Copenhagen
- EEA (2008) Article 17 database. http://biodiversity.eionet.europa.eu/ article17/chapter9
- Ellenberg H (1996) Vegetation Mitteleuropas mit den Alpen, 5th edn. Eugen Ulmer, Stuttgart
- Engelhardt KM, Ritchie ME (2001) Effects of macrophyte species richness on wetland ecosystem functioning and services. Nature 411:687–689
- Erisman JW, Bleeker A, Hensen A, Vermeulen A (2008) Agricultural air quality in Europe and the future perspectives. Atmospheric Environment 42:3209–3217
- Eschen R, Mortimer SM, Lawson CS, Edwards AR, Brook AJ, Igual JM, Hedlund K, Schaffner U (2007) Carbon addition alters vegetation composition on ex-arable fields. Journal of Applied Ecology 44:95–104
- European Commission (2006) Assessment, monitoring and reporting under Article 17 of the Habitats Directive: explanatory notes and guidelines. European Commission
- Fagerli H, Aas W (2008) Trends of nitrogen in air and precipitation: model results and observations at EMEP sites in Europe, 1980–2003. Environmental Pollution 154:448–461
- Falkengren-Grerup U (1995) Long-term changes in flora and vegetation in deciduous forests of southern Sweden. Ecological Bulletins 44:215–226
- Fowler D, Smith R, Muller J, Cape JN, Sutton M, Erisman JW, Fagerli H (2007) Long term trends in sulfur and nitrogen deposition in Europe and the cause of non-linearities. Water Air and Soil Pollution Focus 7:41–47
- Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: recent trends, questions and potential solutions. Science 320:889–892
- Galvánek D, Janák M (2008) Management of Natura 2000 habitats. 6230 \*Species-rich Nardus grasslands. European Commission
- Gauger T, Anshelm F, Schuster H, Erisman JW, Vermeulen AT, Draaijers GPJ, Bleeker A, Nagel H-D (2002) Mapping of ecosystem specific long-term trends in deposition loads and concentrations of air pollutants in Germany and their comparison with critical loads and critical levels. Institut fur Navigation, University of Stuttgart, Stuttgart
- Gidman EA, Stevens CJ, Goodacre R, Broadhurst D, Emmett B, Gwynn-Jones D (2006) Loss of forb diversity in relation to nitrogen deposition in the UK: regional trends and potential controls. Global Change Biology 12:1823–1833
- Hautier Y, Niklaus PA, Hector A (2009) Competition for light causes plant biodiversity loss after eutrophication. Science 324:636–638
- Hejcman M, Klaudisová M, Hejcmanová P, Pavlů V, Jones M (2009) Expansion of *Calamagrostis villosa* in sub-alpine *Nardus stricta* grassland: cessation of cutting management or high nitrogen deposition? Agriculture Ecosystems and Environment 129: 91–96
- Hejcman M, Schellberg J, Pavlů V (2010) Long-term effects of cutting frequency and liming on soil chemical properties, biomass production and plant species composition of Lolio-Cynosuretum grassland after the cessation of fertilizer application. Applied Vegetation Science 13:257–269
- ICP Modelling and Mapping (2004) Manual on methodologies and criteria for mapping critical levels/loads and geographical areas where they are exceeded. Report UBA-Texte 52/04. http:// icpmapping.org/

- Jansen AJM, Roelofs JGM (1996) Restoration of Cirsio-Molinietum wet meadows by sod cutting. Ecological Engineering 7:279–298
- Johnston AE, Goulding KWT, Poulton PR (1986) Soil acidification during more than 100 years under permanent grassland and woodland at Rothamstead. Soil Use and Management 2:3–10
- Jones AT, Hayes MJ (1999) Increasing floristic diversity in grassland: the effects of management regime and provenance on species introduction. Biological Conservation 87:381–390
- Krahulec F (1985) The chorologic pattern of European nardus-rich communities. Vegetatio 59:119–123
- Mace G, Masundire H, Baille J (2005) Biodiversity, Millennium Ecosystem Assessment. Island Press, Washington
- Maskell LC, Smart SM, Bullock JM, Thompson K, Stevens CJ (2010) Nitrogen deposition causes widespread species loss in British habitats. Global Change Biology 16:671–679
- NEGTAP (2001) Transboundary air pollution: acidification, eutrophication and ground-level ozone in the UK. CEH, Edinburgh
- Netherlands Environmental Assessment Agency (2005) Environmental data compendium: nitrogen deposition in the Netherlands, 2001. http://www.mnp.nl/mnc/i-en-0189.html
- Nilsson J, Grennfelt PE (1988) Critical loads for sulphur and nitrogen. UNECE/Nordic Council of Ministers, Copenhagen
- Olff H, Bakker JP (1991) Long-term dynamics of standing crop and species composition after the cessation of fertiliser application to mown grassland. Journal of Applied Ecology 28:1040–1052
- Pieterse G, Bleeker A, Vermeulen AT, Wu Y, Erisman JW (2007) High resolution modelling of atmosphere-canopy exchange of acidifying and eutrophying components and carbon dioxide for European forests. Tellus 59B:412–424
- Poschlod P, Biewer H (2005) Diaspore and gap availability are limiting species richness in wet meadows. Folia Geobotanica 40:13–34
- Pywell RF, Bullock JM, Hopkins A, Walker KJ, Sparks T, Burke MJW, Peel S (2002) Restoration of species-rich grassland on arable land: assessing the limiting processes using a multi-site experiment. Journal of Applied Ecology 39:294–309
- Roelofs JGM, Bobbink R, Brouwer E, De Graaf MCC (1996) Restoration ecology of aquatic and terrestrial vegetation on noncalcareous sandy soils in The Netherlands. Acta Botanica Neerlandica 45:517–541
- Rosegrant MW, Leach N, Gerpacio RV (1999) Alternative futures for world cereal and meat consumption. Proceedings of the Nutrition Society 58:219–234
- Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Biodiversity—global biodiversity scenarios for the year 2100. Science 287:1770–1774
- Schwickerath M (1944) Das Hohe Venn und seine Randgebiete. Pflanzensoziologie 6:1–278
- Sikor T (2003) The commons in transition: agrarian and environmental change in Central and Eastern Europe. Environmental Management 34:270–280
- Silvertown J, Dodd ME, McConway K, Potts J, Crawley M (1994) Rainfall, biomass variation, and community composition in the Park Grass experiment. Ecology 75:2430–2437
- Smith RI, Fowler D, Sutton MA, Flechard C, Coyle M (2000) Regional estimation of pollutant gas dry deposition in the UK: model description, sensitivity analyses and outputs. Atmospheric Environment 34:3757–3777
- Soons MB, Messelink JH, Jongejans E, Heil GW (2005) Habitat fragmentation reduces grassland connectivity for both shortdistance and long-distance wind-dispersed forbs. Journal of Ecology 93:1214–1225

- Stevens CJ, Dise NB, Mountford JO, Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. Science 303:1876–1879
- Stevens CJ, Maskell LC, Smart SM, Caporn SJM, Dise NB, Gowing DJ (2009) Identifying indicators of atmospheric nitrogen deposition impacts in acid grasslands. Biological Conservation 142:2069–2075
- Stevens CJ, Duprè C, Dorland E, Gaudnik C, Gowing DJG, Bleeker A, Diekmann M, Alard D, Bobbink R, Fowler D, Corcket E, Mountford JO, Vandvik V, Aarrestad PA, Muller S, Dise NB (2010a) Nitrogen deposition threatens species richness of grasslands across Europe. Environmental Pollution 158:2940–2945
- Stevens CJ, Thompson K, Grime JP, Long CJ, Gowing DJG (2010b) Contribution of acidification and eutrophication to declines in species richness of calcifuge grasslands along a gradient of atmospheric nitrogen deposition. Functional Ecology 24:478–484
- Stevens CJ, Dupre C, Gaudnik C, Dorland E, Dise NB, Gowing DJ, Bleeker A, Alard D, Bobbink R, Fowler D, Corcket E, Vandvik V, Mountford JO, Aarrestad PA, Muller S, Diekmann M (2011a) Changes in species composition of European acid grasslands observed along a gradient of nitrogen deposition. Journal of Vegetation Science 22:207–215
- Stevens CJ, Duprè C, Dorland E, Gaudnik C, Gowing DJG, Bleeker A, Diekmann M, Alard D, Bobbink R, Fowler D, Corcket E, Mountford JO, Vandvik V, Aarrestad PA, Muller S, Dise NB (2011b) The impact of nitrogen deposition on acid grasslands in the Atlantic region of Europe. Environmental Pollution 159: 2243–2250
- Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Grennfelt P, van Grinsven H, Grizzetti B (2011) The European nitrogen assessment. Cambridge University Press, Cambridge
- ter Braak CFJ, Smilauer P (2002) CANOCO 4.5, 4.5 ed. Biometris, Wargeningen
- Tilman D (1999) Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. Proceedings of the National Academy of Sciences of the United States of America 96:5995–6000
- Török K, Szili-Kovacs T, Halassy M, Toth T, Hayek Z, Paschke MW, Wardell LJ (2000) Immobilization of soil nitrogen as a possible method for the restoration of sandy grassland. Applied Vegetation Science 3:7–14
- Tyler G, Olsson T (2001) Concentrations of 60 elements in the soil solution as related to soil acidity. European Journal of Soil Science 52:151–165
- van den Berg LJL, Dorland E, Vergeer P, Hart MAC, Bobbink R, Roelofs JGM (2003a) Decline of acid-sensitive plant species in heathland can be attributed to ammonium toxicity in combination with low pH. New Phytologist 166:551–564
- van den Berg LJL, Vergeer P, Roelofs JGM (2003b) Heathland restoration in The Netherlands: effects of turf cutting depth on germination of *Arnica montana*. Applied Vegetation Science 6:117–124
- Van Jaarsveld JA (1995) Modelling the long-term atmospheric behaviour of pollutants on various spatial scales. University of Utrecht, Utrecht
- Van Jaarsveld JA (2004) The operation priority substances model. National Institute for Public Health and the Environment (RIVM), Bilthoven
- Walker KJ, Stevens PA, Stevens DP, Mountford JO, Manchester SJ, Pywell RF (2003) The restoration and re-creation of species rich lowland grassland on land formerly managed for intensive agriculture in the UK. Biological Conservation 119:1–18
- Wilson EJ, Wells TCE, Sparks TH (1995) Are calcareous grasslands in the UK under threat from nitrogen deposition?—an experimental determination of a critical load. Journal of Ecology 83:823–832

