Conception d'un web service pour la fouille de données de génomique : application à la caractérisation de la myogenèse et de l'adipogenèse

Nicolas Kaspric

Effects of patchy nitrogen inputs and soil nitrogen heterogeneity on grassland structure and function

Nian-Xun Xi

UNIVERSITE BLAISE PASCAL
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THESE

Présentée à l'Université Blaise Pascal
Pour l'obtention du grade de DOCTEUR D'UNIVERSITE
Spécialité: Ecologie

Par
Nian-Xun XI

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Composition du Jury

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Jean-Christophe LATA Maitre de Conférence, Université Pierre et Marie Curie	Rapporteur
Bernard AMIAUD Professeur, Université Lorraine	Examinateur
Valerie LEGUE Professeure, Université Blaise Pascal	Examinateur
Juliette BLOOR Chargé de Recherche, INRA Clermont-Ferrand	Co-encadrante de Thèse
Pascal CARRERE Ingénieur de Recherche, INRA Clermont-Ferrand	Directeur de Thèse

Laboratoire d'accueil : INRA – Unité de Recherche sur l'Ecosystem prairial (UR 874)
5, Chemin de Beaulieu. 63039 CLERMONT FERRAND cedex 2

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'Innovation is not the product of logical thought, even though the final product is tied to a logical structure.'

Albert Einstein (1879-1955)

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XI Nian-Xun

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31/10/2014

Abstract

Grasslands provide a variety of important ecological and economic services worldwide. Improved understanding of grassland structure and function is necessary for the development of sustainable management and maintaining the provision of multiple ecosystem services in a changing environment. However, predicting grassland structure and function is a challenge because grasslands are dynamic, heterogeneous systems. In grazed grasslands, large herbivore activities promote heterogeneity in soil nutrients via excretion, but the effects of patchy nutrient inputs and soil spatial heterogeneity on grassland structure and function remain unclear.

This thesis addresses effects of spatial heterogeneity in soil nitrogen (N) for grassland ecosystem structure and function, with particular emphasis on community responses. A combination of experimental and modelling approaches are used to study impacts of a number of different patch attributes (N form, patch size, patch contrast), as well as possible interactions with rainfall regime and timing of N inputs. We find that patchy N inputs enhance within-plot plant production and biomass variability irrespective of N form, but do not modify whole-plot plant production in the short term. Nevertheless, patchy organic N promotes spatial and temporal asynchrony in plant-soil responses, with implications for longer-term grassland function. Unlike plant production, community structure responds significantly to patchy N inputs, with increased community dominance and a shift in the rank of subordinate species. Contrary to expectations, rainfall quantity does not modify heterogeneity effects on either plant production or community structure. Modelling work shows that heterogeneity effects on field-scale production vary depending on patch size and patch contrast. For a fixed total N input, field-scale grassland production responds positively to patch size, but decreases in high- versus low-patch contrast conditions. Patch size does not interact with patch contrast or timing of N inputs on grassland production. Overall, our results highlight the importance of N heterogeneity for plant and soil processes at different spatial scales, and demonstrate that heterogeneity effects vary depending on patch attributes. Biotic interactions (competition) appear to play a relatively greater role than abiotic factors (chronic rainfall changes) for heterogeneity effects. Impacts of N heterogeneity on plant and soil processes may have significant implications on plant-soil feedbacks involved with the regulation of biogeochemical cycling, and provide useful information for the development of efficient N management strategies.

Key words: Climate change, Community structure, Grassland modelling, Nitrogen, Patch attributes, Production, Plant-soil interactions, Soil Microorganisms, Spatial scale, Spatial variability, Temperate grassland

Mots-Clefs: Azote, Attributs de patches, Changement climatique, Communauté végétale, Echelle spatiale, Interactions plante-sol, Microorganismes du sol, Modèle de production prairial, Prairies tempérées, Variabilité spatiale

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Chapter one

General Introduction

1.1 Context

Developing sustainable agricultural systems is increasingly urgent to satisfy rising demand for food without significantly damaging environmental health in the 21st century (Pretty et al. 2010). On a global scale, the increase in food production has been mainly attributed to intensive agriculture practices, such as increased applications of fertilizer, pesticide, irrigation and agriculture machinery (Tilman 1999, Pretty 2008). However, these types of intensification can cause substantial environmental harm. For example, huge consumption of nitrogen fertilizers promotes nitrogen (N) leaching and gaseous N losses, increasing pollution in aquatic ecosystems and reducing biological diversity in terrestrial ecosystems (Tilman et al. 2002, Lamarque et al. 2011). Changing agricultural policy and socio-economic conditions emphasize the importance of best practices for the management of N and the need to achieve low-input – lower-output systems. In this context, the EU nitrates Directive aims to protect waters against pollution caused by nitrates from agricultural sources, by establishing balanced N fertilization practices and N application limits in vulnerable areas (Sutton et al. 2011). Moreover, there is growing recognition that agroecology can provide a valuable framework for agricultural systems by integrating ecological processes and principles to the design of sustainable management practices (Vandermeer 1995, Gliessman 2000, Wezel et al. 2014).

Enhancing productivity and resource-use efficiency is of particular interest for grazed grasslands, which support grazing animals and livestock production systems world-wide. Grazed grasslands are complex ecosystems that are commonly characterized by intense human managements of grazers/ livestock (such as cattle and sheep) and fertilizer applications (Haynes and Williams 1993). On a global scale, grazed grasslands cover 3.4 billion hectares (McGilloway 2005) and provide key goods and services such as foods, forage, carbon storage, biodiversity and landscape services (White et al. 2000, MEA 2005). Indeed, grasslands are at the heart of current debates on multi-functionality, which aim to reconcile environmental benefits and production services (Carrère et al. 2012). However, grassland systems face threats from changes in land use and increasingly-common extreme weather events associated with climate change (IPCC 2007). Improved understanding of grassland structure and function is critical for the development of sustainable management and maintaining provision of multiple ecosystem services, particularly under global change.

Predicting grassland structure and function in a changing environment is a challenge, because grasslands are dynamic, heterogeneous ecosystems (Jackson and Caldwell 1993, Adler et al. 2001, Dumont et al. 2002). In grazed grasslands, animals contribute to a high spatial heterogeneity in soil nitrogen via excretion, as well promoting spatial heterogeneity in vegetation structure via non-uniform patterns of defoliation (Haynes and Williams 1993,

Marriott and Carrère 1998). Many studies have examined the impacts of defoliation and heterogeneous vegetation intake on plant- and community-level processes (see Milchunas et al. 1988, Semmartin and Oesterheld 2001, Díaz et al. 2007, Mikola et al. 2009 for some examples). By comparison, fewer studies have addressed impacts of patchy nutrient inputs of varying quantity/quality/distribution on plant N uptake and biotic interactions in multispecies grasslands. Previous work shows that soil nutrient heterogeneity may influence ecological processes at individual and population levels. For example, spatial heterogeneity in soil N significantly modifies root foraging and biomass of plant individuals (Campbell et al. 1991, Birch and Hutchings 1994, Einsmann et al. 1999, Wijesinghe and Hutchings 1999), and affects mortality and trait distributions in plant populations (Casper and Cahill 1998, Day et al. 2003a, b). However, relatively few studies have investigated community or ecosystem responses to spatial heterogeneity in soil nutrient or N (Hutchings et al. 2003).

Animal excretion (urine, dung) consists of several N forms, and can promote spatial heterogeneity in soil inorganic and organic N. Numerous studies indicate that both plants and soil microorganisms have the capacity to take up and compete for soil organic N as well as inorganic forms in grassland (Näsholm et al. 2000, Weigelt et al. 2005, Harrison et al. 2008). Ecosystem/ community responses to nutrient heterogeneity may vary depending on the form and accessibility of the nutrient in question, via differences in diffusion or nutrient mineralization rates or plant/soil competition for N. However, interactions between spatial pattern and form of N inputs for plant and soil components in grassland ecosystems have faced little attention to date. Ecosystem/ community responses to soil N heterogeneity may also depend on abiotic factors. For instance, rainfall regimes are expected to influence impacts of nutrient heterogeneity via soil N-water interactions (Borken and Matzner 2009, St. Clair et al. 2009). Increased intensity and duration of drought reduce mineralization rates of organic N and diffusion rates of inorganic N, which in turn decrease plant-available N. In contrast, heavy rainfall increases soil water moisture which may promote mineralization of N in soil, but is likely to increase risks of nutrient leaching losses. Little is known about plant and soil responses to spatial heterogeneity in soil N under changing rainfall regimes.

My thesis aims to improve understanding of effects of spatial heterogeneity in soil N for grassland ecosystem structure and function, with particular emphasis on community or ecosystem responses and plant-soil interactions. Specifically, my research addresses the three following questions:

i) What are the interactive effects of nitrogen form and spatial pattern on local-scale above- and below-ground processes?

ii) How does spatial N pattern interact with rainfall quantities on grassland structure and function at the local scale?

iii) How do patch attributes and timing of N inputs interact with spatial pattern in soil N to affect plant production at the field scale?

1.2 State of the art

1.2.1 Grasslands as a model ecosystem

On a global scale, grasslands represent the largest terrestrial biome and encompass a wide range of different community types (Table 1-1, White et al. 2000). Grasslands can be defined as a plant community with low-growing plant cover of non-woody species, dominated by grasses (*Poaceae*). They include steppes, savannahs, rangelands and tall-grass prairies across all of the world's continents, with the exception of Antarctica. In natural systems, the grasslands are maintained in an open, herbaceous state due to climatic constraints (aridity, cold temperatures, wind) and/or disturbances (fire, herbivory, drought). Belowground carbon dominates in grassland, mainly in roots and soil organic matter, and is driven by energy flow and productivity. For a given climate regime, grassland often has higher soil carbon contents than other vegetation types (Figure 1-1, White et al. 2000).

Table 1-1 Grassland types and areas in the world (redrawn from White et al. 2000).

Grassland type	Area (million km^2)	Percent of total land area[a]
Savanna	17.9	13.8
Shrubland	16.5	12.7
Non-woody grassland[b]	10.7	8.30
Tundra	7.40	5.70
World total	52.5	40.5

[a] Total land area used for the world is 129476000 km^2 — excludes Greenland and Antarctica.
[b] Includes non-woody grassland.

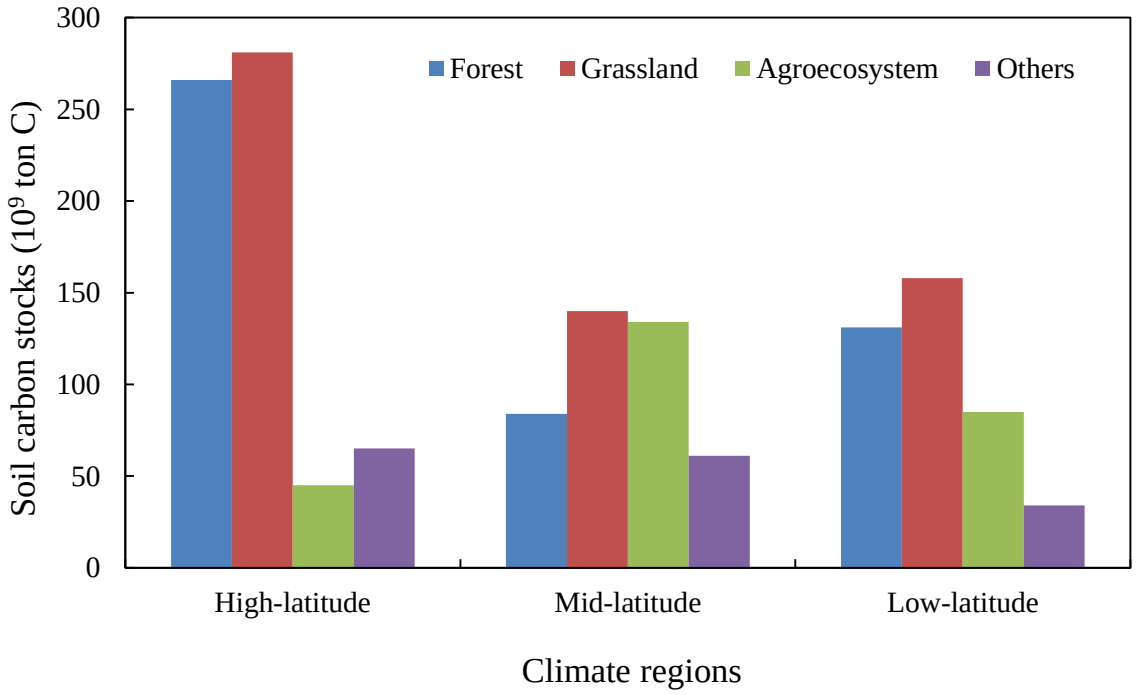

Figure 1-1 Soil carbon stocks in each terrestrial ecosystem at high, mid and low latitudes. Latitudinal designations roughly correspond with boreal and tundra ecosystems ('High-latitude', 50 to 90° N and S), temperate ecosystems ('Mid-latitude', 25 to 50° N and S), and tropical and subtropical ecosystems ('Low-latitude', 25° S to 25° N). Temperate West European countries that extend north of 50° N are included in the mid-latitude range. Data are taken from White et al. (2000).

Grasslands and rangelands cover 50% of the arable lands in Europe, contributing significantly to regional identity (Eurostat 2010). In Western Europe, grasslands are strongly influenced by human activity to support livestock. These semi-natural grasslands are maintained and shaped by management practices (mowing, grazing, fertilizer inputs) which aim to increase their productivity and forage quality. They provide most of the energy and protein required for milk and meat, two important agricultural outputs. In addition, grasslands host a tremendous diversity of plants, animals and microorganisms of functional and/or patrimonial interest (White et al. 2000).

Soil nutrient availability (in particular, nitrogen) has a determinant effect on grassland function and its capacity to provide both supporting services (primary production, nitrogen cycling) and regulating services (carbon sequestration, maintenance of soil fertility). Moreover, under grazing, herbivores induce heterogeneity in soil and vegetation structure (Figure 1-2), with consequences for local-scale community structure and function (Marriott and Carrere 1998), as well as for plant-soil interactions (Haynes and Williams 1993). Below we consider the importance of soil N availability and heterogeneity for grassland function and dynamics.

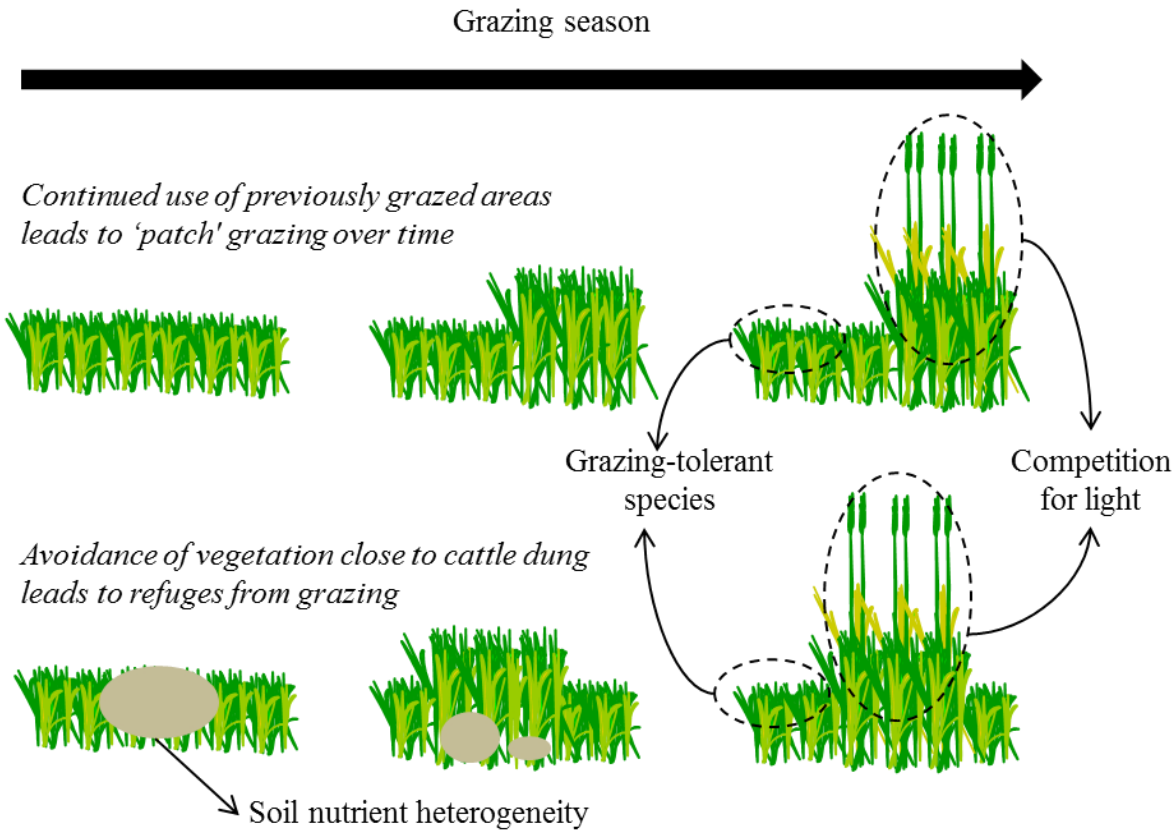

Figure 1-2 Spatial heterogeneity of vegetation and soil nutrient in grazed grassland (based on Haynes and Williams 1993, Adler et al. 2001).

1.2.2 Nitrogen: a key resource for plants and microbes

Nitrogen is an essential element for organisms to grow, maintain and reproduce, and is the main limiting nutrient in most terrestrial ecosystems (Vitousek and Howarth 1991). Organisms use N to synthesize a number of complex organic compounds such as protein and enzymes that drive the key metabolic processes involved in the life cycle. Nitrogen exists in a wide variety of different forms in natural ecosystems, with N transformations and fluxes between organisms driven by a combination of abiotic and biotic factors. In managed systems, N inputs modify plant growth, with direct effects on plant community composition and diversity and plant-soil interactions in grassland ecosystems (Tilman 1987, Bardgett et al. 1999, Hodge et al. 2000). This section describes the N cycle in grasslands, plant-microbe interactions for N and plant N strategies which mediate the rates of N fluxes in ecosystems.

The N cycle in grasslands

As in all ecosystems, the N cycle in grasslands is characterized by inputs, internal cycling between plant/soil pools and outputs (leaching, gaseous losses). Four main processes are involved in the N cycle: N fixation, mineralization (conversion of organic N to ammonia), nitrification (oxidation of ammonia to nitrates/nitrite), and denitrification (reduction of

nitrates to gaseous N) (Figure 1-3). Microorganisms, particularly bacteria, play major roles in all of the principal nitrogen transformations (Chapin et al. 2002). Moreover, internal cycling of N is closely linked to net primary productivity and decomposition rates (Thornton et al. 2009).

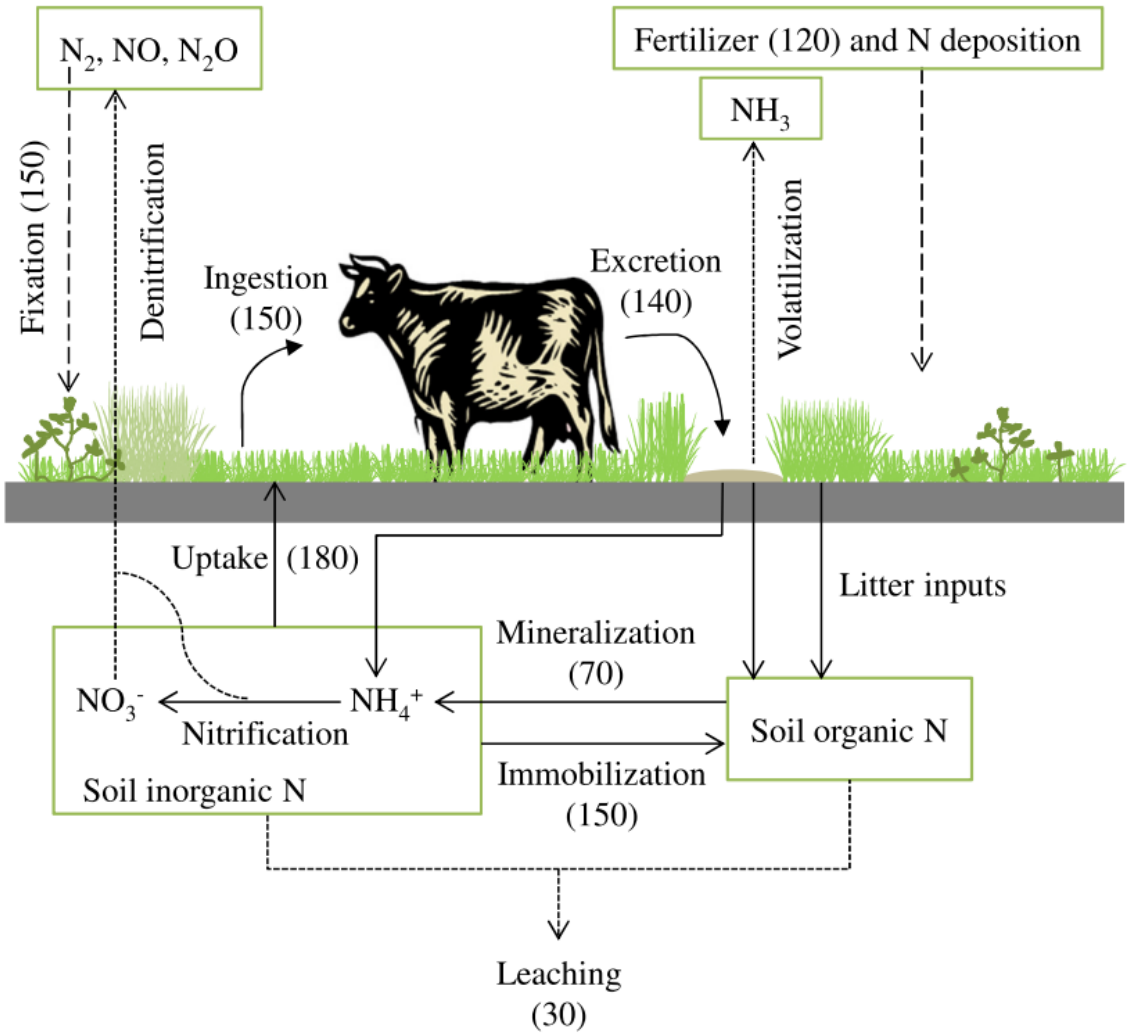

Figure 1-3 Nitrogen cycling in grazed grasslands. Solid arrows indicate internal N cycle within plant-soil-animal systems. Dash arrows indicate N inputs into grazed grasslands, and dot arrows indicate N losses from grazed grasslands. Estimations of N fluxes (kg ha^{-1} $year^{-1}$) are shown in brackets (data from Hodgson 1990). Total N losses to atmosphere via denitrification and volatilization are 50 kg ha^{-1} $year^{-1}$.

In natural and managed grasslands, external N inputs occur via biological N_2 fixation, N deposition and addition of fertilizers (Chapin et al. 2002). Indeed, fertilization is a very necessary source of N inputs in managed grasslands, where cutting or livestock productions annually remove a substantial amount of N from grassland ecosystems (Di et al. 1998). Biological N_2 fixation is the biological transformation from atmosphere N_2 to ammonium by

free-living bacteria, cyanobacteria and symbiotic legume-*Rhizobium* bacteria through the general chemical reaction:

$$N_2 + 4H_2 \rightarrow 2NH_4^+.$$

Biological N_2 fixation represents a large part of N inputs to grasslands, with 200 - 400 kg N ha^{-1} $year^{-1}$ fixed by leguminous species such as *Trifolium repens* in temperate pastures (Whitehead 2000). Nitrogen fixed by nitrogen-fixing plants subsequently becomes available to other plants through the production and decomposition of organic matter. By comparison, inputs from N deposition are low, ranging from 6 - 20 kg N ha^{-1} $year^{-1}$ in Western Europe, but these can nevertheless cause strong changes in the composition of species-rich temperate grasslands (Vitousek et al. 1997, Stevens et al. 2004, Bobbink et al. 2010).

With the exception of N derived from artificial fertilizers, most soil nitrogen is contained in dead organic matter derived from plants, animals and microbes (Chapin et al. 2002). Organic matter is not directly taken up by plants, and needs to be depolymerized by extracellular enzymes of fungi and bacteria to release soluble monomers such as amino acids and peptides (Jackson et al. 2008, Geisseler et al. 2010). Although a variety of amino acids and peptides can be directly used by plants and microbes (Kaye and Hart 1997, Näsholm et al. 2009), plants predominantly take up inorganic N for growth (Näsholm et al. 2000, Weigelt et al. 2005). Monomers/ dissolved organic matters are mineralized by soil fungi and bacteria to form ammonium (NH_4^+), a plant-available N form. Ammonium may be either absorbed by plants or microbes, adsorbed to negatively-charged soil particles, volatilized to ammonia gas or oxidized by nitrifying bacteria and fungi as part of a complex series of soil N transformations (Figure 1-4). These soil N transformations have received increasing attention in recent years since they result in the production of N_2O, an influential greenhouse gas (Wrage et al. 2001, Ravishankara et al. 2009, Klumpp et al. 2011).

In the particular case of grazed grasslands, large herbivores provide an additional dimension to the N cycle (Figure 1-3). Grazers exert a significant effect on fluxes of nutrients primarily through ingestion of plant material and excreta (dung and urine) (Augustine and Frank 2001, Bakker et al. 2004, Hutchings et al. 2007). Ingestion and animal returns promote the presence of readily-available nutrients, although the overall impact of grazers on the N cycle may vary depending on soil fertility, animal feeding behavior and rates of N loss from excretal patches (Haynes and Williams 1993, Bakker et al. 2004). For example, N-rich urine patches generally represent hotspots of N loss via gaseous emissions (NH_3, N_2O) or leaching of nitrate (Di and Cameron 2000, Wachendorf et al. 2005, de Klein et al. 2014), and grazing livestock contributes significantly to N_2O and NH_3 emissions at a global scale (Seinfeld et al. 2006).

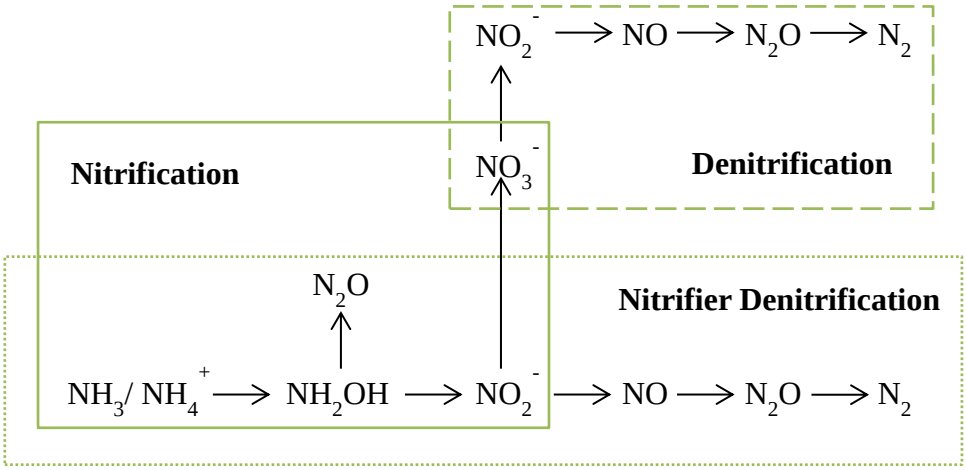

Figure 1-4 Transformations between forms of inorganic nitrogen in soil (redrawn from Wrage et al. 2001).

Plant-microbe interactions for N

The fate of soil N varies depending on a combination of abiotic factors and biotic interactions between plant and soil components. The traditional view of plant-soil interactions for N considers that: i) plants are able to use only inorganic N produced by microbial mineralization (Schimel and Bennett 2004); ii) plant-available N depends on the microbial immobilization-mineralization dynamics that are determined by microbial carbon status and substrate C: N ratios (Figure 1-5, Hodge et al. 2000a); iii) ; plants and microorganisms are both limited by N, promoting competition for N between these two groups (Kaye and Hart 1997); (iv) microbes are superior competitors than plants for inorganic N due to the higher ratios of surface area to volume, rapid growth rates and higher uptake affinities of microbes compared with plant roots (Jackson et al. 1989, Lipson and Näsholm 2001). More recently, authors have emphasized the importance of nutrients sequestered in recalcitrant soil organic matter in plant/soil interactions; labile carbon released by roots stimulates microbial growth in the rhizosphere and promotes the mining of additional N from soil organic matter, particularly when soluble soil N is low (Figure 1-6, Fontaine et al. 2011, Kuzyakov and Xu 2013). In addition, laboratory and field studies with $^{13}C^{15}N$-labelling techniques have demonstrated the capacity of plants to assimilate intact organic N molecules such as amino acids, urea and proteins (Streeter et al. 2000, Merigout et al. 2008, Paungfoo-Lonhienne et al. 2008). Consequently, it is suggested that organic N may have been underestimated in the plant N balance (Näsholm et al. 2009).