ORIGINAL PAPER

# Importance of semi-natural habitats for the conservation of butterfly communities in landscapes dominated by pine plantations

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Abstract While the area of plantation forests continues to increase worldwide, their contribution to the conservation of biodiversity is still controversial. There is a particular concern on the central role played by natural habitat remnants embedded within the plantation matrix in conserving species-rich insect communities. We surveyed butterflies in maritime pine plantation landscapes in south-western France in 83 plots belonging to seven habitat types (five successional stages of pine stands, native deciduous woodlands and herbaceous firebreaks). The effect of plot, habitat and landscape attributes on butterfly species richness, community composition and individual species were analysed with a General Linear Model (GLM), partial Canonical Correspondence Analysis (CCA) and the IndVal method. The most important factors determining butterfly diversity and community composition were the presence of semi-natural habitats (deciduous woodlands and firebreaks) at the landscape scale and the composition of understorey vegetation at the plot scale. Pure effects of plot variables explained the largest part of community variation (12.8%), but landscape factors explained an additional, independent part (6.7%). Firebreaks were characterized by a higher species richness and both firebreaks and deciduous woodlands harboured species not or rarely found in pine stands. Despite the forest-dominated landscape, typical forest butterflies were rare and mainly found in the deciduous woodlands. Threatened species, such as Coenonympha oedippus and Euphydryas aurinia, were found in pine stands and in firebreaks, but were more abundant in the latter. In the studied plantation forest, the conservation of butterflies depends mainly on the preservation of semi-natural habitats, an adequate understorey management and the maintenance of soil moisture levels.

**Keywords** Butterflies  $\cdot$  Communities  $\cdot$  Deciduous woodlands  $\cdot$  Firebreaks  $\cdot$  Habitat  $\cdot$  Landscape  $\cdot$  *Pinus pinaster*  $\cdot$  Plantation forests

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#### Introduction

Plantation forests with their intensive silvicultural management and simplified structure and composition are often considered less valuable for biodiversity conservation than natural forests (Hartley 2002). Many, but not all studies comparing plantations to more natural forests have indeed shown an impoverished flora and fauna in plantations (Moore and Allen 1999; Lindenmayer and Hobbs 2004; Carnus et al. 2006). However, forest management in plantation forests is not incompatible with biodiversity conservation and possibilities exist to enhance their biodiversity (Kerr 1999; Hartley 2002; Carey 2003; Carnus et al. 2006). Apart from providing a habitat, plantation forests can also have beneficial effects as landscape matrix elements by increasing the connectivity of natural forest remnants (Aberg et al. 1995) or by acting as a buffer to mitigate negative edge effects for forest interior species (Aune et al. 2005; Fischer et al. 2006). Biodiversity conservation in plantation landscapes will however also depend on the presence of more natural habitat elements, such as wetlands or late successional stages of remnant forest, within the plantation matrix (Lindenmayer and Hobbs 2004; Fischer et al. 2006).

Conserving biodiversity in plantation forests implies the identification of explanatory, environmental factors that determine patterns of species occurrences. Since species respond to environmental factors at different, interacting scales (from the micro-habitat and habitat to the landscape and regional scale) multi-scale approaches are required to analyse these causal mechanisms (Wiens 1989; Cushman and McGarigal 2002). In this study we analysed the effect of factors at both the local and landscape scale on butterfly diversity in pine plantation landscapes. Butterflies were chosen because they are easy to identify in surveys and include species with different habitat preferences and dispersal capacities and show therefore different responses to habitat and landscape features (Dennis 1992; Thomas 1995). Moreover, a large number of butterfly species are declining at an alarming rate through substantial parts of their European range and conservation measures are urgently needed (Van Swaay and Warren 1999).

During their life cycle most butterfly species need complementary resources (hostplants for larvae, nectar plants for adults, roosting-, resting- and overwintering-sites, favourable micro-climatological conditions) resulting in very direct relationships with habitat characteristics such as vegetation composition and management (Dennis et al. 2003). For many taxa, including butterflies, habitat characteristics alone are often insufficient to predict species presence or abundance and landscape characteristics can provide additional explanatory information (Mazerolle and Villard 1999; Jeanneret et al. 2003a; Krauss et al. 2003; Bergman et al. 2004; Stefanescu et al. 2004). Many butterfly studies conducted at the landscape scale have focussed on the effect of patch size and isolation and have used the equilibrium theory of island biogeography (Mac Arthur and Wilson 1967) or the metapopulation theory (Hanski 1999) to explain species richness or population dynamics, respectively (Thomas and Harrison 1992; Baguette et al. 2000; Steffan-Dewenter and Tscharntke 2000; Anthes et al. 2003). Both theories assume clearly delimited habitats surrounded by uniformly unsuitable habitat (the landscape matrix). However landscape matrices are not entirely hostile and the 'mosaic concept' (Wiens 1995; Duelli 1997) can offer an alternative to explain species richness. In this concept species richness increases with the number of biotope types per unit area, the number of patches, the edge length and the proportion of natural and semi-natural areas (Duelli 1997). Many butterfly species are found along edges or use resources in different vegetation types (Dennis et al. 2006) thus supporting the mosaic concept. Positive effect of patch density (PD) on butterfly diversity has been demonstrated by Schneider and Fry (2001) and Debinski et al. (2001). Dunning et al. (1992) described these effects of landscape context in terms of landscape complementation and supplementation, corresponding to the use of patches with nonsubstitutable or substitutable resources, respectively. Landscape effects found to be linked with species diversity will also depend on the scale of the analysed landscape; shorter distances will be more related to landscape complementation/supplementation and mosaic concepts (Weibull et al. 2000; Schneider and Fry 2001) and larger scales to metapopulation functioning and habitat thresholds (Bergman et al. 2004).

The aim of this study was therefore to identify key factors at both the habitat and landscape level that drive butterfly diversity in plantation forests, and that can be used by forest managers and landscape planners to maintain or restore butterfly diversity. We address the following questions:

- Do habitat types in a pine plantation landscape differ in butterfly species richness and composition?
- What is the contribution of semi-natural and open habitats such as oak woodland remnants and herbaceous firebreaks to butterfly diversity in pine plantation landscapes?
- What is the relative importance of understorey vegetation composition, habitat-type and landscape attributes on butterfly community composition?

#### Methods

Study area and plot selection

The study was carried out in South West France in the 'Landes de Gascogne' (Fig. 1), a region covering one million ha and dominated by plantations of native maritime pine (*Pinus pinaster*). Silvicultural management of the pine stands is intensive, including soil preparation and fertilization before seeding or planting, mechanical understorey removal and four thinning operations within the 40–50 years rotation cycle (Trichet et al. 1999). Deciduous woodlands are rare and found along rivers or as scattered patches of a few hectares. They are generally dominated by *Quercus robur*, on dry sites by *Q. pyrenaica* and along rivers by *Alnus glutinosa* and *Q. robur*. Open areas in the landscape are mainly represented by large maize fields, pine clearcuts, firebreaks and powerlines.

The whole region is covered by nutrient poor, acid podzol soils with a pH of 3.5–5.5 (Trichet et al. 1999). Differences in soil moisture have an important effect on the understorey vegetation composition in forest stands: in wet conditions *Molinia caerulea* is dominant with presence of *Erica tetralix*, intermediate conditions are characterized by dominance of *Pteridium aquilinum* and *Ulex europaeus* and in dry condition *Calluna vulgaris* and *Erica cinerea* dominate (Timbal and Maizeret 1998). Firebreaks and powerlines can have a heathland vegetation as described above or a grassland vegetation, dominated by for example *Holcus lanatus* or *Anthoxanthum odoratum*. Management of firebreaks and powerlines fires is considered doubtful and only firebreaks that are classified in a fire prevention scheme are mown once a year in summer. Other, private firebreaks are mown less often (every 4–8 years) or are progressively transformed to pine plantations. Management of powerlines is in general extensive (every 4–8 years) and aims at suppressing the regrowth of woody species, but in some cases they are mown annually. Firebreaks and powerlines typically have a width of about 15–100 m.

Within the 'Landes de Gascogne' two study sites were selected: Tagon (5,000 ha), situated 35 km southwest of Bordeaux and Solferino (10,500 ha), located 65 km to the south of



**Fig. 1** Map of the two study sites in the south-west of France and location of the sampled plots within each site. The polygon boundaries represent the edges of landscape elements such as pine stand edges or edges of roads. Firebreaks are the very narrow polygons between some stands or along some roads and at the scale of the figure cannot be separately indicated since they occupy only a small percentage of the total landscape

Tagon (Fig. 1). Both sites are dominated by maritime pine plantations, present similar types of soils, and include different degrees of landscape fragmentation and heterogeneity. A total of 83 plots were selected in the two sites (Fig. 1, Table 1) belonging to seven different habitat types. These seven habitat types were defined a priori and represent the main land-use types within the forested landscape as well as being habitat types of ecological relevance to butterflies. Five of them were related to successional stages of maritime pine plantations: herbaceous clearcuts, shrubby clearcuts, young pines (canopy height <7 m), mid-class pines (canopy height 7–15 m) and older pines (canopy height >15 m). The two other habitat types were deciduous woodlands (isolated patches or riparian forests) and firebreaks or powerlines (hereafter called firebreaks). Plots with different types.

## Butterfly sampling

Butterflies were recorded in the 83 plots using the line-transect method (Pollard and Yates 1993). In each plot a transect of eight sections of 50 m long was laid out and butterflies were counted within 2.5 m on each side of the transect line and 5 m ahead of the recorder. Species were identified by sight or caught and released for species difficult to identify (e.g. *Thymelicus* species). Each plot was visited four times between May 14th and September 4th 2004. Surveys were conducted between 10:00 and 18:00 h and only during appropriate

A	Description	ED	DD1	002	DD2	DD4	DD5	DW
Actollylli	Description	гв	FF I	FF2	FF 3	FF4	FF3	DW
		12 plots	10 plots	10 plots	10 plots	11 plots	11 plots	19 plots
Plot variable	?S							
Nectar	Log (number of flowers +1)	7.8	6.0	6.9	6.8	5.4	6.1	3.5
%Soil	% Bare soil	13.7	29.1	28.2	9.6	26.1	16.4	33.4
%Paqu	% Pteridium aquilinum	1.5	12.5	3.0	17.2	7.3	16.8	20.2
%Mcae	% Molinia caerulea	28.3	42.3	22.7	29.2	35.6	52.5	10.4
%Grass	% Other grass species	40.0	14.7	10.4	20.9	8.3	8.9	19.7
%Dicots	% Herbaceous dicotyledons	7.2	2.1	1.7	0.9	1.4	1.0	1.0
%Erica	% Ericaceae <0.7 m	6.9	5.1	15.7	15.4	12.7	7.4	1.0
%Umin	% Ulex minor <0.7 m	1.3	1.5	1.5	9.0	1.4	2.0	0.1
%Oth-her	% Other plants herbaceous layer	1.5	1.4	3.8	1.8	4.0	1.1	14.8
%Ueur	% Ulex europaeus >0.7 m	1.2	1.4	14.1	3.1	7.6	2.7	0.3
%Faln	% Frangula alnus >0.7 m	0.8	3.0	6.9	3.9	7.5	5.0	1.1
%Esco	% Erica scoparia >0.7 m	0.6	0.3	1.9	1.2	3.8	6.5	0.8
%Oth-shrub	% Other shrubs >0.7 m	3.2	1.7	5.8	0.5	2.5	1.5	20.3
Moisture	Soil moisture at 50 cm (classes 0–4)	1.7	2.2	1.4	1.7	1.5	1.8	1.3
Landscape v	ariables							
%FB	% Firebreaks	7.2	1.1	1.9	2.2	3.2	2.0	2.1
%PP1	% Herbaceous clearcuts	1.7	27.0	3.1	2.1	1.7	4.4	6.4
%PP2	% Shrubby clearcuts	16.0	7.9	22.2	8.1	4.4	9.2	8.0
%PP3	% Young pine stands (<7 m)	26.8	11.1	15.6	33.2	10.0	18.2	14.6
%PP4	% Mid-class pine stands (7–15 m)	26.2	28.6	21.1	33.5	55.7	21.0	16.5
%PP5	% Older pine stands (>15 m)	10.6	17.4	27.8	14.0	13.9	32.1	17.6
%DW	% Deciduous/mixed woodland	6.9	2.6	4.7	1.5	3.0	2.4	16.5
SHDI	Shannon diversity index	1.6	1.4	1.5	1.4	1.2	1.5	1.6
SHEI	Shannon eveness index	0.75	0.71	0.74	0.68	0.60	0.75	0.73
PRD	Patch richness density	15.9	14.5	14.5	14.7	14.1	14.8	17.8
SHAPE	SHAPE index	2.1	1.9	2.0	1.9	1.9	1.9	2.0
ED	Edge density	229.3	180.5	203.1	193.3	184.9	193.2	224.8
PD	(edge length in m/ha) Patch density (patches/100 ha)	56.0	41.8	49.3	46.9	40.7	44.5	57.0

 Table 1
 Plot and landscape variables used in GLM and CCA analyses

Mean values are given per habitat type. Abbreviations for habitat types: *FB* firebreaks, *PP1* herbaceous clearcuts, *PP2* shrubby clearcuts, *PP3* young pine stands (<7 m), *PP4* mid-class pine stands (7–15 m), *PP5* older pine stands (>15 m), *DW* deciduous woodlands. Landscape variables were measured in a 50-hectare circle (including the inventoried plot)

weather conditions (temperature >20°C, cloudless or just a few clouds and wind force <5 Beaufort). The order of the plots and habitat types surveyed was randomized per visiting period and visits to the two sites alternated. For data analysis the total number of individuals per species was pooled over the four visits and eight sections for each plot.

Butterfly species were classified as typical forest or non-forest species. Limits between forest and non-forest species are not strict, but we defined as forest species those species whose adults and immature stages are more often found within forests than in open habitats (Ebert and Rennwald 1991). We also attributed a European and national threat status to all

native species, excluding migrants such as *Vanessa cardui* (Van Swaay and Warren 1999). For the European status we used the list of threatened species cited in the Red Data Book of European Butterflies (Van Swaay and Warren 1999). In this Data Book the IUCN criteria, which are based on population declines over a 10-year period, were adjusted to butterfly data using a roughly equivalent distribution decline over a 25-year period. Species with a decrease of at least 20% are classified as threatened, and depending on their total decrease and present distribution classed as critically endangered, endangered or vulnerable. Species with a decrease of 15–20% and a present distribution of <1% of Europe are also classified as vulnerable. For the French national status we calculated the distribution trend by dividing the number of departments where a species was not seen after 1980, but was present before 1980, by the total number of departments where the species was ever seen (Lafranchis 2000). Species with a distribution decrease of at least 30% were classified as 'nationally threatened', assuming that these species are vulnerable at a national scale. We used a less severe threshold to compensate for the lack of data in several departments (Lafranchis 2000). Butterfly species are named in the text according to Karsholt and Razowski (1996).

#### Plot variables

We measured a set of potential explanatory variables at both the plot and landscape scale in order to relate butterfly species richness and community composition to environment. At the plot scale three types of variables were measured: flower abundance, vegetation composition of the herbaceous and shrub layer and soil moisture (Table 1). Flower abundance was measured to estimate the availability of nectar, the most common food source for adult butterflies in temperate areas (Shreeve 1992; Ebert and Rennwald 1991). Flower abundance was estimated during each of the four butterfly surveys using the method described by Clausen et al. (2001). Only plant species known to be used by butterflies as nectar plants were noted (Ebert and Rennwald 1991; van Halder, personal observations). Flower abundance was estimated per plant family or per species for abundant and easily identifiable species. The number of flower units was estimated in every section using the following abundance classes: 1-25, 26-50, 51-100, 101-200, 201-400, 401-800 and 800-1,600 flower units (Clausen et al. 2001). For data analysis the mid-values of each class were summed over the eight sections, the four visits and the different flower species or families. Total flower abundance was log-transformed to reduce the effect of outliers and because we hypothesized a non-linear relation between butterfly and flower abundance.

At the end of the field season understorey vegetation composition was recorded in a representative section within each plot. The vegetation was divided into a herbaceous layer (<0.7 m) and a shrub layer (0.7–7 m) and for each layer the % cover of the main vegetation components was estimated as the relative foliage area projected on a horizontal plane.

Soil moisture was estimated once between May and July 2004 at two points in each plot at a depth of 50 cm using a relative scale from 0 to 4, based on tactile and visual criteria. We used this estimation method because it can be used on soil samples extracted with an auger, a very easy and quick method, whereas measurements with a probe at 50 cm depth would have needed to dig a soil profile of at least 50 cm deep. We measured volumic soil moisture using a Theta Probe type ML2x (Delta-T Devices Ltd., Cambridge, UK) with 12 replications per class to test the relationship between moisture estimates and measures. There were significant changes in measured soil moisture between our relative classes (ANOVA, F = 78.4, P < 0.001). The scale from 0 to 4 corresponds to a mean soil moisture of 4.0, 12.8, 19.6, 44.4 and 62.6%, respectively.