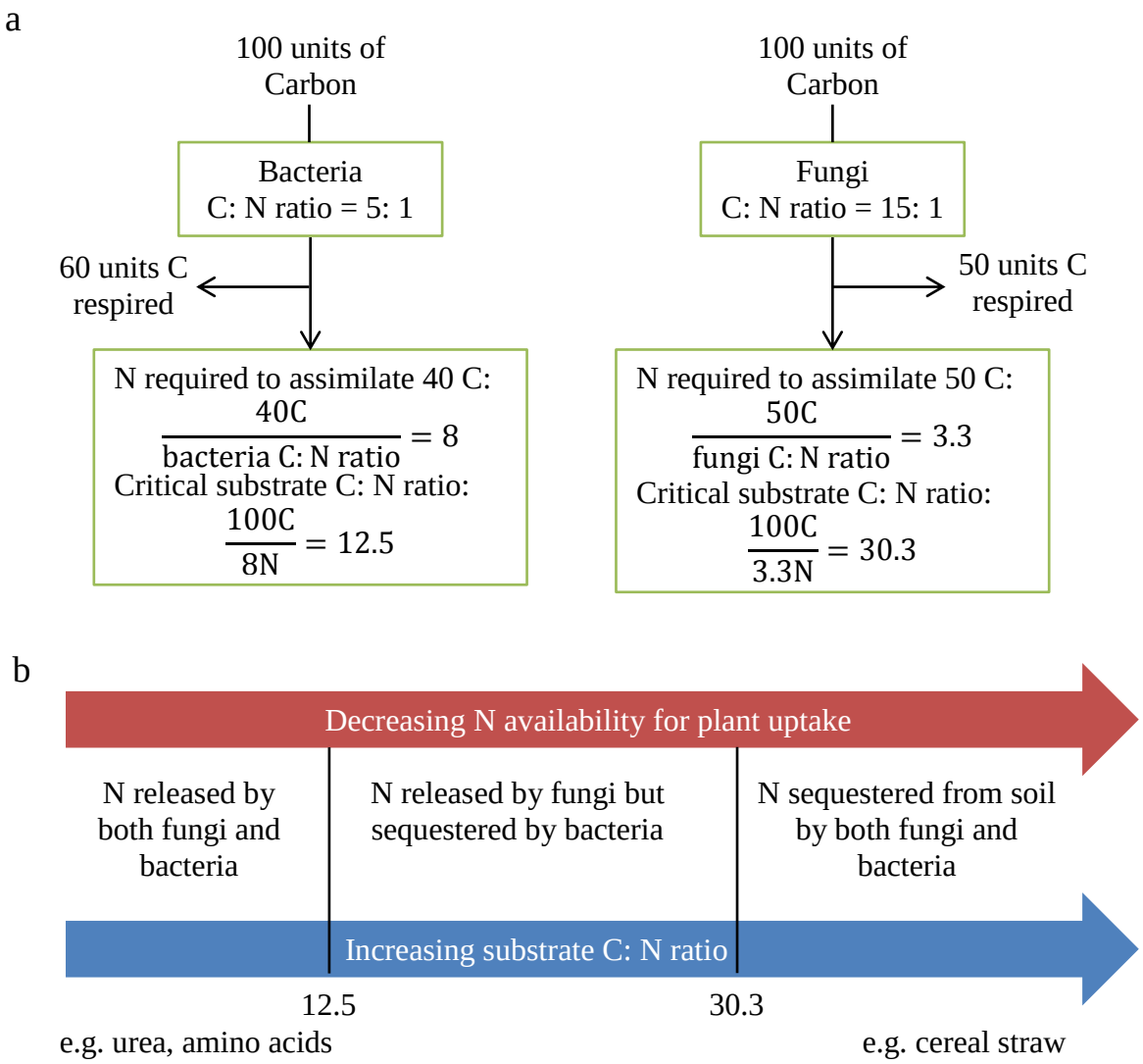

Figure 1-5 The critical substrate C: ratio for fungi or bacteria under which net mineralization rate is zero (a); plant-available N controlled by soil microbes and substrate C: N ratio (b). The figure is redrawn from Hodge et al. (2000).

Although microbes are often found to out-compete plants for simple organic N forms, studies on inorganic N partitioning between plants and microbes have generated conflicting results (Bardgett et al. 2003, Burger and Jackson 2004, Harrison et al. 2008, Bloor et al. 2009). The intensity of plant–microbial competition for N is known to vary depending on microbial activity, resource availability, ecosystem productivity and study length (Kaye and Hart 1997, Hodge et al. 2000a, Bardgett et al. 2003, Dunn et al. 2006). Unlike the seasonality of plant N uptake, soil microbes are active (albeit at reduced levels) throughout the year. This asynchrony between plant and soil activity can modify the outcomes of competition for N (Hodge et al. 2000a). Moreover, plants can become more effective competitors for N in the longer term since they have a greater capacity to sequester N for longer compared with

microbes; rapid microbial turnover, microbial death induced by microbial-feeding fauna and environmental fluctuation (e.g. drying-wetting cycles) release N into the soil and may then be 'locked away' in plant material (Harrison et al. 2007, Hodge et al. 2000b, Mansson et al. 2014).

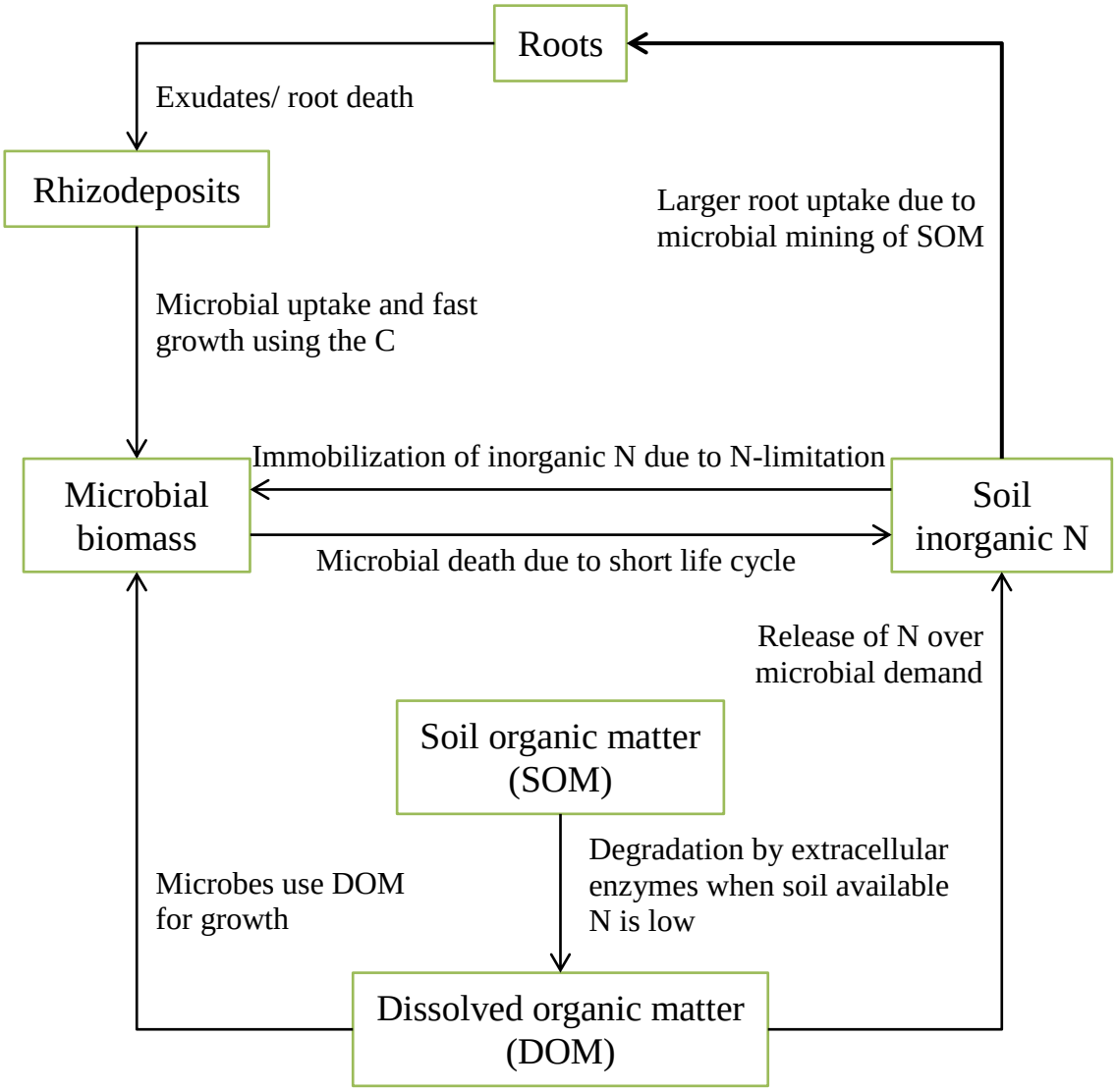

Figure 1-6 Interaction between roots and soil microbes for carbon and nitrogen uptake (modified from Kuzyakov and Xu (2013).

Plant N strategies

Plant nutrient uptake, use and loss are key controls over the cycling of N in grassland ecosystems. It has long been recognised that plant communities in low N environments are dominated by species that conserve nutrients and reduce nutrient loss through low growth rates, low rates of tissue turnover and high N use efficiency (Chapin 1980). Berendse and Aerts (1987) divided N use efficiency (NUE) into two components: N productivity (NP) i.e. the instantaneous rate of C fixation per unit N in the plant, and the mean residual time of N

(MRT) i.e. the time period during which N captured is used for C fixation. Pot experiments show NUE consistently decreases with soil N availability (Aerts and de Caluwe 1994, de Aldana and Berendse 1997). Studies focusing on interspecific differences also demonstrate a trade-off between NP and MRT among species in response to resource availability (de Aldana and Berendse 1997, Eckstein and Karlsson 1997, Silla and Escudero 2004). Thus, N-poor habitats favour slow-growing species with low N loss rates from plant biomass (low NP and long MRT), whereas N-rich habitats favour fast-growing species with high N losses (high NP and short MRT).

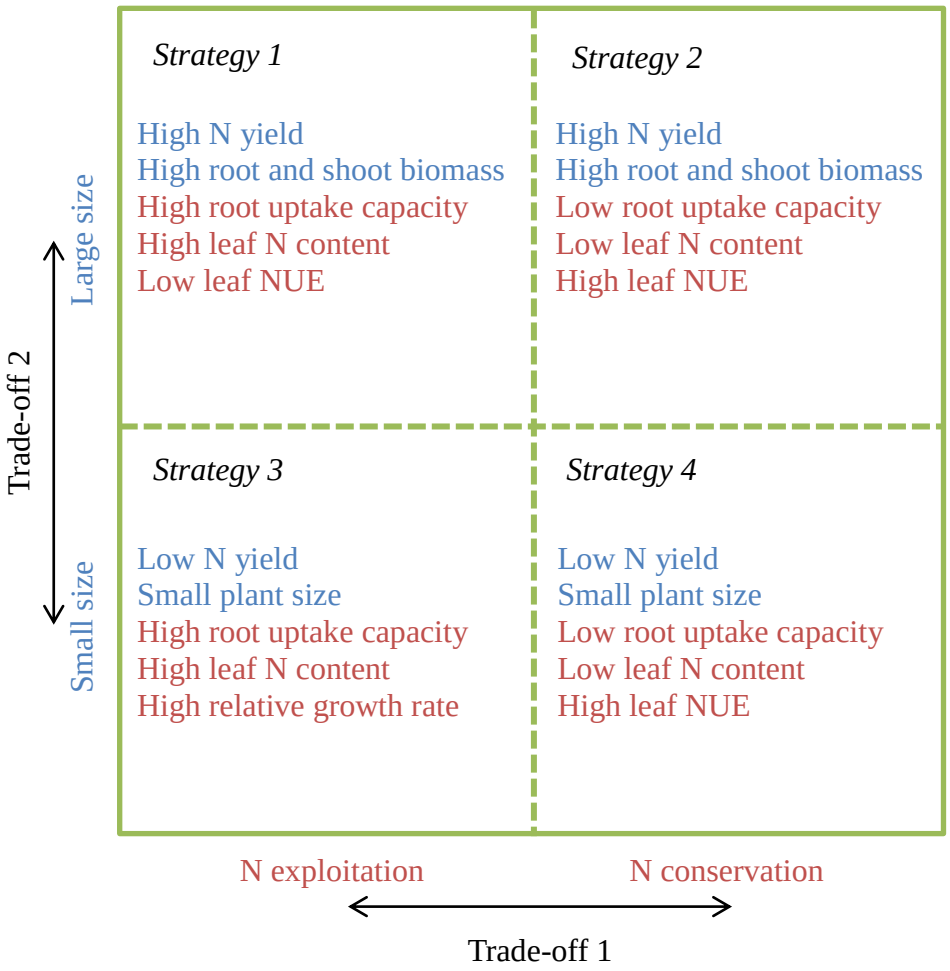

Figure 1-7 Plant nitrogen strategies reflecting N uptake and use for grasses based on trade-offs between root and shoot traits (following Maire et al. 2009). Traits associated with N exploitation versus N conservation are given in red font, and blue font for size-related traits.

Within a given habitat and plant community, co-occurring plant species and functional groups may meet their N demand via different N strategies. For example, legumes acquire large amounts of N from atmosphere through symbiotic N_2 fixation. Within plant community legumes commonly facilitate non-legume species through transfer of N fixed from legumes to non-legumes, usually resulting in over-yielding (i.e. facilitation, Bessler et al. 2012, Frankow-

Lindberg and Dahlin 2013). Aside from legumes, a growing body of studies suggest niche differentiation among species and complementary N use (Gross et al. 2007, Roscher et al. 2008, Maire et al. 2009). Pot studies show some species-specific preferences for chemical N forms for temperate grassland plants (Weigelt et al. 2003, 2005), although plant preference for chemical N forms may shifts depending on competitive environments (Miller et al. 2007, Ashton et al. 2010). Plant N strategies have also been identified using trade-offs between traits within and between varying habitats (Figure 1-7, Maire et al. 2009).

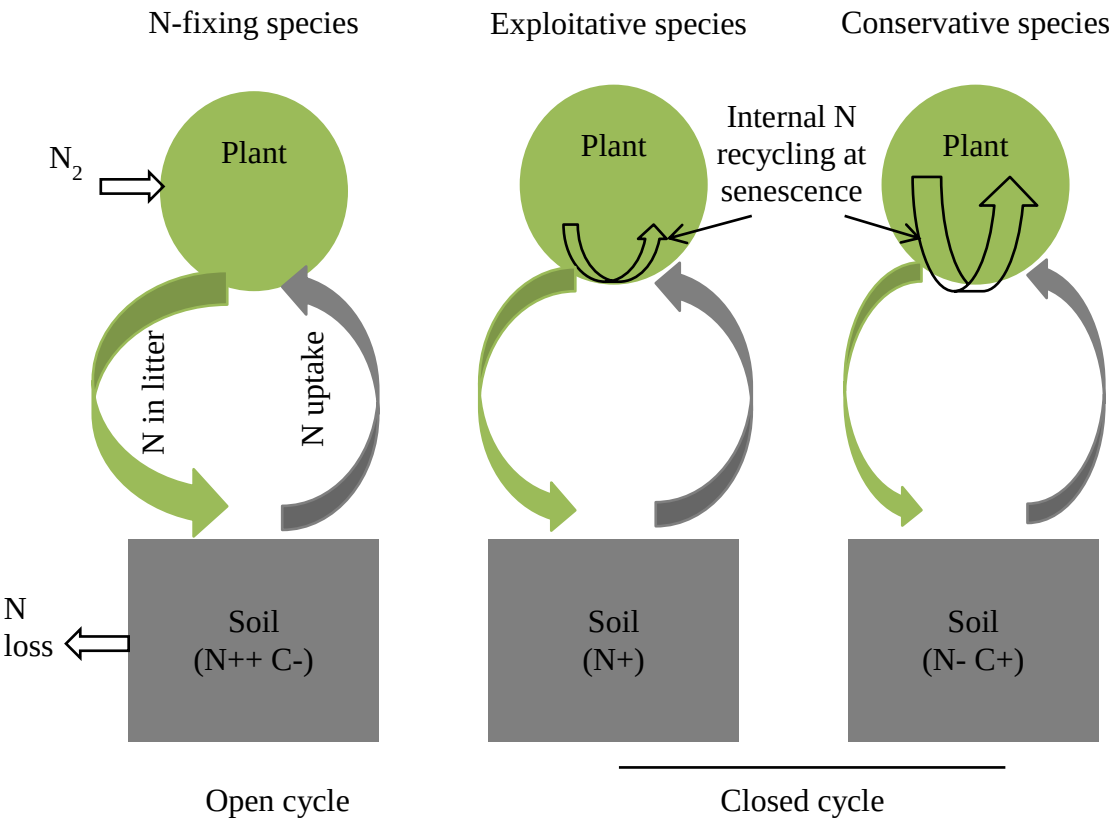

Figure 1-8 Linkages between species strategies and the soil nitrogen pool (redrawn from Amiaud and Carrere 2012). Size of arrows reflects the magnitude of N fluxes.

At the global scale, plants can be grouped into two primary strategies based on morphological and physiological traits: exploitative and conservative strategies (Wright et al. 2004, Grassein et al. 2010). Exploitative species are characterized by traits linked to fast tissue turnover, high resource capture and fast growth ability (high NP, leaf N content, special leaf area, root uptake capacity but short leaf life span and MRT), whereas conservative species have the opposite trait suites (Silla and Escudero 2004, Maire et al. 2009, Grassein et al. 2010). The presence of N-fixers, exploitative and conservative species within habitats generates a dynamic system, with fluctuations in community composition linked to variation in the soil N pool (Figure 1-8, Amiaud and Carrère 2012). Recent work shows that variation

in plant traits and N-use strategy are strongly linked to microbial traits and belowground processes (García-Palacios et al. 2011, Grigulis et al. 2013). Root traits (in particular, specific root length) appear to be closely associated with soil nutrient cycling and belowground biomass in heterogeneous conditions. Thus, plant N strategies may play an important role for plant-soil feedbacks and ecosystem services such as production and nutrient retention (Grigulis et al. 2013).

1.2.3 Spatial heterogeneity: what and how?

Spatial heterogeneity is a ubiquitous feature of natural ecosystems. As emphasized by Levin (1992), '*all ecological systems exhibit heterogeneity and patchiness on a broad range of scales, and this patchiness is fundamental to population dynamics, community organization and element cycling*'. Over the last decade many studies have addressed the causes of soil spatial heterogeneity and resource patches in grassland systems and investigated the ecological consequences at different biological organization levels (see Adler et al. 2001, Huber-Sannwald and Jackson 2001, Hutchings et al. 2003, Gillet et al. 2010 for some examples). This section describes concepts and definitions for spatial heterogeneity in ecological studies, and reviews the causes of soil nutrient heterogeneity in grazed grasslands.

Definitions and quantification of spatial heterogeneity

Heterogeneity is a confusing term in ecological literature, and can be viewed with different perspectives (Kolasa and Rollo 1991). Indeed, studies on ecological heterogeneity often refer to different aspects of heterogeneity (Milne 1991, Wiens 2000), blocking effective comparison among studies. To over this problem, Li and Reynolds (1995) defined spatial heterogeneity as the complexity and variability of a system property in space. A system property can be anything of ecological interest, such as soil resources, light, plant biomass or temperature. Complexity and variability refer to qualitative (categorical) and quantitative (numerical) descriptors of the system property respectively. An environment is considered to be homogeneous if the system property shows no variation across space.

Spatial heterogeneity can be quantified either directly, by measuring complexity and variability, or indirectly by measuring departure from homogeneity which is usually defined as the random distribution of a system property (Li and Reynold 1995, Adler et al. 2001). Statistical techniques and metrics to quantify spatial heterogeneity depend on data types (qualitative versus quantitative), and whether geographical coordinates are available for the measurements (spatially-explicit versus non-spatial or spatially-implicit data) (Dutilleul and Legendre 1993, Adler et al. 2001). In the case of truly spatial data, measurements can provide

either point pattern data where variables distribute at discrete locations (e.g. individual of species in space) or regionalized data where variables distribute continuously in space (e.g. soil nutrient availability). For point pattern data, spatial heterogeneity can be measured using methods such as second-order statistics and joint-count statistics (Fortin and Dale 2005). For regionalized quantitative data, spatial heterogeneity can be quantified in terms of spatial dependence using trend surface analysis, anisotropy analysis and autocorrelation analysis (Legendre and Fortin 1989, Li and Reynolds 1995). For regionalized qualitative data (e.g. categorical maps), spatial heterogeneity consists of complexity in composition and configuration of patches. Composition includes richness and relative abundance of patch types that can be analyzed using diversity indices. Configuration includes spatial arrangement of patch, patch shape, patch contrast, connectivity and anisotropy that can be analyzed using methods such as patchiness index, contagion index, fractal analysis and connectivity analysis (Figure 1-9, Li and Reynolds 1995, Gustafson 1998).

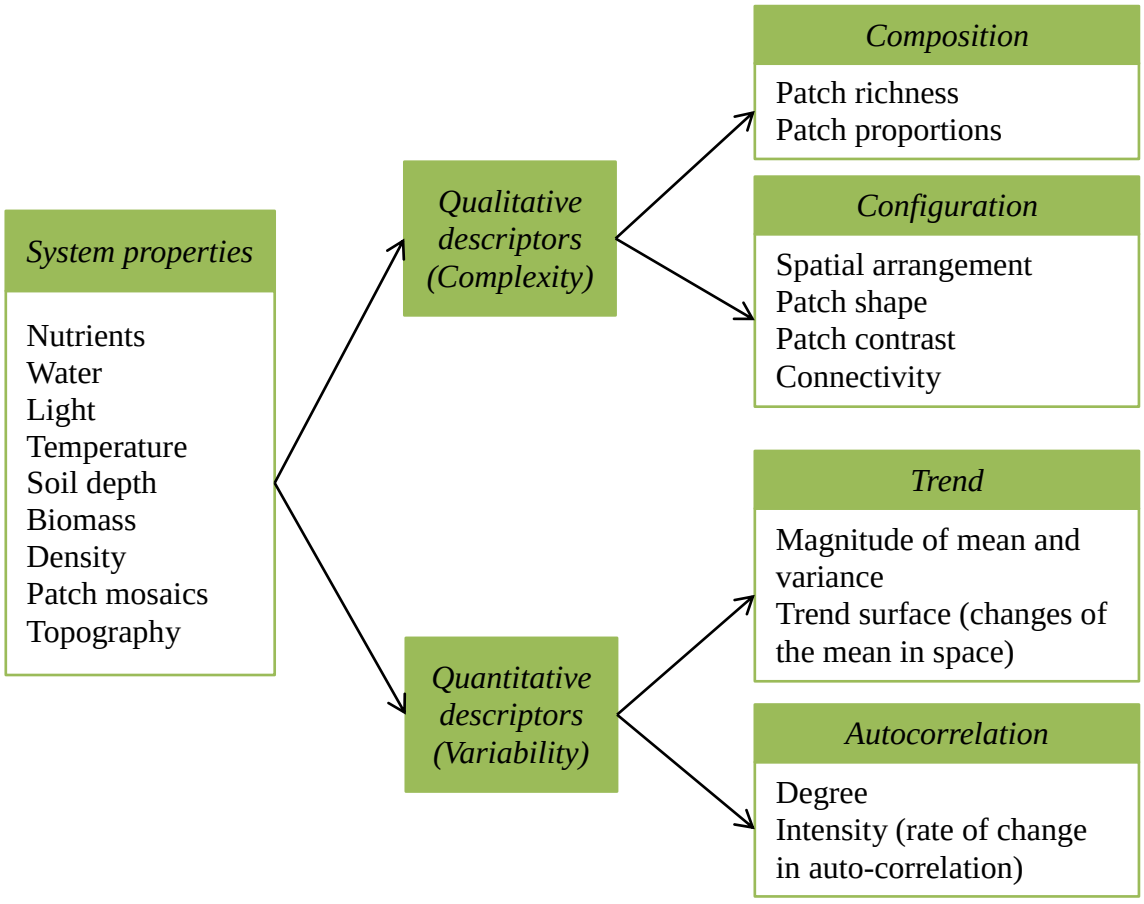

Figure 1-9 Key descriptors of spatial heterogeneity (based on Li and Reynolds 1995).

Detecting spatial heterogeneity is a function of scale, number of sampling units, and distance between sampling units (Fortin and Dale 2005). Moreover, observational scale is

determined by extent and grain. Extent is the overall study area, and grain is the size of individual sampling units in an experiment such as a quadrat or pixel (Figure 1-10, Wiens 1989). Extent and grain define the upper and lower limits of resolution of a study; extent size must be larger than scale of patchiness or heterogeneity otherwise significant spatial heterogeneity cannot be detected.

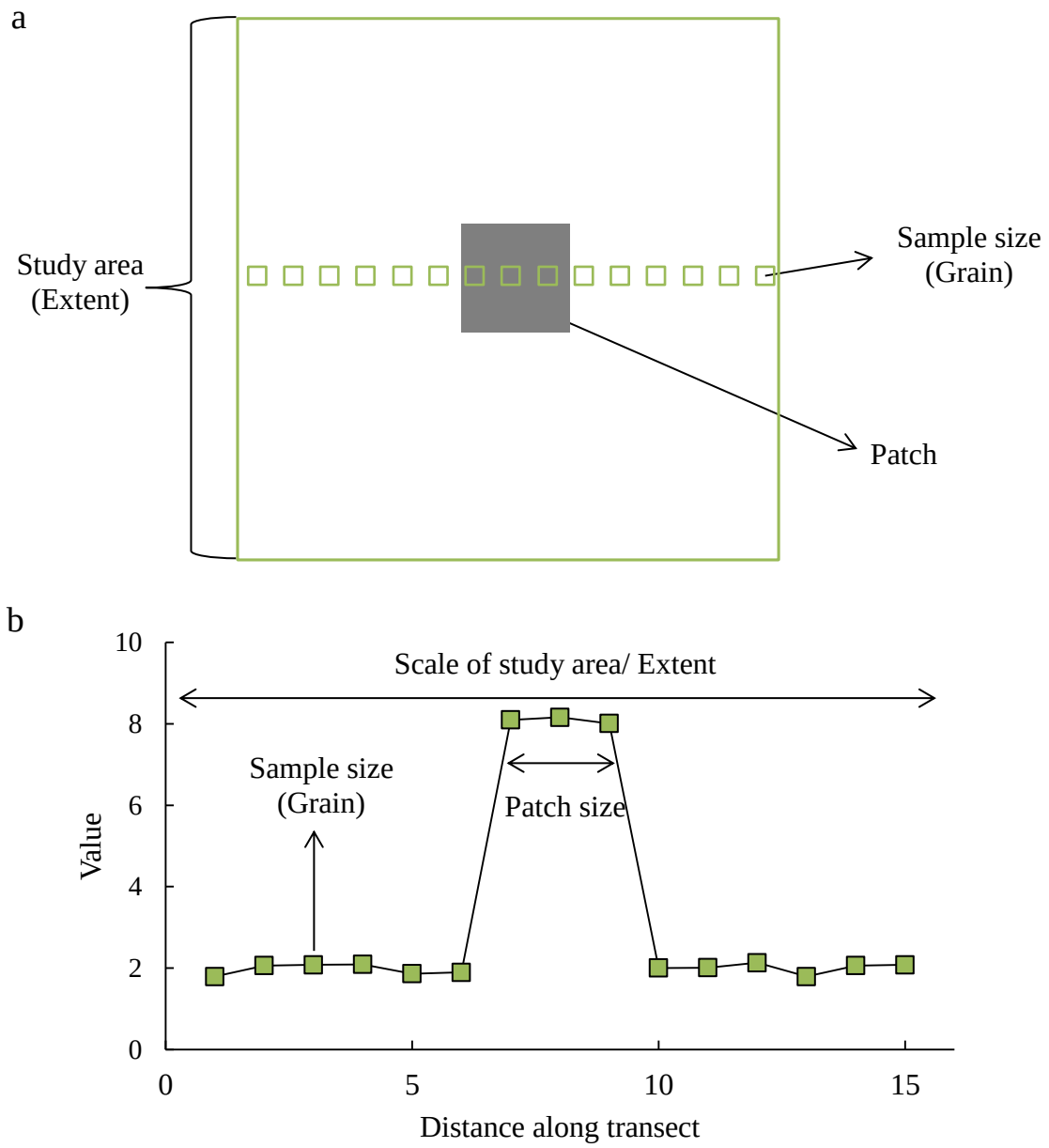

Figure 1-10 An example of extent and grain in a patchy environment.

Causes of spatial heterogeneity in grazed grasslands

Grasslands are dynamic and heterogeneous ecosystems shaped by animal activities (defoliation, excretion, trampling) which affect the paddocks in a non-uniform manner across space (Figure 1-2, Marriott and Carrère 1998, Augustine and Frank 2001). Impacts of grazing

animals are superimposed onto a background of existing site variability due to microtopography, and interact with abiotic factors at a local-scale such as soil physical and chemical characteristics (e.g. soil pore volume, soil texture) and microclimate (e.g. seasonal changes in rainfall and temperature). These abiotic factors influence nutrient availability and spatial distribution by controlling nutrient release into soil solution via mineralization, spatial diffusion/ movement of nutrients (Stark 1994, Burke et al. 1999, Hook and Burke 2000). Plants also promote within-site variability due to variation in plant traits which can impact on N cycling; where vegetation shows patchy distributions, plants can generate spatial nutrient heterogeneity by depleting nutrients around plant roots and returning them beneath plant canopy through litter fall (Hook et al. 1991, Jackson and Caldwell 1993, Kelly and Burke 1997).

Large grazing herbivores directly redistribute nutrients by excretion, creating high-concentration nutrient patches across the paddock. In the case of N, only a small fraction of the ingested N is assimilated, and up to 90% of N is excreted into grasslands, resulting in N application rates of around 1000 kg N ha^{-1} within cattle dung and urine patches (Haynes and Williams 1993). Chemical properties of dung and urine are highly distinctive, creating different types of nutrient patches. The average N content of dung is 2.0 - 2.8% of dry dung matter (Whitehead 1970). Dung consists of only small amounts of inorganic N (mainly ammonium), and the bulk of dung-N is in organic forms including amino sugars, proteins, amino acids and amides (van Faassen and van Dijk 1987, Bosshard et al. 2011). In contrast, urine contains over 60% of excreted N; the majority of urine-N occurs in the form of urea, and the remainder mainly consists of amino acids and peptides (Bathurst 1952). These excretal patches are non-randomly distributed in fields, with greater concentrations near watering points or resting zones (Haynes and Williams 1993, Auerswald et al. 2010). Species and body size of grazers may influence the scale of N redistribution via contrasting excretions and selective diet (Bakker et al. 2009, Bloor et al. 2012). For example, large grazers, such as cattle, return dung in large amounts in few patches, whereas smaller grazers, such as sheep and rabbits, defecate small pellets that are distributed over a wide area.