#### Landscape variables

Land-use types in the two study sites were mapped in a GIS (Arcview 3.3, ESRI) using aerial colour photos with a resolution of 50 cm as background layer. Photos dated from 2000 and 2002 for the Tagon and Solferino region, respectively, and patch attributes were verified in the field. Twelve different land-use types that could be identified on these aerial photos were mapped: the seven surveyed habitat types and five rarer land-use types: hedgerows, meadows, crops, roads and urban areas. Landscape metrics were calculated within circular buffers with a radius of 400 m (circa 50 ha), from the centre of the sampled plots, using Fragstats 3.3 in raster version and a cell size of 2.5 m (McGarigal et al. 2002). Since the surveyed patch is (partly) included in our buffer the calculated metrics represent a combination of patch and landscape characteristics (Fahrig 2003). For most butterfly species in temperate areas 400 m is considered a moderate dispersal distance (Maes and Van Dyck 1999) and could therefore reveal ecologically relevant landscape relations. Larger buffers were not analysed because overlap between buffers would increase collinearity of data. Within each buffer the percentage cover of the seven main habitat types was calculated as well as several metrics reflecting landscape heterogeneity and fragmentation (Table 1). We used the Shannon Diversity Index (SHDI), the Shannon Eveness Index (SHEI) and the Patch Richness Density (PRD) as metrics of landscape heterogeneity and the SHAPE index, the PD and Edge Density (ED) as metrics for landscape fragmentation (McGarigal et al. 2002). Studies on birds, spiders and carabids in the same area have shown an effect of landscape composition and landscape structure (patch size, ED and SHDI) on species composition and richness (Barbaro et al. 2005). Landscape effects on butterflies have been analysed in several studies showing an effect of landscape composition (Schneider and Fry 2001; Söderström et al. 2001; Jeanneret et al. 2003a; Stefanescu et al. 2004), landscape fragmentation (Schneider and Fry 2001) and landscape heterogeneity (Weibull et al. 2000; Jeanneret et al. 2003a; Krauss et al. 2003).

#### Data analysis

Analyses were performed at different levels of biodiversity (species richness, single species abundances and composition of species assemblages) using hierarchical sets of explanatory variables: habitat type, plot variables and landscape variables. The effect of habitat type on number of species and total abundance of butterflies was tested by a one-way ANOVA, followed by Tukey's post hoc test. Total abundance of butterflies was log-transformed to improve normality of residuals. Species richness was analysed with a general linear model (GLM), using site, habitat and their interaction as categorical variables, and plot and landscape variables as continuous variables. Quadratic terms of plot and landscape variables were added to examine the possibility of curvilinear relationships between explanatory variables and species richness. We used a forward stepwise selection procedure (P < 0.05 for inclusion) to build the model. The possible interaction between selected categorical and continuous variables was tested in a forward procedure with the selected variables and their interaction.

To identify species characteristic for a habitat type or a group of habitat types we used the Indicator Value (IndVal) method (Dufrêne and Legendre 1997). Indicator species can be defined as species found mostly in a certain habitat type and present in the majority of sites of that type. To incorporate these two criteria the IndVal index multiplies the relative abundance of a species in a habitat (mean abundance in a habitat divided by the sum of mean abundances in all habitats) with the frequency of occurrence in that habitat. The index is calculated for each habitat and the IndVal of a species corresponds to the largest IndVal value observed over the

different habitats. The index is maximum (100%) when the individuals of a species are only observed in one habitat type and in all sites of that habitat. The IndVal of a species can be calculated for each level of a hierarchic site typology. The level where the species reaches its maximum IndVal index can be considered as the 'best' clustering level for that species (Dufrêne and Legendre 1997). This procedure distinguishes between generalist species (maximum IndVal at a higher cluster level) and stenotopic species (maximum at lower levels). In our Indval analysis we used a hierarchic site typology based on the habitat types we had distinguished a priori. The clustering of groups in the hierarchy was based on their stand structure similarity. The first level groups all sites and permits identification of species that have higher IndVals for all samples than for any sample subset (generalist species). The second level separates open habitats from forested habitats, in the next steps the open habitats are separated in herbaceous and shrubby habitats, the forested habitats in pine stands and deciduous woodlands and so on (see Fig. 3 for separations in further steps). This classification tests if species are characteristic for a specific clustering of predefined habitat types. The statistical significance of the index was estimated at each level of the hierarchy by a random reallocation procedure of plots among plot groups based on 999 permutations (Dufrêne and Legendre 1997). Species present with <5 individuals were excluded from analysis.

Canonical Correspondence Analysis (CCA) was used to relate environmental variables to species assemblages (Ter Braak 1986; Palmer 1993). CCA is an ordination technique for multi-variate direct gradient analysis in which the ordination axes of a Correspondence Analysis (CA) are constrained to be linear combinations of the environmental variables (Ter Braak 1986). The % of variance in the species data set that is 'explained' by the environmental variables can be calculated by dividing the inertia of the canonical axes by the total inertia of the CA and this % represents an overall method of CCA fit. We tested the explanatory effects of three sets of variables in separate CCA analyses: the seven habitat types, the 14 plot variables and the 13 landscape variables (see Table 1). For each set significant variables were selected in a forward stepwise procedure based on the additional variation explained by each variable (P < 0.05 for inclusion). Next, we combined the selected variables in one CCA model and calculated the variation explained independently and jointly by the different sets of variables by performing several partial CCA analyses (Borcard et al. 1992; Cushman and McGarigal 2002). In a partial CCA the pure effect of a variable or a group of variables is calculated after eliminating the variance due to other variables (the covariables). The different parts of the variation partitioning were calculated following the formulas given by Cushman and McGarigal (2002). Finally, we determined for each variable if it explained a significant part of variation when the variables in the two other subsets were used as covariables. Significance of the additional effect of each variable during the forward selection procedure and of the different (partial) CCA models was tested with 999 Monte Carlo permutations. In CA and (partial) CCA rare species represented by <5 individuals were omitted (Jongman et al. 1995). ANOVA and GLM were calculated with STATISTICA 7.1, CA and (partial) CCA with CANOCO 4.5 software.

#### Results

Species richness and abundance of individual species

A total of 2,750 individuals belonging to 44 species were recorded in the 83 plots (see Table 2). The number of species varied from 2 to 22 per plot and the number of individuals from 2 to 154. Most abundant species, with more than 200 individuals, were in decreasing

Species	Total abund- ance	FB	PP1	PP2	PP3	PP4	PP5	DW	European threat status	National threat status	Forest species
Pyronia tithonus	469	11.5	1.4	2.2	9.7	6.7	5.6	3.3			
Maniola jurtina	239	7.8	0.3	0.3	0.7	2.5	0.5	5.2	CD	m	
Coenonympha oedippus	236	5.3	5.5	2.2	3.6	0.6	4.5	0.2	CR	Т	
Lycaena phlaeas	227	6.4	0.7	1.6	0.9	4.7	4.1	1.1			
Minois dryas	218	4.8	3.9	3.6	3.6	1.7	2.6	0.1		Т	
Coenonympha pamphilus	217	5.8	3.1	4.5	3.5	0.8	1.6	0.5			
Cupido argiades	202	11.3	1.7	1.5	2.8	0.1	0.5	0.1			
Pararge aegeria	181	0.0	0.0	0.0	0.1	0.3	0.1	9.3			Forest
Euphydryas aurinia	118	7.2	0.7	0.2	0.6	0.1	0.5	0.6	VU		
Gonepteryx rhamni	95	1.3	1.5	0.8	1.2	1.2	0.6	1.3			Forest
Hipparchia statilinus	79	3.2	0.3	1.2	0.3	0.3	1.7	0.1		Т	
Heteropterus morpheus	56	1.9	0.6	0.7	1.0	0.5	0.4	0.1		Т	
Aricia agestis	46	0.4	0.3	0.0	0.0	0.7	0.9	1.1			
Ochlodes venata	46	0.2	0.2	0.1	1.0	0.5	1.2	0.6			
Coenonympha arcania	45	0.0	0.1	0.0	0.7	0.6	0.9	1.1			
Polyommatus icarus	43	2.7	0.0	0.3	0.1	0.1	0.2	0.2			
Thymelicus lineola	37	2.8	0.0	0.0	0.1	0.0	0.0	0.2			
Hipparchia semele	30	0.2	0.2	0.0	0.1	0.9	0.2	0.7		Т	
Melitaea cinxia	17	1.4	0.0	0.0	0.0	0.0	0.0	0.0			
Colias croceus	16	1.2	0.0	0.2	0.0	0.0	0.0	0.0			
Limenitis reducta	15	0.4	0.1	0.2	0.0	0.0	0.0	0.4			Forest
Boloria selene	12	0.8	0.0	0.0	0.0	0.0	0.0	0.2		Т	
Argynnis paphia	11	0.0	0.0	0.1	0.0	0.0	0.0	0.5			Forest
Lycaena alciphron	11	0.1	0.1	0.1	0.0	0.0	0.5	0.1			
Brintesia circe	10	0.3	0.1	0.1	0.0	0.0	0.2	0.1			
Thymelicus sylvestris	8	0.5	0.0	0.0	0.2	0.0	0.0	0.0			
Callophrys rubi	7	0.2	0.0	0.1	0.2	0.2	0.0	0.0			
Melitaea athalia	7	0.1	0.0	0.0	0.0	0.0	0.3	0.2			
Pyrgus malvoides	7	0.5	0.1	0.0	0.0	0.0	0.0	0.0			
Satyrium ilicis	7	0.1	0.0	0.1	0.1	0.0	0.4	0.0			Forest
Arethusana arethusa	5	0.3	0.0	0.0	0.1	0.0	0.0	0.0		Т	
Neozephyrus quercus	5	0.0	0.0	0.0	0.0	0.0	0.0	0.3			Forest
Pieris rapae	5	0.1	0.0	0.1	0.0	0.0	0.0	0.2			
Lampides boeticus	4	0.1	0.2	0.0	0.1	0.0	0.0	0.0			
Erynnis tages	3	0.2	0.0	0.0	0.0	0.0	0.0	0.1			
Pieris napi	3	0.0	0.0	0.0	0.0	0.0	0.0	0.2			
Vanessa atalanta	3	0.0	0.0	0.0	0.0	0.0	0.0	0.2			
Boloria dia	2	0.2	0.0	0.0	0.0	0.0	0.0	0.0			
Celastrina argiolus	2	0.0	0.0	0.0	0.0	0.1	0.0	0.1			
Iphiclides podalirius	2	0.2	0.0	0.0	0.0	0.0	0.0	0.0			
Lasiommata megera	1	0.0	0.0	0.0	0.0	0.0	0.0	0.1			
Limenitis camilla	1	0.0	0.0	0.0	0.0	0.0	0.0	0.1			Forest
Pieris brassicae	1	0.0	0.0	0.0	0.0	0.0	0.0	0.1			
Vanessa cardui	1	0.0	0.0	0.0	0.0	0.0	0.0	0.1			