Grazers may indirectly influence soil nutrient heterogeneity by altering distribution, quantity and quality of litter inputs and soil abiotic/ biotic environments for litter decomposition (Bardgett and Wardle 2003, Sørensen et al. 2009, Schrama et al. 2013). Grazing affects vegetation stand structure, typically creating patches of short and tall stands of vegetation when vegetation biomass exceeds grazer intake (Adler et al. 2001). Grazing also affects plant community composition, typically promoting species with shorter stature, clonal rather than sexual reproduction or rosette growth forms (Milchunas and Lauenroth 1993).

Both grazing-induced changes in vegetation height and community composition may have indirect effects on microclimate and local soil conditions by altering plant growth rates, the quality/quantity of above- and belowground litter inputs and root exudation (Olff and Ritchie 1998, Semmartin and Oesterheld 2001, Bardgett and Wardle 2003). In fertile systems, grazing may induce positive plant-soil feedbacks by increasing vegetation compensatory growth and litter quality (high N content) which in turn enhance soil mineralization rates via shifts in microbial communities and food webs for decomposition (Figure 1-11, Bardgett and Wardle 2003, Wardle et al. 2004).

1.2.4 Consequences of soil N heterogeneity on plant function

In recent years, there has been considerable interest in plant responses to soil nutrient heterogeneity at a range of spatial scales and biological levels (individuals, populations, communities; Robinson 1994, Hutchings et al. 2003, García-Palacios et al. 2012). This section reviews morphological and physiological responses of individual plants to spatial soil heterogeneity/ patchy N inputs, and then considers how heterogeneity in soil N modifies population- (yield, size structure, mortality) and community-level responses (production, community structure).

Soil N heterogeneity and individual plants

When plants are provided with non-uniform (or patchy) nutrients, the roots often grow and proliferate in the nutrient-rich zone, a phenomenon known as root foraging (Robinson 1994). Plant roots adjust their morphology and architecture by increasing the elongation and growth of existing roots within nutrient patches and increasing the production of long and fine lateral roots with a high absorbing surface (Jackson and Caldwell 1989, Larigauderie and Richards 1994, Bilbrough and Caldwell 1995, Farley and Fitter 1999), potentially enhancing root system size (i.e. the whole root biomass) or the allocation to nutrient patches within root systems (also known as root precision). Previous studies with individual plants have found a trade-off between the whole root size and root precision of grassland species (Campbell et al. 1991, Wijesinghe et al. 2001, but see Einsmann et al. 1999). Increased N foraging may also stem from physiological alterations in N uptake kinetics i.e. higher N influx rates per unit root biomass/ root surface (Hodge 2004). Physiological plasticity is generally considered to be less energy/ C-consuming compared to morphological responses (Grime 2001), and usually occurs before morphological responses by plants, in particular when nutrient patches are dominated by mobile inorganic N (van Vuuren et al. 1996, Cui and Caldwell 1997).

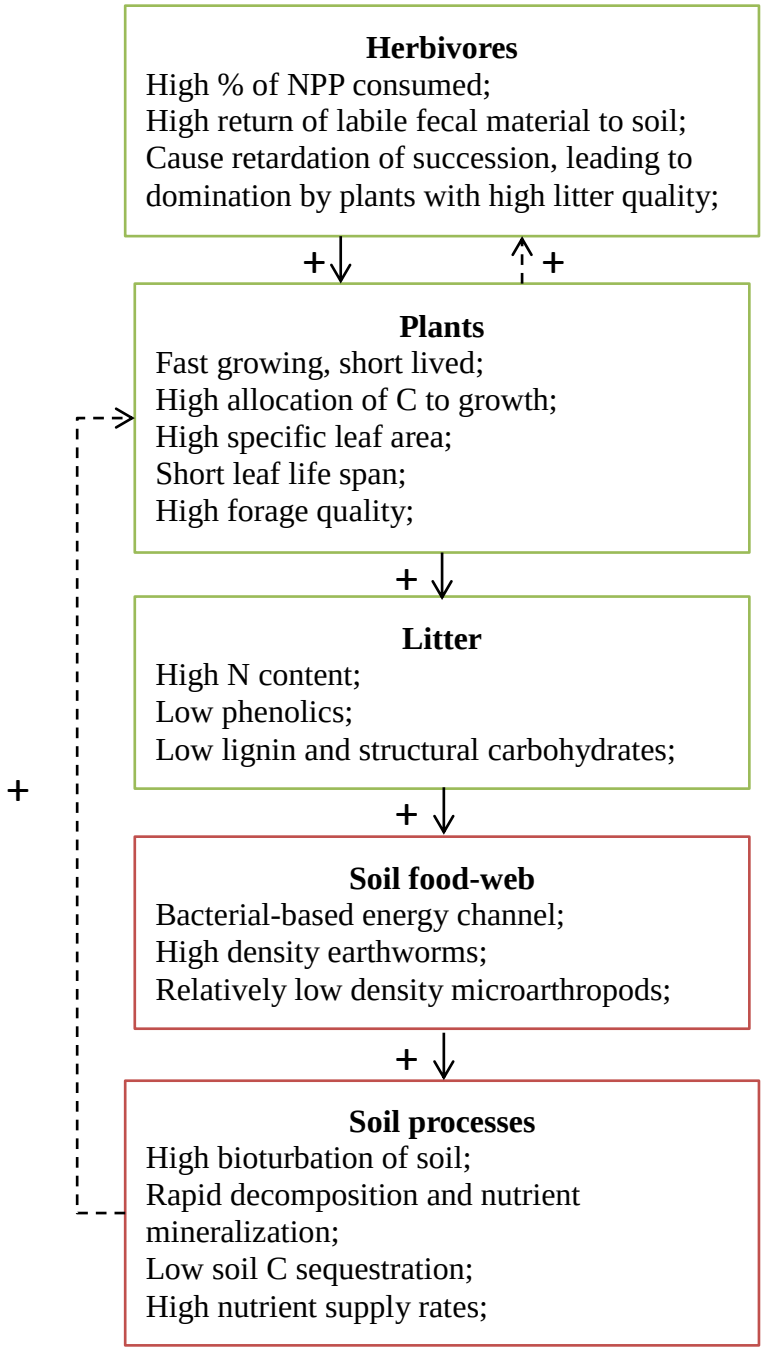

Figure 1-11 In fertile and productive ecosystems, herbivores induce positive plant-soil interactions (redrawn from Wardle et al. 2004). Green boxes represent aboveground processes; red boxes represent belowground processes.

Aside from localized responses by root systems, patchy nutrient inputs may also induce responses of individual plants as a whole, including changes in the biomass allocation between root and shoot and the whole plant production. For example, studies on the herbs or grasses observe that the whole plant production and root: shoot ratio of the whole plant is significantly enhanced by the heterogeneous N supply compared to the homogeneous treatments with the equivalent N supply (Birch and Hutchings 1994, Cahill and Casper 1999).

This response pattern is driven by higher root: shoot ratio of plant parts located in nutrient patches compared to the background soil (Wijesinghe and Hutchings 1997, 1999). Whole-plant responses to nutrient patches may vary depending on patch contrast (the contrast between nutrient patches and background soil) and/or patch size relative to plant root systems (Wijesinghe and Hutchings 1999, Hutchings and Wijesinghe 2008). When nutrient patches have low N concentration compared to the background soil, plants are less able to detect nutrient patches and thus possess little selectivity in root placement patterns. Moreover, when plants are exposed to small-scale N heterogeneity (nutrient patches are small relative to the size of plant root systems, and plant roots cross several patches), plant growth is less enhanced than nutrient patches are large or similar in size to plant root systems (Hutchings et al. 2003).

Population-level responses

Morphological and physiological responses of individual plants to nutrient patches have consequences for the performance of plant populations in heterogeneous nutrient conditions. Plants located in (or within foraging distance of) nutrient-rich patches will have access to more nutrients than plants in nutrient-poor zones, resulting in larger plants in N-rich patches. Indeed, pot experiments support the idea that patchy N conditions should promote size inequalities between plants and greater size variation within populations (Fransen et al. 2001, Day et al. 2003a, b). Plant size is critical for plant competitive ability. In homogeneous N conditions, belowground competition is considered to by root-size symmetric (i.e. nutrient capture is proportional to root system size) (Weiner 1986, Cahill and Casper 2000). In patchy N conditions however, belowground competition tends to be size-asymmetric, with plants that first access nutrient patches obtaining a size-disproportionate advantage over their competitors (Schwinning and Weiner 1998). Increased intraspecific competition intensity in patchy nutrient conditions is confirmed by work with grasses (Day et al. 2003c). Population yield has been shown to increase when nutrients are supplied heterogeneously, driven by enhanced nutrient foraging by individuals within patches (Day et al. 2003a).

Despite strong short-term responses of populations to soil nutrient heterogeneity/ patchy N inputs, differences in population yields between heterogeneous and homogeneous N treatments may decrease over time due to intense intraspecific competition/ self-thinning in nutrient patches and small-scale changes in plant mortality (Day et al. 2003b). However, overall mortality rates of plant populations may be lower in heterogeneous compared to homogeneous conditions if nutrient-poor locations are less exploited by roots and thus provide refuges for plants from intense root competition (Casper and Cahill 1996, Day et al.

2003b). In contrast, plant competition may be uniformly intensified in homogeneous conditions as nutrients are depleted by plants, resulting in increased overall population mortality. Outcomes of plant-plant competition in patchy N environments may be mediated by plant size (and hence plant perception of environmental heterogeneity), nutrient patch size and patch contrast (Hutchings et al. 2003), as well as species' differences in N-use strategy. Small, high-contrast patches are expected to promote intense and size-asymmetric competition between plants. Large, high-contrast patches are expected to increase plant competition intensity primarily at patch boundaries when root system of a single plant cannot completely dominate a nutrient patch.

Community-level responses

Compared to the high number of studies published on individual- and population-level responses to soil N heterogeneity, work on community-level responses is relatively limited. Shifts in plant growth and biomass allocation pattern induced by heterogeneous nutrient inputs may have cascading effects on plant-plant interactions and belowground competition (Hutchings et al. 2003, Schenk 2006). Heterogeneous nutrient supply can alter competitive hierarchies between plant species (Fransen et al. 2001), and increase competition intensity experienced by plants (Day et al. 2003c). Nevertheless, previous work suggests community biomass is enhanced in patchy compared to uniform conditions, possibly due to an increase in root biomass and higher community below-ground: above-ground biomass ratios in the heterogeneous treatments (Maestre et al. 2005, Wijesinghe et al. 2005).

The classical niche theory predicts that resource heterogeneity should promote species richness, because coexisting species occupy patches of different quality and thus intense interspecific competitions are effectively avoided (Pacala and Tilman 1994, Chesson 2000). However, this classic theory has not been always supported by observational or experimental work (Reynolds et al. 2007, Lundholm 2009, Tamme et al. 2010). A number of studies suggest that effects of heterogeneous nutrient additions on species diversity may vary depending on the plant size, presence of clonal species or buffering effects of dominant species (Baer et al. 2004, Eilts et al. 2011). Aside from species richness, resource heterogeneity may modify community diversity via shifts in species relative abundance and hence dominance (or evenness) patterns. Two recent studies have demonstrated that small-scale heterogeneity of soil resources increases community dominance, possibly due to increased asymmetric competition for both above- and belowground resources (Rajaniemi 2011, Gazol et al. 2013).

Discrepancy between the results of different studies may in part reflect different experimental conditions and application of nutrient patches. In particular, when plants compete for a single limiting nutrient and patch scales are small relative to the size of individual plants, nutrient heterogeneity may reduce species richness compared to homogeneous conditions when the same amount of nutrients are supplied, because single plants of species that can efficiently exploit separate nutrient patches may be capable of dominating both nutrient patches and nutrient-poor locations, excluding inferior competitors (Hutchings et al. 2003). To date, however, most studies have used artificial plant communities (where community composition differs from with natural communities), and have carried out experiments outdoors or in greenhouse at very small spatial scales. Little remains known about responses of natural communities to soil nutrient heterogeneity/ patchy nutrient inputs at larger spatial scales and in field conditions.

1.2.5 Factors controlling impacts of soil N heterogeneity

Although grassland responses to soil N heterogeneity are relatively well described, the importance of interacting biotic and abiotic factors associated with global change has faced little attention. A number of recent studies suggest that elevated CO_2 or rainfall regime may modify impacts of spatial soil heterogeneity on grassland ecosystems (Maestre and Reynolds 2006 and 2007a, Garcia-Palacios et al. 2012). This section considers how biotic and abiotic factors may mediate impacts of soil N heterogeneity, and proposes that interactions between these factors and soil N heterogeneity deserve future research.

Biotic factors: plant and soil microbe community composition

Plant community structure has the potential to modify impacts of soil nutrient heterogeneity on grassland ecosystem function directly due to the presence of different species or species combinations which modify patterns of soil N uptake due to their different N-use strategies and plant traits. For instance, fast-growing species exploit nutrient-rich patches rapidly and hence possess stronger biomass responses compared to slow-growing species (Hodge et al. 1999, Robinson et al. 1999). In homogeneous N conditions, high species diversity has been shown to enhance community productivity, in part due to complementary N use (Figure1-12, Fargione et al. 2007, Roscher et al. 2008). Previous studies with model grassland communities have indicated that community composition interacts with heterogeneous nutrient inputs to influence community production, with enhanced aboveground and belowground biomass in heterogeneous conditions when communities contains plant species that effectively use nutrient patches (Maestre et al. 2006, Maestre and Reynolds 2007b).

These studies observed that species richness (simply the number of species occurring in communities) had no significant effects on community biomass, and did not interact with soil nutrient heterogeneity, underlining the importance of plant traits for ecosystem function.

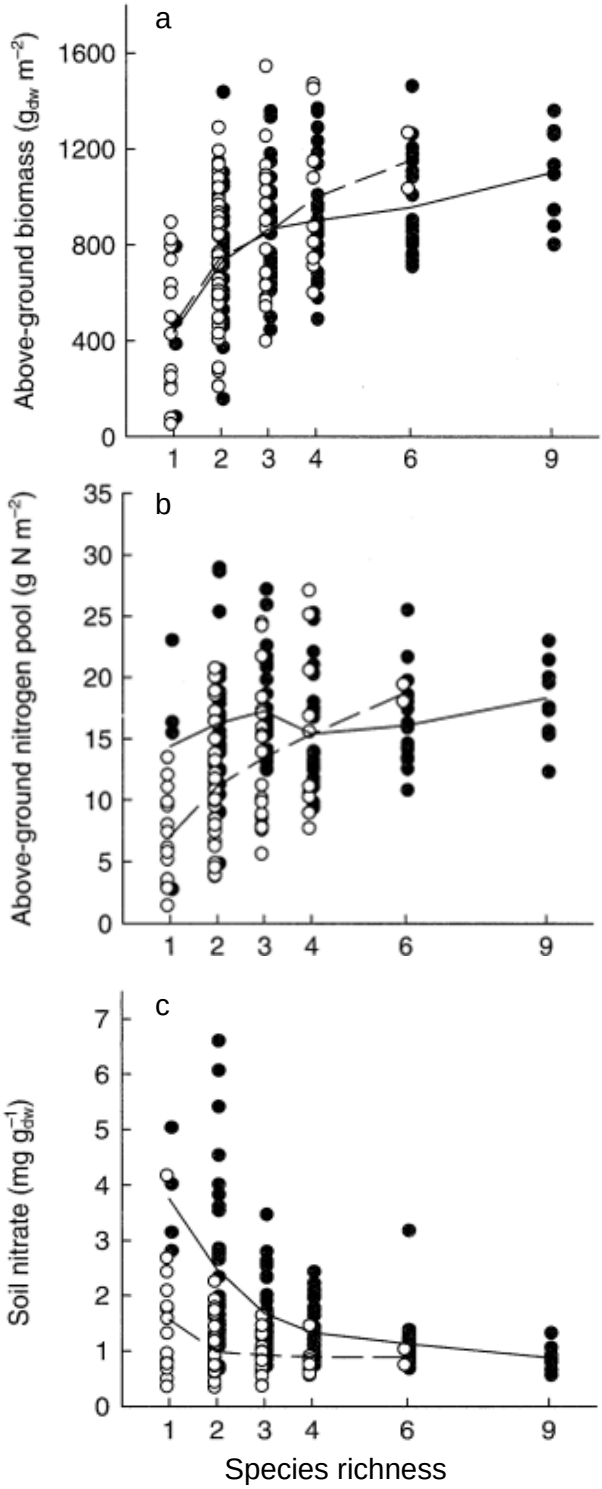

Figure 1-12 Aboveground biomass (a) and aboveground nitrogen pool (b) significantly increase with species richness within grassland plant assemblages in the second year after establishment, irrespective of 'with legumes' (closed cycles) or 'without legumes' (open cycles). In contrast, soil nitrate decreases with species richness, suggesting increased efficiency in N acquisition and complementary N use (taken from Roscher et al. 2008).

Apart from these direct impacts, plants can indirectly influence impacts of soil nutrient heterogeneity on grassland ecosystem function via their effects on microbial communities. For example, previous work has shown that fast-growing plant species with a high litter quality select for a bacterial-based microbial community, whereas slow-growing plant species tend to be associated with fungal-dominated microbial communities (Orwin et al. 2010, Grigulis et al. 2013). Microbial community composition (in particular, fungi: bacteria ratios) may influence impacts of soil N heterogeneity since they play a major role in controlling loss and retention of N (de Vries et al. 2006). Microbial communities dominated by bacteria generally show faster rates of N cycling, whereas fungal-dominated communities typically slow down rates of N cycling and are associated with decreased soil N leaching (Wardle et al. 2004, de Vries et al. 2006, Fontaine et al. 2011). High nitrogen inputs may also modify the relative abundance of bacterial groups (Ramirez et al. 2012) and lower fungi: bacteria ratios (de Vries and Bardgett 2012), with implications for N leaching and N retention. Shifts in microbial community structure induced by patchy N inputs could therefore have feedback effects on plant growth and plant/soil competition for N. Limited evidence from pot experiments suggests that soil spatial heterogeneity may influence outcomes of plant-microbe competition (Wang and Bakken 1997), but the importance of spatial heterogeneity for plant-soil interactions for N remains unclear.

Abiotic factors: soil water availability

Water and N are known to be important co-limiting resources in grasslands (Harpole et al. 2007, Lamb et al. 2007). Changes in precipitation regime have impacts on soil water availability and hydrological process in natural systems (Knapp et al. 2002, Fay et al. 2003). Soil N availability is strongly determined by water availability, because soil water influences N mineralization via soil microbial activities, N mobility in soil solution and uptake by plant roots (Fierer and Schimel 2002, Gordon et al. 2008, Borken and Matzner 2009). Moreover, soil water availability exerts significant influences on plant performance and plant traits (Figure 1-13, Chaves et al. 2003), with consequences for community structure and species composition in grassland ecosystems (Lauenroth and Dodd 1978, Morecroft et al. 2004, Evans et al. 2011). Typically, water deficits result in a decrease in plant growth and an increase in mortality, modifying regeneration dynamics and selecting for more stress-tolerant genotypes or species in the longer term (Walck et al. 2011, Volaire et al. 2014). Excess water is also known to create oxygen deficit that promotes fine root mortality and affects microbial activities for mineralization (Crawford and Braendle 1996, Kreyling et al. 2008). Thus water availability may be expected to interact with soil N heterogeneity via direct effects on soil N

transformations and transfers, as well as indirect effects via changes in plant species growth and community structure.

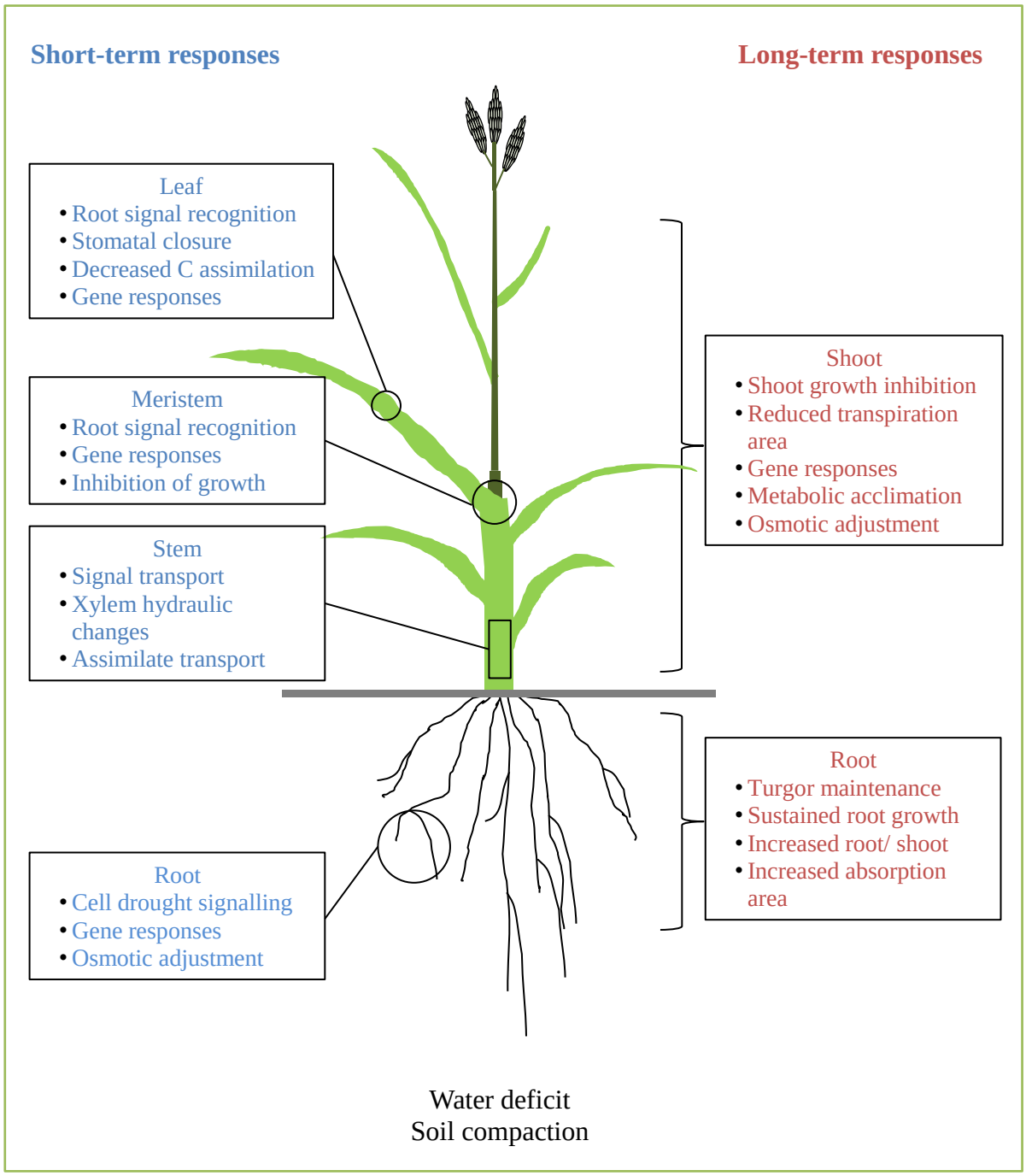

Figure 1-13 Whole-plant responses to drought stress in the short and longer term (based on Chaves et al. 2003).

Studies with homogeneous water and N addition show grassland plant responses to N availability are often modified by water availability (Harpole et al. 2007, St Clair et al. 2009, but see Lamb et al. 2007). To date very few experiments have simultaneously manipulated water availability and soil N heterogeneity, as emphasized in a recent review of plant

responses to soil nutrient heterogeneity and global change drivers (Garcia-Palacios et al. 2012). Results from one microcosm experiment showed that shoot biomass was considerably higher at high water availability when nutrients were heterogeneously supplied, indicating a significant spatial N pattern x water availability interaction (Maestre and Reynolds 2007a). However, interactions between N heterogeneity and water/ rainfall regimes have yet to be demonstrated for complex grassland communities.

1.3 Thesis plan

Despite the large body of published work on nutrient heterogeneity, few studies have considered how patch attributes and abiotic conditions may modify effects of N heterogeneity on plant and soil processes at the community and ecosystem levels. My thesis addresses this knowledge gap by focusing on a number of different patch attributes (N form, patch size, patch contrast), as well as possible interactions with rainfall regime. My thesis comprises of three results chapters which examine impacts of N heterogeneity (via heterogeneous N inputs) on grassland structure and function at different spatial scales, and using a variety of complementary approaches. Throughout these chapters, I use a temperate grassland dominated by fast-growing grass species as a model system. Heterogeneous N treatments are representative of N deposited on grasslands in cattle excreta.

In Chapter Two, I use a field experiment to examine the interactions between N form and N pattern on a five-year old grassland dominated by *Dactylis glomerata*. Inorganic and organic N forms were applied to replicate grassland plots in a uniform or patchy pattern at the start of May 2012, and plant/soil measurements were carried out at peak biomass and at the end of the growing season. The following hypotheses are addressed: i) patchy N addition will enhance community-level plant production due to increased root biomass and nutrient-use efficiency; ii) plant biomass will respond more strongly to inorganic compared with organic N forms, whereas carbon-limited microbial biomass will respond more strongly to organic N; iii) plant and soil responses to patchy N addition will occur over a wider area within inorganic N plots compared to organic N plots, due to the higher mobility of inorganic N in soil; iv) plant biomass responses to patchy N addition will occur over a wider area within plots compared to microbial biomass responses, due to plant root distribution and root foraging.

In Chapter Three, I use an outdoor mesocosm experiment to examine the interactions between N application pattern (homogeneous, heterogeneous) and rainfall regime (control; wet, +50 compared to control; dry, -50% compared to control) on a multi-species grassland community. Short-term species- and community-level responses to heterogeneous N are examined, focusing on biomass and dominance patterns at peak biomass (two months after N

application). The following hypotheses are addressed: i) patchy N inputs promote both species-level and community-level biomass variability due to increased plant growth in/adjacent to N patches; ii) patchy N inputs modify plant community structure and dominance patterns due to species-specific variation in N responses; iii) dry conditions buffer plant biomass responses to patchy N inputs due to lower rates of N mineralization, decreased N transfers in soil and decreased plant N uptake; iv) wet conditions buffer plant biomass responses to patchy N inputs due to higher rates of N mineralization and increased N losses (leaching, gaseous emissions) from N patches.

In Chapter Four, I use a spatially explicit model simulating field-scale grassland production to examine whether patch attributes mediate effects of heterogeneous nitrogen supply on grassland production. Patchy nutrient inputs in model simulations were generated by an increase in soil nitrogen availability index, modifying patch size, patch contrast and timing of N inputs in order to address the following hypotheses: i) field-level grassland production increases with larger nutrient patch sizes due to smaller patch perimeter/ area ratios and hence lower occurrence of intense competitive effects at patch boundaries; ii) high contrast N patches reduce field-level grassland production i.e. lower biomass in high- versus low-contrast patch treatments; iii) timing of patchy N inputs modifies grassland production, due to shifts in potential plant growth rates and intensity of plant competition; iv) timing of patchy N inputs mediates patch size effects on field-level grassland production due to phenology-driven shifts in competitive effects at patch boundaries.

In Chapter five, I bring together key results from the three data chapters in order to address three overarching questions and propose a conceptual model that aims to better understand impacts of spatial nutrient heterogeneity and climate factors in terrestrial ecosystems. Directions for future work are proposed after analysis of study limitations.

Chapter Two

Nitrogen Form and Spatial Pattern Promote Asynchrony in Plant and Soil Responses to Nitrogen Inputs in a Temperate Grassland

Homogeneous and patchy inputs of inorganic and organic nitrogen (N) are common in grazed grasslands, but little is known about the interactions between spatial pattern and form of N inputs for plant and soil processes. This chapter set out to investigate this topic using a six-month, *in situ* grassland experiment. Impacts of uniform and patchy N addition on plant and soil properties were examined using three N forms of increasing complexity (inorganic N; a simple amino acid, glycine; a complex protein, BSA). Plant and microbial biomass was recorded at two harvest dates (June, September), and plant/soil N was determined along with dissolved organic carbon (DOC). For this study, designed by J. Bloor and P. Carrère, I was responsible for performing the experiment (carrying out regular measurements, plant/ soil sampling at harvests, carrying out soil extractions, grinding and preparing plant samples for CN analysis), with some help at harvests from J. Bloor and P. Carrère. I also carried out all of the data analysis.

One month after N addition, patchy N treatments increased plant production but decreased biomass produced per gram nitrogen (a proxy of N use efficiency) compared with uniform N treatments. Contrary to expectations, plant production showed limited differences among N form treatments. However, microbial biomass and DOC showed significant N form x pattern interactions, with strongest responses to patchy inputs of complex organic N. Irrespective of N form, plant responses to patchy N inputs occurred over a larger spatial area than soil microbe responses, consistent with optimal foraging by plant roots. Unlike plants, microbial responses to patchy N inputs were still observed after six months. Overall, our results indicate that patchy inputs of N promote the uncoupling of plant and soil properties, with greatest differences observed for complex organic N inputs. The spatial and temporal asynchrony between plant production and microbial biomass observed may have significant implications for the competitive balance of plants and soil microbes in space, as well as for plant-soil feedbacks involved with the regulation of biogeochemical cycling.

This chapter has been published in *Soil Biology & Biochemistry* (Xi, Carrère and Bloor, 2014, volume 71, page 40-47; Appendix 1).