 Table 2
 List of butterfly species observed in the 83 plots, the total number of observed individuals per species, the mean number of individuals per habitat type, their European and national threat status and their classification as a forest species

Species are ordered by their total abundance. Abbreviations for habitat types: *FB* firebreaks, *PP1* herbaceous clearcuts, *PP2* shrubby clearcuts, *PP3* young pine stands (<7 m), *PP4* mid-class pine stands (7–15 m), *PP5* older pine stands (>15 m), *DW* deciduous woodlands. European threat status: *CR* critically endangered, *VU* vulnerable, National threat status (in France): *T* threatened

order Pyronia tithonus, Maniola jurtina, Coenonympha oedippus, Lycaena phlaeas, Minois dryas, C. pamphilus and Cupido argiades. Among the 44 recorded species seven can be characterized as typical forest-species: Gonepteryx rhamni, Neozephyrus quercus, Satyrium ilicis, Limenitis camilla, Limenitis reducta, Argynnis paphia and Pararge aegeria (Ebert and Rennwald 1991). Only two of them (P. aegeria and G. rhamni) were relatively abundant (181 and 95 individuals, respectively), for all others <15 individuals were observed. Two species are listed as threatened in Europe: C. oedippus (critically endangered) and Euphydryas aurinia (vulnerable) (Van Swaay and Warren 1999). With 236 and 118 individuals, respectively, these two species belong to the ten most common species observed in this study. Seven species can be considered as nationally threatened: Heteropterus morpheus, Boloria selene, Arethusana arethusa, Hipparchia statilinus, Hipparchia semele, M. dryas and C. oedippus (Table 2).

The mean species richness was significantly higher in the firebreaks than in all other habitat types (ANOVA, N = 83, F = 5.32, P < 0.001, Fig. 2). The total abundance showed the same pattern with a significantly higher mean number of individuals in the firebreaks  $[81.6 \pm 11.7 \text{ individuals/plot (mean } \pm \text{SE})]$  than in the other habitats (mean abundance varying from  $20.2 \pm 4.4$  to  $30.7 \pm 7.9$ ; ANOVA, N = 83, F = 5.53 and P < 0.001). The deciduous woodland patches had the highest mean richness of typical forest species (Fig. 2), which was significantly higher than that of the other habitat types (ANOVA, N = 83, F = 4.37 and P < 0.001), with the exception of the firebreaks. The number of threatened species was significantly higher in the firebreaks than in the deciduous woodland patches (ANOVA, N = 83, F = 3.09, P = 0.009, Fig. 2), but did not differ significantly from that in the pine stands. Forward selection of variables in GLM resulted in the selection of both habitat and landscape variables that explained 47.1% of species richness variation (F = 7.22, P < 0.001). The mean species richness per plot depended on the habitat type and was positively correlated with the availability of nectar in the understorey vegetation of the plot and the % cover of young pine stands (quadratic term) in the surrounding landscape and negatively correlated with the % herbaceous clearcuts in the landscape. In a forward selection procedure of these variables and the three interactions between habitat and the continuous variables no interaction terms were selected.



**Fig. 2** Mean species richness ( $\pm$ standard error) per habitat type of all butterfly species, forest species and threatened species. *Bars* of the same colour sharing no *letter* are significantly different (Tukey test, P < 0.05). Habitat types: *FB* firebreaks, *PP1* herbaceous clearcuts, *PP2* shrubby clearcuts, *PP3* young pine stands, *PP4* mid-class pine stands, *PP5* older pine stands, *DW* deciduous woodlands



**Fig. 3** Indicator species for the *different levels* of the hierarchic site typology. Species are only mentioned at the level where they have their maximum, significant indicator value (indicator value between parentheses)

Among the 33 species analysed 18 had a significant IndVal index at one or several levels of the typology and eight species had their maximum value at the first level regrouping all plots (Fig. 3). Three species had their maximum value in deciduous woodlands and ten species in firebreaks. Three species were characteristic for open sites and two for forested sites, but no species were characteristic for pine stands at lower levels of the hierarchic typology (Fig. 3).

#### Composition of species communities

The eigenvalues of the first two axes of a CA on a 33 species  $\times$  83 plots matrix, were, respectively, 0.62 and 0.45; further axes had an eigenvalue of 0.29 or less (total inertia of CA was 3.83). The first axis separated the deciduous woodland plots from the other plots; the second axis did not show a clear separation between the different pine stands, clearcuts and firebreaks (Fig. 4).

In the CCA with seven habitat types as environmental variables, three significant variables were retained (Table 3), which explained together 17.4% of total CA inertia (P = 0.001). The first axis (eigenvalue 0.44) was correlated with the deciduous woodland habitat; the second axis was mainly related to firebreaks and had an eigenvalue of only 0.16.

In the CCA with 14 plot variables as environmental variables, six significant variables (Table 3) were retained by the forward selection procedure, which explained 27.5% of total variation (P = 0.001). The eigenvalues of the first two axes were 0.45 and 0.33, respectively.



Axis 1 (eigenvalue 0.62)

**Fig. 4** Correspondence Analysis ordination of the 83 inventoried plots (axes 1 and 2). The position of the name of each habitat type indicates the mean position of plots belonging to that habitat type. *Different symbols* indicate the position of the different plot types. Plot types: *FB* firebreaks (*white circles*), *PP1* herbaceous clearcuts (*white diamonds*), *PP2* shrubby clearcuts (*black diamonds*), *PP3* young pine stands (*white triangles*), *PP4* mid-class pine stands (*grey, inversed triangles*), *PP5* older pine stands (*black triangles*), *DW* deciduous woodlands (*black squares*)

The second axis opposed sites dominated by *M. caerulea* to sites with a high cover of herbaceous dicotyledons and *U. europaeus*, and a higher nectar abundance.

Forward selection procedures of landscape variables in CCA resulted in the selection of four significant landscape variables (Table 3), which explained 18.0% of total variation (P = 0.001). The first two CCA axes had eigenvalues of 0.28 and 0.19. The first axis was correlated with the amount of deciduous woodlands in the landscape, the second axis opposed landscapes with a high cover of firebreaks to landscapes with a high cover of shrubby clearcuts.

Canonical Correspondence Analysis with these 13 selected variables combined explained 41.7% of species variation (P = 0.001). Examination of the CCA plot (Fig. 5) shows that the first axis opposed forest species such as *P. aegeria*, *A. paphia*, *N. quercus* and *L. reducta* which were associated with deciduous woodlands (DW and %DW) to species found in pine stands and firebreaks (e.g. *C. oedippus*, *M. dryas* and *C. argiades*). Best correlated with the second axis were the percentage cover of *M. caerulea* (%Mcae) in the plot and the percentage shrubby clearcuts (%PP2) in the landscape on the positive side of this axis and the percentage cover of herbaceous dicotyledons (%Dicots), of *U. europaeus* (%Ueur), the flower abundance (Nectar) and the percentage of firebreaks in the landscape (%FB) on the negative side. The second axis is therefore mainly correlated with the vegetation composition in pine stands and firebreaks. Species such as *C. oedippus*, *H. morpheus* and *M. dryas* were found in open pine stands and firebreaks with a high cover of *M. caerulea* and located in landscapes with a high cover of clearcuts. Species at the opposite end of the

Variable	Additional % explained in forward selection	% Variation explained per variable set	% Explained when two other subsets used as covariables	Р
Habitat type		17.4		
DW (deciduous woodlands)	11.2		1.9	0.002
FB (firebreaks)	4.2		2.2	0.001
PP4 (mid-class pine stands)	1.9		1.0	ns
Plot variables		27.5		
%Mcae (% Molinia caerulea)	9.3		5.1	0.001
%Oth-shrub (% other shrubs)	8.0		1.8	0.018
Nectar	4.0		1.9	0.007
%Ueur (% Ulex europaeus)	2.4		2.7	0.001
%Paqu (% Pteridium aquilinum)	2.1		2.1	0.006
%Dicots (% herbaceous dicotyledons)	1.7		2.5	0.002
Landscape variables		18.0		
%DW (% deciduous/mixed woodlands)	6.8		2.4	0.003
%PP2 (% shrubby clearcuts)	5.1		1.0	ns
%FB (% firebreaks)	3.9		1.6	0.025
ED (edge density)	2.2		2.3	0.002

**Table 3** Selected variables per variable subset in order of selection during the stepwise selection procedure in CCA, the additional variance explained by each variable at the time of inclusion, the % variance explained by each variable subset and the % variation explained by each variable using the variables in the two other subsets as covariables (partial CCA) and the associated probability (*P*)

*ns* Not significant (P > 0.05)

second axis were more abundant in stands with *U. europaeus* or were found in plots (mostly firebreaks) with higher % dicotyledons. The third axis (eigenvalue 0.21) opposed the firebreaks (axis positively correlated with FB, %FB and ED) to the pine stands (correlated with PP4, %Ueur). Positively associated with this axis were for example *E. aurinia*, *T. lineola* and *Melitaea cinxia*.

The decomposition of the variation in independent and confounded effects of the three variable subsets is shown in Fig. 6. The pure effects of plot variables, habitat-types and landscape features accounted for 12.8, 5.1 and 6.7% of variation, respectively (all significant P = 0.001). All variable subsets provided an independent, additional contribution to the explained variation, but the independent effect of plot variables was the most important. Analysis of the independent effect of each variable, after controlling for the variation explained by the other two subsets, revealed a significant effect of most variables (Table 3). Only the habitat type mid-class pines (PP4) and the landscape variable % shrubby clearcuts (%PP2) were no longer significant.

### Discussion

Conserving biodiversity in plantation forests is becoming increasingly necessary because the area of planted forests continues to increase worldwide. In Europe, for example, the area of plantation forests augmented from 8.6 to 10.5 million hectares in the period of 1990–2005 (FAO 2007). One conservation option is to improve biodiversity within stands by adapting stand management (Kerr 1999; Lindenmayer and Hobbs 2004). However,



Axis 1 (eigenvalue 0.51)

Fig. 5 Canonical Correspondence Analysis ordination biplot (axes 1 and 2) with plot, habitat and landscape variables represented by *arrows* and butterfly species by *diamonds*. Names of butterfly species are indicated only for species that are explained for more than 25% by the CCA. For legend of environmental variables see Table 1. Butterfly species: Apap Argynnis paphia, Bcir Brintesia circe, Carc Coenonympha arcania, Carg Cupido argiades, Ccro Colias croceus, Coed Coenonympha oedipuus, Cpam Coenonympha pamphilus, Eaur Euphydryas aurinia, Grha Gonepteryx rhanni, Hmor Heteropterus morpheus, Hsta Hipparchia statilinus, Lphl Lycaena phlaeas, Lred Limenitis reducta, Mcin Melitaea cinxia, Mdry Minois dryas, Mjur Maniola jurtina, Nque Neozephyrus quercus, Paeg Pararge aegeria, Pica Polyommatus icarus, Pmal Pyrgus malvoides, Prap Pieris rapae, Ptit Pyronia tithonus, Tlin Thymelicus lineola

large-scale intensive stand management may impede the presence of many species, and the role of semi-natural habitat remnants within plantation landscapes may be essential (Lindenmayer and Hobbs 2004; Fischer et al. 2006).

Importance of semi-natural habitats in plantation landscapes

This study confirms the importance of semi-natural habitats for butterflies in pine plantation landscapes. Both herbaceous firebreaks and deciduous woodlands were characterized by the presence of butterfly species not or rarely found in pine stands. Firebreaks were more species-rich than the other habitat types and several butterfly species were almost exclusively found in firebreaks (e.g. *M. cinxia, P. icarus, T. lineola* and *E. aurinia*). The higher species richness of firebreaks might be largely attributable to their more diverse herbaceous vegetation, providing a greater and more diverse flower abundance (nectar) and hostplants



**Fig. 6** Decomposition of the variance in butterfly community structure explained by plot variables, habitat type and landscape variables in independent and confounded effects using several partial CCA's. Parts A, B and C represent the independent effects of plot, habitat and landscape variables, respectively, parts D, E, F and G indicate the joint effects. Significance levels are based on 999 Monte Carlo permutations: \*\*\**P* = 0.001. The area of each *circle* is proportional to the variance explained by that group of variables. The total variance explained by the three sets of variables is 41.7%

not or rarely found in forest stands, such as *Plantago lanceolata* and herbaceous Fabaceae. Micro-climate and especially high insolation alone does not seem to explain differences in butterfly richness since open areas such as clearcuts had a lower species richness than firebreaks.

Deciduous woodlands were also characterized by the presence of several characteristic species. P. aegeria was very typical for deciduous plots and together with A. paphia and N. quercus formed a group of species associated with deciduous woodlands. Although our study was performed in a well-forested region the number and abundance of typical forest species was low and these species were mainly present in deciduous woodland patches. This study confirms thereby the fact that coniferous forests do not represent a suitable habitat type for most forest butterflies (Ebert and Rennwald 1991). Deciduous woodlands, on the contrary, provide hostplants for butterfly species feeding on broadleaved trees (e.g. Quercus sp. for N. quercus), have a more diverse herbaceous vegetation (with Viola sp. for A. paphia), offer a more varied structure for mate finding behaviour and probably provide more spatial variation in micro-climate than pine plantations. These differences between deciduous woodlands and pine stands are due to their different tree composition but also to their different management. Plantation stands are typically characterized by a uniform and intensive management, whereas management of deciduous woodlands is more variable in time and space allowing a greater structural diversity.

Although the butterfly communities of pine stands seem to resemble to those of firebreaks (Fig. 4), they harbour only half the number of species compared to firebreaks and no characteristic species. Apparently butterfly communities of pine stands represent an impoverished version of firebreak communities. The different successional stages of pine stands show no clear differences in butterfly community composition but several species (e.g. *C. oedippus*, *M. dryas* and *O. venata*) were less abundant in mid-class pines than in young and older pine stands because of higher canopy cover.

#### Relative effects of plot and landscape variables

Comparing the independent effect of plot, habitat and landscape variables revealed clearly the important effect of understorey vegetation on butterfly communities. Butterfly species show preferences for certain vegetation types and specific growing conditions of their hostplants and it is therefore logical that local factors are the most important for this taxonomic group (Thomas et al. 2001). The composition of understorey vegetation explained differences in butterfly community structure that were not explained by habitat type. The most important plot variable was the M. caerulea cover. This grass species dominates in sites with a high soil moisture and the second axis of the CCA is explained by a gradient in vegetation composition related to soil moisture. A group of species (C. oedippus, H. morpheus and M. dryas) was positively associated with sites dominated by M. caerulea, their main hostplant in the studied region. Cover by U. europaeus and by dicotyledons were best correlated with the opposite side of the second axis. The U. europaeus cover is however not directly related to the butterfly species (as hostplant or nectar plant) but moderate cover by this shrub characterizes drier pine stands, with butterfly species such as L. phlaeas and H. statilinus. Cover by dicotyledons, that may be nectar- or hostplants for several species, was higher in herbaceous firebreaks than in other habitat types and was associated with the presence of L. phlaeas, P. icarus, T. lineola, M. cinxia and C. croceus.

The proportion of explained variance in CCA is low, but this is a common feature in multi-variate analysis of ecological communities (e.g. Jeanneret et al. 2003b; Titeux et al. 2004; Aviron et al. 2005; Schweiger et al. 2005). The aim of CCA is to identify important environmental variables and even low percentages might be informative (Ter Braak 1986). By introducing more environmental variables, the proportion of explained variance will necessarily increase, but for a meaningful analysis the number of environmental variables should not be more than c.10% of the number of plots (Lebreton et al. 1988). A part of the unexplained variance in our study may be due to variables that were not measured, such as intra-plot variation in vegetation composition and canopy cover, or differences in management regimes.

Landscape attributes explained an independent part both in partial CCA analysis and in GLM modelling, thereby confirming that different organization levels should be considered when explaining species abundance patterns (Wiens 1989). In partial CCA the only significant landscape composition variables were the percentage cover of deciduous woodlands and firebreaks. Since the surveyed plot was included in the calculation of landscape metrics, this effect of habitat amount can either be an effect of habitat patch size or an effect of landscape supplementation, i.e. the use of several, similar patches within a landscape (Dunning et al. 1992). Larger patches or more patches tend to supply a greater diversity of environmental conditions and support more species. A positive effect of woodland area within 1 km on butterfly diversity was also demonstrated by Shreeve and Mason (1980) and by Baz and Garcia-Boyero (1995). Edge density explained also a significant, independent part of community variation. A high ED may be positive for species using herbaceous strips along stand edges or for multi-habitat species (habitat complementation) (Duelli 1997). A positive or negative effect of increased ED and the associated fragmentation will however also depend on the observed species and the studied landscape type. Butterfly species characteristic for large woodlands are probably sensitive to fragmentation, but these species are very rare or absent in our study area.