2.1 Introduction

Nitrogen (N) inputs play a key role for primary productivity, plant community composition, soil microbial diversity and plant-soil interactions in grassland ecosystems (Tilman 1987, Bardgett et al. 1999, Hodge et al. 2000a). However, a growing body of evidence suggests that grassland responses to N addition may depend not only on the quantity and form of N (organic and inorganic), but also on the spatial pattern of N (Hutchings et al. 2003, Maestre and Reynolds 2006, Orwin et al. 2009). This is of particular interest for managed grasslands where both evenly-distributed and patchy inputs of N are common due to fertilizer management and grazer activity respectively.

During grazing, up to 90% of ingested N from herbage is returned to the pasture in dung and urine patches which cover a small area but have high concentrations of N (equivalent to application rates of around 1000 kg N ha^{-1} for dung patches, Haynes and Williams 1993). Such spatial variation in the quantity of soil N has the potential to influence plant processes from the individual to the community level (Hutchings et al. 2003, García-Palacios et al. 2012). For example, root systems of individual plants may respond to local increases in soil N by the production of new lateral roots in N patches, increased growth of existing roots or plasticity in nutrient uptake kinetics, enhancing efficient capture of N (Campbell et al. 1991, Robinson 1994, Hodge 2006). Root foraging responses and improved nutrient capture by plants in heterogeneous N conditions may also modify plant population structure and enhance yield (Day et al. 2003a). In theory, species-differences in N foraging efficiency and/or N complementarity should enhance community-level production in heterogeneous conditions (Wijesinghe et al. 2005, Kahmen et al. 2006). In practice, evidence from field experiments on natural plant communities is lacking.

In contrast to the large number of studies addressing plant responses to patchy increases in soil N, very few studies have considered the impacts of soil heterogeneity on the plant-soil system as a whole (Day et al. 2003c, Maestre and Reynolds 2006, but see Orwin et al. 2009). Soil N availability is a driver for microbial processes such as nitrification and denitrification in terrestrial ecosystems (Booth et al. 2005, Barnard et al. 2006), and recent work indicates that N inputs may also modify microbial community structure (Allison et al. 2008, Ramirez et al. 2012). In the absence of plants, soil respiration and microbial biomass have been shown to decrease under N addition (Ramirez et al. 2012). However, in the presence of plants, responses of microbial biomass to soil N enrichment are mixed (Hodge et al. 2000b, Allison et al. 2008, Treseder 2008). Recent advances indicate that interactions at the above-belowground interface may provide important feedbacks regulating ecosystem processes and ecosystem N retention (de Vries and Bardgett 2012). To date though, the

effects of patchy increases in soil N on coupled plant-soil responses remain poorly understood.

Aside from modifying the quantity of soil N distributed across space, large herbivores alter the relative abundance of N forms in the soil via their animal returns (Augustine and Frank 2001). The majority of N in urine is present as urea, which is rapidly hydrolyzed to inorganic N (NH_4^+), whereas the bulk of N in large herbivore feces is in organic form (Haynes and Williams 1993). Previous studies have shown that both plant species and microbial communities are capable of direct uptake of inorganic and organic N forms (Bardgett et al. 2003, Weigelt et al. 2005, Harrison et al. 2008, Näsholm et al. 2009). Work from pot and field experiments also suggests that plant and soil responses to homogeneous nutrient inputs may vary depending on whether inorganic or organic N is added (Dunn et al. 2006, Harrison et al. 2008). However, interactions between N form and spatial pattern are largely unknown. Ecosystem responses to heterogeneous N inputs could be modified by N form due to differences in diffusion, mineralization rates or availability for uptake between different N compounds (Hodge et al. 2000a, Jan et al. 2009). In addition, shifts in plant-soil competition for N over time (Hodge et al. 2000b) could promote variation in short- and long-term responses to patchy inorganic and organic inputs.

Here, we investigated plant and soil responses to N form and N application pattern using a factorial *in situ*, grassland experiment. We focus on impacts of uniform or patchy N addition on the soil-plant system in the short and longer term (one and six months respectively), using N forms of increasing complexity (inorganic N, simple amino acid, complex protein) to represent the range of N inputs that commonly occur in grasslands (Jones et al. 2004). We addressed four main hypotheses: (i) patchy N addition will enhance community-level plant production due to increased root biomass and nutrient-use efficiency; (ii) plant biomass will respond more strongly to inorganic compared with organic N forms, whereas carbon-limited microbial biomass will respond more strongly to organic N; (iii) plant and soil responses to patchy N addition will occur over a wider area within inorganic N plots compared to organic N plots, due to the higher mobility of inorganic N in soil; (iv) plant biomass responses to patchy N addition will occur over a wider area within plots compared to microbial biomass responses, due to plant root distribution and root foraging.

2.2 Material and methods

2.2.1 Study site

The study was conducted in a five-year old sown grassland located at INRA-Clermont-Ferrand, France (45°47' N, 03°05' E, 350m a.s.l.). The climate is temperate with a mean annual precipitation of 575mm and a mean annual temperature of 12.4 °C. The plant community is dominated by the grass *Dactylis glomerata*. Additional grass species include *Lolium perenne* and *Festuca rubra*. Other species include legumes (*Trifolium repens*, *Lotus corniculatus*) and forbs (*Taraxacum officinale*, *Achillea millefolium*). The soil type is silty clay loam (2.55% C, 0.23% N), with a pH_{H2O} of 7.8. Prior to this experiment, the site was mown three times a year and not fertilized.

2.2.2 Experimental design

In order to investigate the interactive effects of N form and N application pattern on plants and soil microbes, two spatial pattern treatments (homogeneous, HOM; heterogeneous, HET) were crossed with three N form treatments. The three N forms were ammonium nitrate (NH_4NO_3, inorganic N, hereafter abbreviated as IN), glycine ($C_2H_5NO_2$, a simple amino acid, abbreviated as GLY), and bovine serum albumen (a model protein, abbreviated as BSA). These represent the range of N forms present in the soil soluble N pool of temperate grasslands; NH_4NO_3 is commonly applied in fertilised grasslands, GLY is the dominant amino acid in hydrolysed cow urine (Bathurst 1952), and BSA was used to represent complex organic N forms with high molecular weight present in cow dung. BSA was used rather than cow dung itself to avoid possible confounding effects of other nutrients present in dung (e.g. phosphorous). In addition, a control treatment without N addition was established (total of seven N treatments x 6 replicates = 42 plots).

In April 2012, 95 cm x 95 cm experimental plots were established across the study site. The botanical composition of all plots was determined using the point quadrat method with 25 points recorded per plot. Principal components analysis was used to identify two classes of plots according to the relative abundance of grasses, legumes and forbs (data not shown). Plots were then assigned to experimental treatments such that each treatment included equal numbers of each vegetation class chosen at random (this ensured no significant difference between treatments in the relative abundance of species at the start of the experiment). Vegetation was cut to 5 cm on 23rd April, in line with local cutting practices. Immediately prior to N application, measurements of vegetation height indicated no significant difference between experimental plots (one-way ANOVA, $P>0.05$).

All N treatments were established on 11th May 2012; N application was in liquid form, combined with a simulated small rainfall event (4 mm). In the homogeneous N treatments, dilute N solution was applied across the whole plot. In the heterogeneous N treatments, concentrated N solution was applied to the central 25 x 25 cm patch of each plot (similar in size to cattle dung or urine patches) with distilled water alone applied to remainder of the plot. Total N and water addition were equal for all plots (N application equivalent to 50 kg N ha^{-1}, consistent with values of urea application and amino acid concentrations in grassland soil). Nitrogen loading in the central patch of the heterogeneous treatments (800 kg N ha^{-1}) was consistent with N from cattle excretion (Haynes and Williams 1993). The control plots without N addition received water alone.

Plant communities were left to grow under natural rainfall conditions. Measurements of vegetation height were carried out at roughly ten-day intervals throughout the experimental period in the centre and 'edge' zone within all experimental plots (see description of within-plot zones below).

2.2.3 Harvests and analyses

Plants and soil were harvested at peak biomass (45 days after N addition) and again at the end of October (164 days after N addition), following autumn regrowth (Figure 2-1). In the second harvest, only 27 out of 42 plots were considered (3-4 replicates per treatment) due to significant rodent damage that occurred in some plots during September. At each harvest, experimental plots were cut to a height of 5 cm. Plant biomass data from a 10cm-wide strip around the perimeter of each plot was discarded to avoid edge effects. The remaining 75 x 75 cm zone of each plot which was divided into three sub-zones: the central 25 x 25 cm patch ('centre'), and two concentric 12.5 cm wide strips extending out from the centre ('middle' and 'edge' respectively, Figure 2-2). Plant samples from each sub-zone were dried (60°C, 48h) prior to weighing to determine dry mass. Dried aboveground plant material was ground and analysed for total N content using an elemental combustion analyser (Flash EA 1112 CNS analyzer, ThermoFinnigan, Milan, Italy). Aboveground N concentrations were used to assess the ratio of aboveground biomass to N content (g dry mass g N^{-1}). Biomass: N ratios can be used as proxy of nitrogen use efficiency (NUE, e.g. Fargione and Tilman 2006, Roscher et al. 2008), although the mean residence time of N in the plant also influences NUE (Berendse and Aerts 1987).

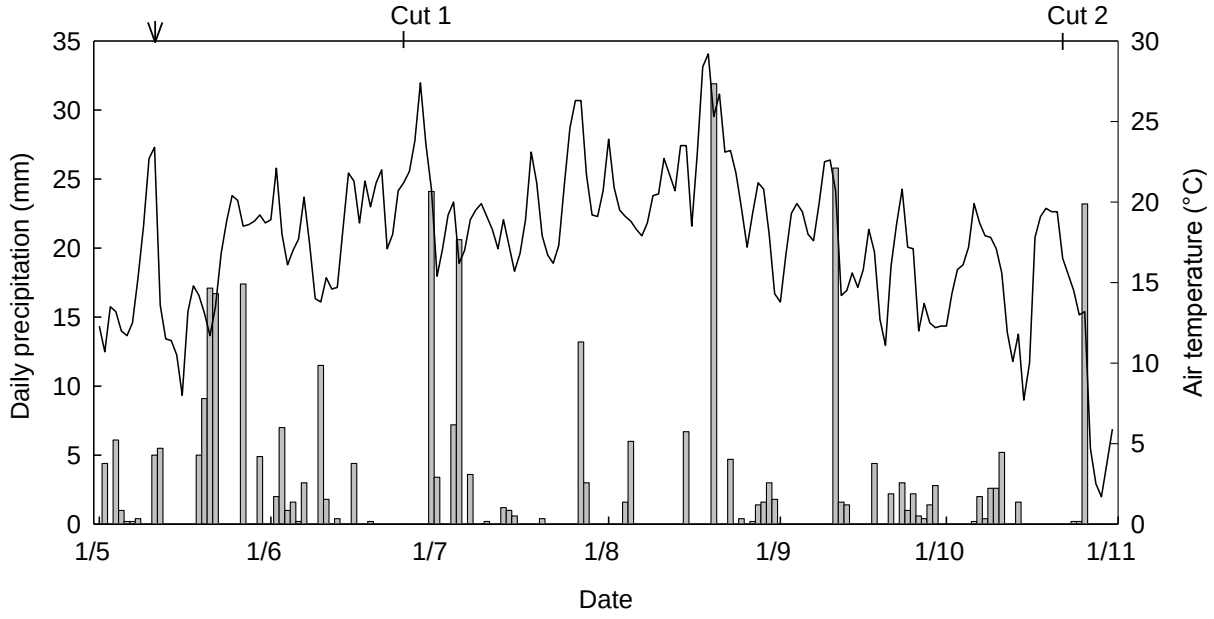

Figure 2-1 Daily precipitation and air temperature recorded over the experimental period. Timing of N application is indicated by an arrow.

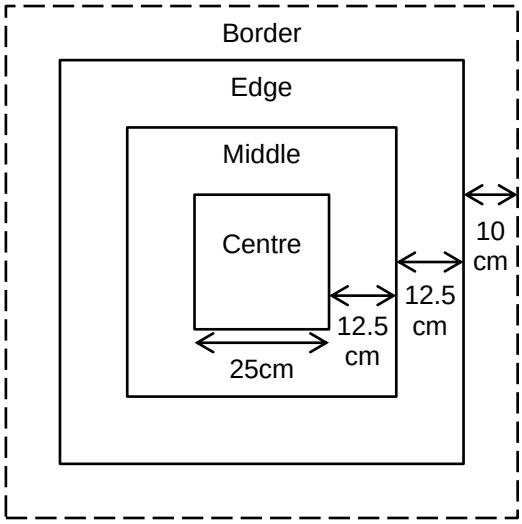

Figure 2-2 Schematic diagram of within-plot sampling zones for each experimental plot.

Two soil cores were taken in each of the three sub-zones (2.5 cm diameter, 15 cm deep); soil cores were pooled per sub-zone and per plot, returned to the laboratory and maintained at <5°C prior to analysis. All soil samples were sieved at 2 mm and the remaining below-ground plant biomass, coarse soil and organic debris were washed to extract and separate roots and rhizomes. Root samples were dried (60°C, 48h) prior to weighing to determine dry mass. Microbial biomass C was measured on 5 g subsamples of the sieved soil using the chloroform fumigation–incubation method (Brookes et al. 1985). Soil samples were

extracted with 25 ml of 0.5 M K_2SO_4 solution, microbial C being calculated as the difference in total C extracted in fumigated and unfumigated soils (k_C = 0.35 as the adjustment factor, Sparling et al. 1990). Non-fumigated extracts were used as an estimate of dissolved organic C (K_2SO_4-extractable DOC). Soil mineral N ($NH_4^+$and NO_3^-) was extracted by shaking 5 g of freshly sieved soil with 25 ml 1M KCl on an orbital shaker for 1 hour. The KCl extracts were filtered through Whatman glass microfibre filters and analyzed by colorimetric measurements and autoanalyser procedures (Bran & Luebbe AutoAnalyser 3, Hamburg, Germany). Additional 5 g soil samples were oven-dried (105°C, 24h) to determine soil moisture content.

2.2.4 Statistical analysis

Comparisons of vegetation height at different within-plot locations (centre, edge) were assessed with one-way ANOVA at each measurement date during the study period. Harvest data were analysed using a mixed model procedure and a repeated measures, split-plot design (Quinn and Keough 2002). Plots were considered as a random factor, with N form and N application pattern as fixed whole-plot factors, and within-plot sampling location (centre, middle and edge) and harvesting dates as fixed sub-plot factors. Differences between treatments were determined with Tukey's HSD post-hoc tests. Since data for the second harvest was only available for a subset of plots due to rodent damage, we checked that this would not introduce bias into the results; paired T-tests indicated no significant difference between the full and partial dataset for the first harvest ($P > 0.05$) so we assume that the partial dataset at the second harvest provides a good representation of treatment effects. We also checked that discarding the biomass from the perimeter strip did not introduce artefacts in the analysis of aboveground biomass data; data from the first harvest indicated no significant differences between the within-plot biomass of 'edge' and 'border' zones on an area-basis in all N treatments (paired T-test, $P > 0.1$), and split-plot ANOVA for the biomass at first harvest gave qualitatively the same results with/ without the perimeter biomass included. We assume this holds true for the second harvest where border data was not available.