The relatively low percentage of variation explained by landscape variables (18.0%, independent effect 6.7%) can be due to several factors. Possible landscape effects may be masked by the important variation in plot types (different stand types and understorey

vegetation) compared to the variation in landscapes. To study landscape effects more accurately, we suggest the selection of the same plot type in a range of landscapes differing in composition and structure (Bergman et al. 2004). This may also permit to separate effects of landscape composition and fragmentation. Analysing the landscape at larger scales might also reveal additional effects. However, butterfly studies that examined the effect of different buffer sizes show contradictory results (Weibull et al. 2000; Krauss et al. 2003; Bergman et al. 2004). Differences in landscape types and their associated key factors may be responsible for these contradictions. Finally, landscape analysis also depends on the accuracy and choice of the patch typology. A patch typology based on a combination of stand type and understorey vegetation might have better described difference in habitat quality for the studied butterfly species. It would also have allowed the establishment of species (or guild) specific habitat maps (Li and Wu 2004) and to reveal more or less isolated habitat patches that do not appear in the current typology. Such a typology can however not be based exclusively on aerial photos.

The presence of threatened species

Threatened butterfly species, such as C. oedippus, M. dryas, H. morpheus and H. statilinus, were observed both in pine stands and firebreaks, but they were more abundant in the latter. Firebreaks can therefore function as an essential reservoir/source in the landscape. Wahlberg et al. (2002) demonstrated in Finland that the continued presence of meadows was necessary for the survival of *E. aurinia*, a species occurring both in meadows and in clearcuts. Firebreaks may play the same role in our dynamic landscape. It seems however likely that the large areas of pine stands play a role as alternative habitat and refugium for species occurring both in firebreaks and pine stands, that they improve landscape connectivity and that they buffer the semi-natural habitats (Aberg et al. 1995; Lindenmayer and Franklin 2002; Lindenmayer and Hobbs 2004; Aune et al. 2005). The presence of threatened butterfly species in a landscape dominated by pine plantations argues for their potential conservation value. The 'Landes de Gascogne' forest is characterized by oligotrophic habitat conditions occurring over large areas; conditions that tend to disappear under agricultural and urbanization pressure elsewhere. Typical butterflies of nutrient poor habitats are therefore threatened in several European countries, but are still occurring regularly in the studied region.

Nevertheless, this study does not show the possible negative effects of pine plantations on butterfly species present before the massive afforestation carried out in the 1850s, when the landscape was dominated by large, mainly wet heathlands. It seems likely that several butterfly species might have seriously declined as a consequence of the huge habitat transformation. Some of these species such as *Maculinea alcon*, *Plebejus argus* and *P. idas* are very rare in the Landes de Gascogne forest and survive nowadays in isolated areas of heathland vegetation (military zones and some firebreaks). The nowadays relatively rich firebreaks may thus represent an impoverished version of the original species pool of large heathlands.

How to improve butterfly diversity in plantation forests

The enhancement of biodiversity within plantation forests should include measures to promote woodland habitats for forest species, but should also include measures to retain rare or specialist species of pre-planting habitats (Oxbrough et al. 2006). In the study area, forest butterflies were mainly found in deciduous woodlands and their presence was

correlated with the extent of these woodlands, suggesting that habitat thresholds may exist. Bergman et al. (2004), for example, showed a sharp increase in occupancy probability for several butterfly species when the cover of deciduous forest/semi-natural grassland was higher than values between 2 to 12%. Our analyses do not provide an estimation of how much deciduous woodland is needed to conserve characteristic species. Species such as *A. paphia* and *L. reducta* were more abundant in larger deciduous woodlands, but these were also the more varied and humid riparian forests so that the effect of habitat quality and quantity are difficult to separate.

In our study area rare or threatened species were most abundant in firebreaks and the conservation and management of open spaces can be considered as an important technique of nature conservation within plantations (Gittings et al. 2006). In the Republic of Ireland, for example, all grant-aided afforestation should contain 5–10% open space (Gittings et al. 2006). Recommendations for an optimal width of firebreaks for butterflies are difficult to give, based on the results of our study. For the 12 surveyed firebreaks, with a width varying from 15–90 m, no significant relation between species richness and firebreak width could be demonstrated. Oxbrough et al. (2006) showed for ground dwelling spiders that open spaces of <15 m wide did not support an open spider fauna due to the influence of the tree canopy. This suggests that firebreaks of 15 m may already be large enough to harbour a fauna of open spaces. The minimal width depends also on the neighbourhood of the firebreak (e.g. bordered by a high-forest stand or by a road) and its orientation, which will affect the light conditions (Ferris and Carter 2000). The actual management of firebreaks in the studied area is very variable (varying from annual mowing to about once every 8 years) and this variation is partly responsible for their diverse butterfly composition. The vegetation diversity within firebreaks can be increased and the temporarily negative impact of management reduced by a more varied management regime within firebreaks. For wide forest rides Ferris and Carter (2000) recommend a system with three different intervention frequencies. Comparable systems could be used in firebreaks, creating a more natural forest edge and by maintaining the largest part of the firebreak as herbaceous vegetation with a varied structure and composition.

The fact that composition of understorey vegetation explained the largest part of butterfly community composition implies that management within pine stands (e.g. removal of shrub layer, thinning, soil preparation before planting) and other habitats will directly affect butterfly diversity. In the Landes de Gascogne forest butterfly composition was most strongly influenced by differences in vegetation composition related to soil humidity. Maintaining existing humidity gradients and conserving the wet areas in the landscape are therefore decisive measures in conserving butterfly diversity in all habitat types, especially because silvicultural and agricultural practices tend to decrease soil moisture. Within-stand variation in canopy cover or understorey vegetation was not measured in this study, but field observations showed that butterflies were more abundant in gaps or in parts of stands with a lower canopy cover. Maintaining this variation within pine stands will therefore be profitable for butterflies. The positive effect of more open pine stands on butterfly species richness and composition has also been demonstrated in *Pinus ponderosa* (Waltz and Covington 2004) and Pinus edulis/Juniperus monosperma forests (Kleintjes et al. 2004). This effect was attributed to a higher light intensity (Waltz and Covington 2004) or to an increase in understorey cover (Kleintjes et al. 2004). Field observations also suggested the importance of variation in understorey vegetation composition. Most of the observed butterflies depend on herbaceous plants as hostplants, but some use shrubs or trees. Stand management trying to create a varied understorey vegetation dominated by herbs but with presence of some shrubs or deciduous trees seems most beneficial for butterflies. A more

#### Conclusion

Three factors appear to have an important effect on butterfly richness and community composition in the studied pine plantation landscape: the presence of deciduous woodlands, the presence of firebreaks and the variation in understorey vegetation, related to both soil moisture and management practices. Explanatory factors measured at the local scale (plot vegetation and habitat type) explained the largest part of community variation, but landscape factors explained an additional, independent part. This confirms the importance of multiscale analyses to explain patterns of biodiversity. Our study demonstrates the importance of interstitial habitats at the landscape level and shows that stand management can influence butterfly diversity, mainly by maintaining a diverse herbaceous layer.

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#### References

- Aberg J, Jansson G, Swenson JE, Angelstam P (1995) The effect of matrix on the occurrence of hazel grouse (*Bonasa bonasia*) in isolated habitat fragments. Oecologia 103:265–269
- Anthes N, Fartmann T, Hermann G, Kaule G (2003) Combining larval habitat quality and metapopulation structure—the key for successful management of pre-alpine *Euphydryas aurinia* colonies. J Insect Conserv 7:175–185
- Aune K, Jonsson BG, Moen J (2005) Isolation and edge effects among woodland key habitats in Sweden: is forest policy promoting fragmentation? Biol Conserv 124:89–95
- Aviron S, Burel F, Baudry J, Schermann N (2005) Carabid assemblages in agricultural landscapes: impacts of habitat features, landscape context at different spatial scales and farming intensity. Agric Ecosyst Environ 108:205–217
- Baguette M, Petit S, Queva F (2000) Population spatial structure and migration of three butterfly species within the same habitat network: consequences for conservation. J Appl Ecol 37:100–108
- Barbaro L, Pontcharraud L, Vetillard F, Guyon D, Jactel H (2005) Comparative responses of bird, carabid, and spider assemblages to stand and landscape diversity in maritime pine plantation forests. Ecoscience 12:110–121
- Baz A, Garcia-Boyero A (1995) The effects of forest fragmentation on butterfly communities in central Spain. J Biogeogr 22:129–140
- Bergman KO, Askling J, Ekberg O, Ignell H, Wahlman H, Milberg P (2004) Landscape effects on butterfly assemblages in an agricultural region. Ecography 27:619–628
- Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological variation. Ecology 73:1045–1055
- Carey AB (2003) Biocomplexity and restoration of biodiversity in temperate coniferous forest: inducing spatial heterogeneity with variable-density thinning. Forestry 76:127–136
- Carnus JM, Parrotta J, Brockerhoff E, Arbez M, Jactel H, Kremer A, Lamb D, O'Hara K, Walters B (2006) Planted forests and biodiversity. J For 104:65–77
- Clausen HD, Holbeck HB, Reddersen J (2001) Factors influencing abundance of butterflies and burnet moths in the uncultivated habitats of an organic farm in Denmark. Biol Conserv 98:167–178
- Cushman SA, McGarigal K (2002) Hierarchical, multi-scale decomposition of species-environment relationships. Landsc Ecol 17:637–646
- Debinski DM, Ray C, Saveraid EH (2001) Species diversity and the scale of the landscape mosaic: do scales of movement and patch size affect diversity? Biol Conserv 98:179–190

Dennis RLH (1992) The ecology of butterflies in Britain. Oxford University Press, Oxford, UK

- Dennis RLH, Shreeve TG, Van Dyck H (2003) Towards a functional resource-based concept for habitat: a butterfly biology viewpoint. Oikos 102:417–426
- Dennis RLH, Shreeve TG, Van Dyck H (2006) Habitats and resources: the need for a resource-based definition to conserve butterflies. Biodivers Conserv 15:1943–1966
- Duelli P (1997) Biodiversity evaluation in agricultural landscapes: an approach at two different scales. Agric Ecosyst Environ 62:81–91
- Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol Monogr 67:345–366
- Dunning JB, Danielson BJ, Pulliam HR (1992) Ecological processes that affect populations in complex landscapes. Oikos 65:169–175
- Ebert G, Rennwald E (1991) Die schmetterlinge Baden-Württembergs. Band 1 Tagfalter I. Verlag Eugen Ulmer, Stuttgart
- Fahrig L (2003) Effects of habitat fragmenation on biodiversity. Annu Rev Ecol Evol Syst 34:487-515
- FAO (2007) State of the Word's Forests 2007. Food and Agriculture Organisation of the United Nations, Rome
- Ferris R, Carter C (2000) Managing rides, roadsides and edge habitats in lowland forests. For Comm Bull 123:1–78
- Fischer J, Lindenmayer DB, Manning AD (2006) Biodiversity, ecosystem function, and resilience: ten guiding principles for commodity production landscapes. Front Ecol Environ 4:80–86
- Gittings T, O'Halloran J, Kelly T, Giller PS (2006) The contribution of open spaces to the maintenance of hoverfly (Diptera, Syrphidae) biodiversity in Irish plantation forests. For Ecol Manage 237:290–300
- Hanski I (1999) Metapopulation ecology. Oxford University Press, New York
- Hartley MJ (2002) Rationale and methods for conserving biodiversity in plantation forests. For Ecol Manage 155:81–95
- Jeanneret P, Schupbach B, Luka H (2003a) Quantifying the impact of landscape and habitat features on biodiversity in cultivated landscapes. Agric Ecosyst Environ 98:311–320
- Jeanneret P, Schupbach B, Pfiffner L, Herzog F, Walter T (2003b) The Swiss agri-environmental programme and its effects on selected biodiversity indicators. J Nat Conserv 11:213–220
- Jongman RGH, Ter Braak CJF, Van Tongeren OFR (1995) Data analysis in community and landscape ecology. Cambridge University Press, Cambridge
- Karsholt O, Razowski J (1996) The Lepidoptera of Europe. A distributional checklist. Apollo Books, Stenstrup
- Kerr G (1999) The use of silvicultural systems to enhance the biological diversity of plantation forests in Britain. Forestry 72:191–205
- Kleintjes PK, Jacobs BF, Fettig SM (2004) Initial response of butterflies to an overstory reduction and slash mulching treatment of a degraded pinon-juniper woodland. Restor Ecol 12:231–238
- Krauss J, Steffan-Dewenter I, Tscharntke T (2003) How does landscape context contribute to effects of habitat fragmentation on diversity and population density of butterflies? J Biogeogr 30:889–900
- Lafranchis T (2000) Les papillons de jour en France, Belgique et Luxembourg et leurs chenilles. Biotope, Mèze (France)
- Lebreton JD, Chessel D, Prodon R, Yoccoz N (1988) L'analyse des relations espèce-milieu par l'analyse canonique des correspondances. I.-Variables de milieu qualitatives. Acta Oecol 9:53–67
- Li HB, Wu JG (2004) Use and misuse of landscape indices. Landsc Ecol 19:389–399
- Lindenmayer DB, Franklin JF (2002) Conserving forest biodiversity: a comprehensive multiscaled approach. Island Press, Washington, Covelo, London
- Lindenmayer DB, Hobbs RJ (2004) Fauna conservation in Australian plantation forests—a review. Biol Conserv 119:151–168
- Mac Arthur RH, Wilson EO (1967) The theory of island biogeography. Princeton University Press, Princeton, NJ
- Maes D, Van Dyck H (1999) Dagvlinders in vlaanderen—ecologie, verspreiding en behoud. Stichting Leefmilieu/Antwerpen i.s.m. Instituut voor Natuurbehoud en Vlaamse Vlinderwerkgroep, Brussel
- Mazerolle MJ, Villard MA (1999) Patch characteristics and landscape context as predictors of species presence and abundance: A review. Ecoscience 6:117–124
- McGarigal K, Cushman SA, Neel MC, Ene E (2002) FRAGSTATS: spatial pattern analysis program for categorical maps. Computer software program produced by the authors at the University of Massachusetts, Amherst. Available at the following web site: www.umass.edu/landeco/research/fragstats/fragstats.html
- Moore SE, Allen HL (1999) Plantation forestry. In: Hunter MLJ (ed) Maintaining biodiversity in forest ecosystems. Cambridge Univesity Press, New York
- Oxbrough AG, Gittings T, O'Halloran J, Giller PS, Kelly TC (2006) The influence of open space on grounddwelling spider assemblages within plantation forests. For Ecol Manage 237:404–417

- Palmer MW (1993) Putting things in even better order—the advantages of canonical correspondence-analysis. Ecology 74:2215–2230
- Pollard E, Yates TJ (1993) Monitoring butterflies for ecology and conservation. Chapman & Hall, London
- Schneider C, Fry GLA (2001) The influence of landscape grain size on butterfly diversity in grasslands. J Insect Conserv 5:163–171
- Schweiger O, Maelfait JP, Van Wingerden W, Hendrickx F, Billeter R, Speelmans M, Augenstein I, Aukema B, Aviron S, Bailey D, Bukacek R, Burel F, Diekotter T, Dirksen J, Frenzel M, Herzog F, Liira J, Roubalova M, Bugter R (2005) Quantifying the impact of environmental factors on arthropod communities in agricultural landscapes across organizational levels and spatial scales. J Appl Ecol 42: 1129–1139
- Shreeve TG (1992) Adult behaviour. In: Dennis RLH (ed) The ecology of butterflies in Britain. Oxford University Press, Oxford, UK
- Shreeve TG, Mason CF (1980) The number of butterfly species in woodlands. Oecologia 45:414-418
- Söderström B, Svensson B, Vessby K, Glimskär A (2001) Plants, insects and birds in semi-natural pastures in relation to habitat and landscape factors. Biodivers Conserv 10:1839–1863
- Stefanescu C, Herrando S, Paramo F (2004) Butterfly species richness in the north-west Mediterranean basin: the role of natural and human-induced factors. J Biogeogr 31:905–915
- Steffan-Dewenter I, Tscharntke T (2000) Butterfly community structure in fragmented habitats. Ecol Lett 3:449–456
- Ter Braak CJF (1986) Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67:1167–1179
- Thomas CD (1995) Ecology and conservation of butterfly metapopulations in the fragmented British landscape. In: Pullin AS (ed) Ecology and conservation of butterflies. Chapman & Hall, London
- Thomas CD, Harrison S (1992) Spatial dynamics of a patchily distributed butterfly species. J Anim Ecol 61:437–446
- Thomas JA, Bourn NAD, Clarke RT, Stewart KE, Simcox DJ, Pearman GS, Curtis R, Goodger B (2001) The quality and isolation of habitat patches both determine where butterflies persist in fragmented landscapes. Proc R Soc Lond B Bio 268:1791–1796
- Timbal T, Maizeret C (1998) Biodiversité végétale et gestion durable de la forêt landaise de pin maritime: bilan et évolution. Rev For Fr 5:403–424
- Titeux N, Dufrene M, Jacob JP, Paquay M, Defourny P (2004) Multivariate analysis of a fine-scale breeding bird atlas using a geographical information system and partial canonical correspondence analysis: environmental and spatial effects. J Biogeogr 31:1841–1856
- Trichet P, Jolivet C, Arrouays D, Loustau D, Bert D, Ranger J (1999) Le maintien de la fertilité des sols forestiers landais dans le cadre de la sylviculture intensive de pin maritime. Revue bibliographique et identification des pistes de recherche. Etude et Gestion des Sols 6:197–214
- Van Swaay CAM, Warren MS (1999) Red data book of European butterflies (Rhopalocera). Council of Europe Publishing, Strasbourg
- Wahlberg N, Klemetti T, Hanski I (2002) Dynamic populations in a dynamic landscape: the metapopulation structure of the marsh fritillary butterfly. Ecography 25:224–232
- Waltz AEM, Covington WW (2004) Ecological restoration treatments increase butterfly richness and abundance: mechanisms of response. Restor Ecol 12:85–96
- Weibull AC, Bengtsson J, Nohlgren E (2000) Diversity of butterflies in the agricultural landscape: the role of farming system and landscape heterogeneity. Ecography 23:743–750
- Wiens JA (1989) Spatial scaling in ecology. Funct Ecol 3:385-397
- Wiens JA (1995) Habitat fragmentation: island v landscape perspectives on bird conservation. Ibis 137:97-104