Analysis of harvest data was conducted using the 'nlme' package in R (R Development Core Team 2013). Remaining analyses were conducted using Statgraphics 4.1 (Statistical Graphics Corp., Rockville, Maryland, USA); data were log-transformed to meet assumptions of homogeneity of variance and normality if necessary.

2.3 Results

2.3.1 Vegetation height dynamics

Vegetation height showed a strong and rapid response to N addition irrespective of N form, but within-plot responses (expressed as a difference in absolute vegetation height between N-fertilized and control plots) varied depending on the spatial pattern of N application (HOM versus HET, Figure 2-3). In HOM treatments, vegetation height responded to N addition in the same way across the experimental plots (centre/ edge) whereas in HET treatments, height responses were only observed in the centre of plots (Figure 2-3). Significant height responses to homogeneous N addition did not persist after the first harvest. In contrast, significant height responses to heterogeneous N addition were observed in the centre of plots after the first harvest (Figure 2-3B). These responses were most pronounced in response to organic N addition (GLY, BSA).

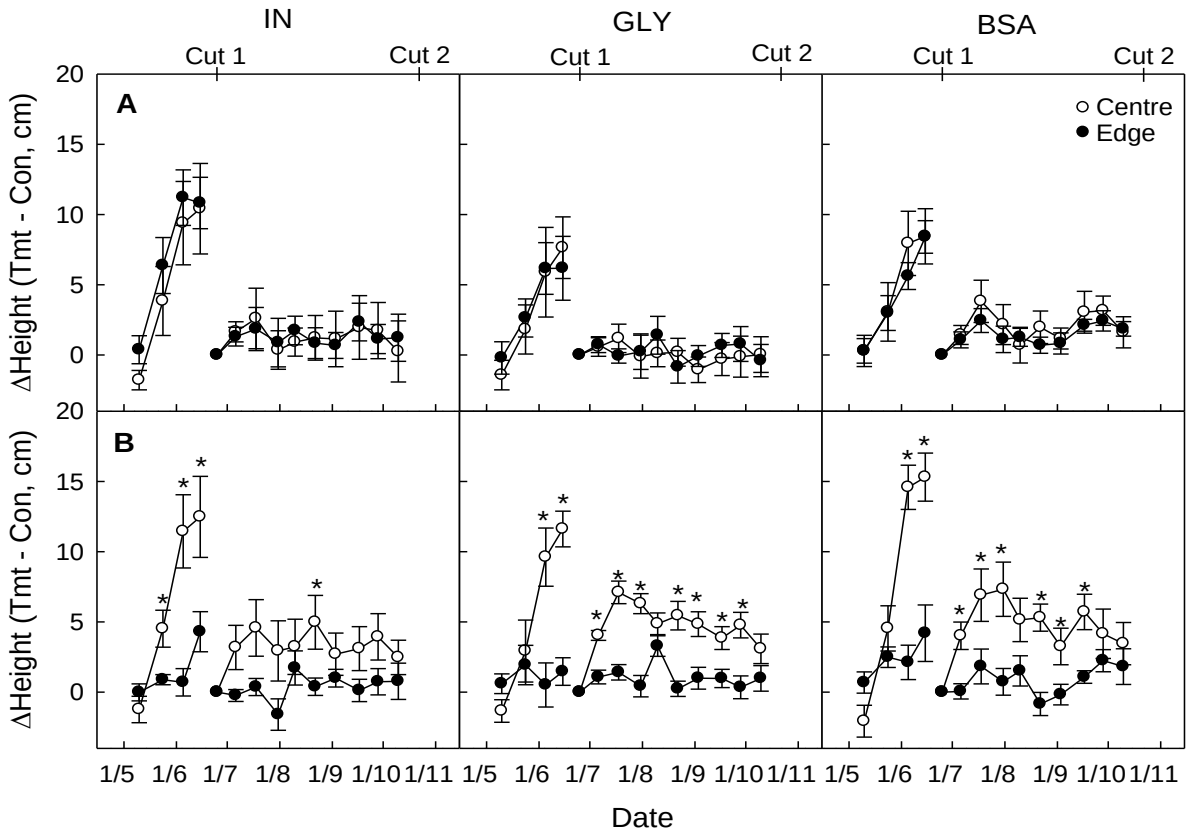

Figure 2-3 The difference in mean vegetation height between N-fertilized and control plots at different plot locations (centre/edge) during the experimental period for homogeneous (A) and heterogeneous (B) nitrogen applications. Nitrogen forms are given by: IN, ammonium nitrate; GLY, glycine; BSA, bovine serum albumin. Means and standard errors are shown (n = 6); significant differences between plot locations per sampling date are indicated by an asterisk ($P<0.05$).

2.3.2 Plant biomass responses to N form and N application pattern within plots

Plant biomass responses to N addition varied with both time and plot location (Table 2-1, Figure 2-4). At the first harvest (45 days after N addition), nitrogen application pattern had a significant overall effect on plant biomass ($F_{1,30}$ = 7.98, $P < 0.05$), with increased plant biomass in heterogeneous N plots (Figure 2-4). However, production increases in the HET treatments varied depending on plot location, and were most pronounced across the centre and middle zones of the plot (Pattern x Location interaction, $F_{2,60}$ = 17.74, $P < 0.05$, Figure 2-4). This pattern was mirrored by belowground biomass in the 0-15cm soil layer (Table 2-2, Figure 2-5). Moreover, aboveground plant biomass showed a significant Form x Location interaction at the first harvest ($F_{4,60}$ = 3.31, $P < 0.05$), driven by strong responses to BSA-Het across the centre and middle zones of the plot (Figure 2-4).

Unlike aboveground biomass, the aboveground biomass: N ratio responded to both N form and N pattern at the first harvest ($F_{2,30}$ = 3.95, $P < 0.05$ and $F_{1,30}$ = 23.65, $P < 0.05$ respectively). Biomass: N was greater in IN/ BSA treatments compared to GLY, and had greater values in HOM compared to HET treatments (Figure 2-6). Biomass: N responses to N treatments did not vary depending on within-plot sampling location.

At the second harvest (164 days after N addition), both aboveground biomass and biomass: N were significantly lower than at the first harvest (Figure 2-4, 2-6). Neither aboveground biomass nor biomass: N showed any response to either N form or pattern of N application, leading to significant Treatment x Time interactions (Table 2-1). Furthermore, experimental treatments had no effect on root biomass in the 0-15 cm soil layer (data not shown).

Table 2-1. Effects of N form, N application pattern, within-plot location, harvesting time and all interactions on aboveground (>5 cm) and belowground (0-15 cm) variables. F values derived from analysis of variance are shown with degrees of freedom (df): significant effects (P<0.05) are given in bold type.

		F values			
Effect	*df*	Aboveground biomass	Biomass: N	Microbial biomass C	DOC[#]
N form (F)	2, 30	0.96	2.06	2.44	**4.70**
N pattern (P)	1, 30	2.72	**15.45**	3.67	**6.62**
Within-plot location (L)	2, 111	**16.50**	**6.09**	**39.40**	**48.74**
Time (T)	1, 111	**172.32**	**89.91**	**60.53**	**46.53**
F x P	2, 30	1.17	1.43	**4.89**	3.05
F x L	4, 111	2.06	0.96	**4.01**	**3.90**
P x L	2, 111	**11.90**	1.51	**24.23**	**15.24**
F x T	2, 111	0.34	**4.07**	**5.62**	0.94
P x T	1, 111	**4.79**	**17.43**	**14.55**	1.95
L x T	2, 111	**11.36**	2.97	**23.33**	**41.86**
F x P x L	4, 111	0.73	1.65	**5.70**	**4.15**
F x P x T	2, 111	0.83	0.42	1.12	2.70
F x L x T	4, 111	**4.13**	0.04	**3.64**	**3.91**
P x L x T	2, 111	**13.53**	0.55	**21.12**	**3.74**
F x P x L x T	4, 111	**2.99**	1.00	**2.71**	0.54

[#]DOC, Dissolved organic carbon.

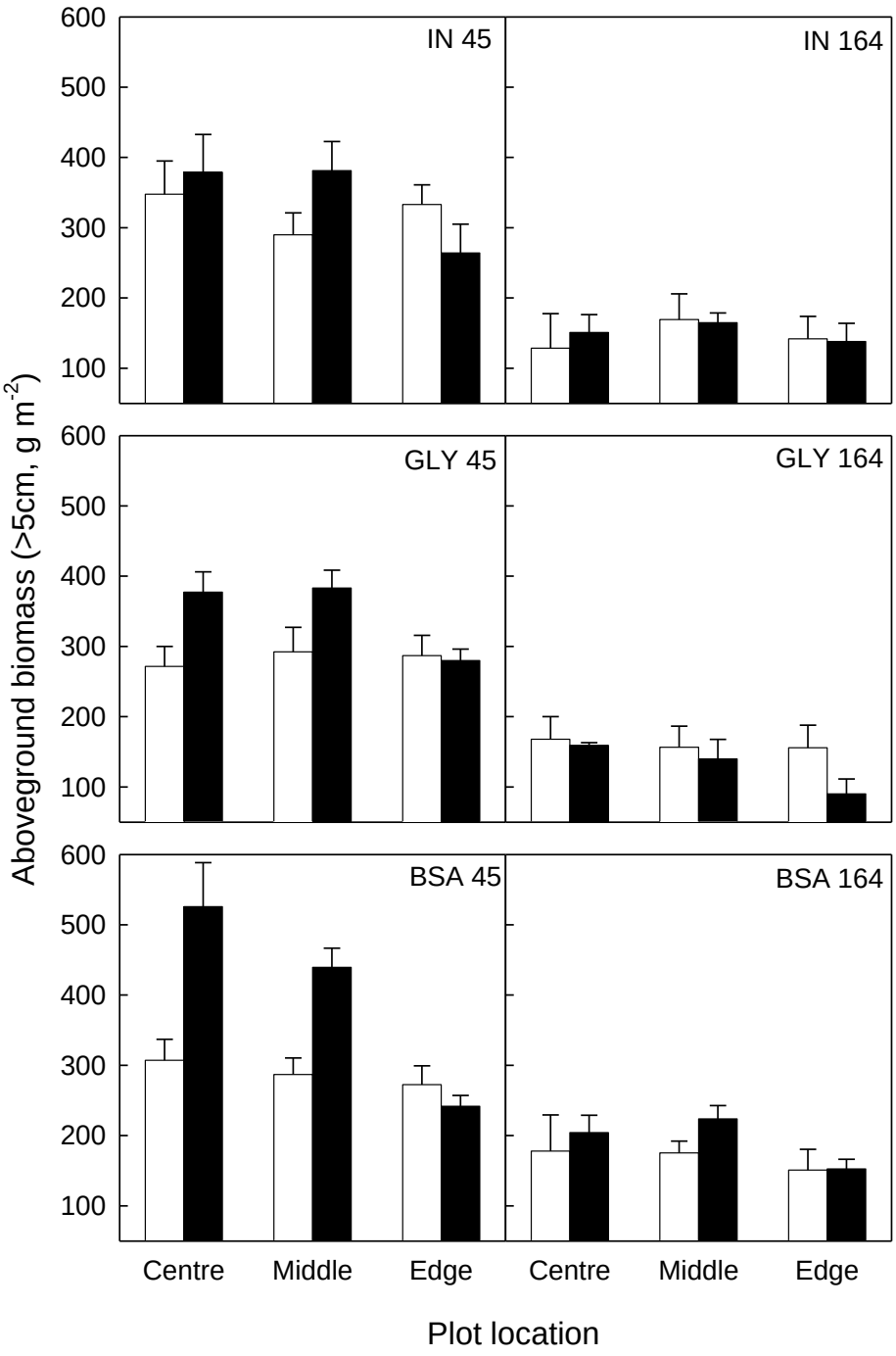

Figure 2-4 Effects of N form and N application pattern on aboveground biomass at different plot locations 45 days and 164 days after N addition. White bars and black bars indicate homogeneous and heterogeneous treatments respectively. N forms are given by: IN, ammonium nitrate; GLY, glycine; BSA, bovine serum albumin. Means and standard errors are shown.

Table 2-2 Effects of N form, N application pattern, within-plot location and all interactions on total soil inorganic N, soil NH_4^+: NO_3^- ratio and root biomass in the 0-15cm soil layer 45 days after N addition. F values derived from analysis of variance are shown with degrees of freedom (df): significant effects (P<0.05) are given by a bold type.

		F values		
Effect	*df*	Total soil inorganic N	NH_4^+: NO_3^- ratio	Root biomass
N form (F)	2, 30	0.29	1.02	0.25
N pattern (P)	1, 30	**43.03**	3.19	**7.37**
Within-plot location (L)	2, 60	**59.07**	**28.67**	**15.97**
F x P	2, 30	**14.19**	0.30	0.24
F x L	4, 60	1.62	**3.46**	0.14
P x L	2, 60	**44.95**	**6.17**	0.38
F x P x L	4, 60	2.27	1.40	1.73

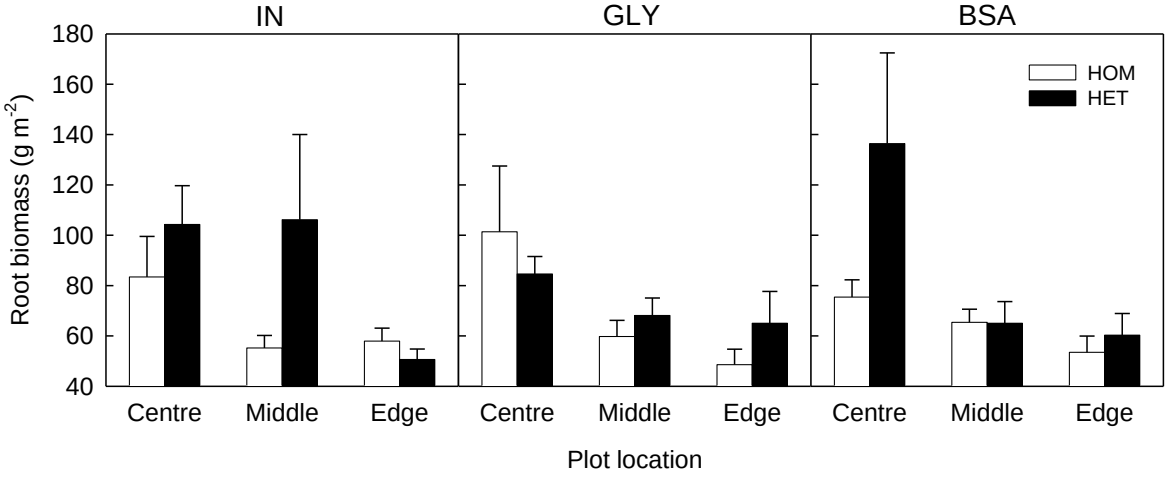

Figure 2-5 Effects of N form and N application pattern on root biomass in the 0-15 cm soil layer at different plot locations 45 days after N addition. Treatments are given by: HOM, homogeneous; HET, heterogeneous; IN, ammonium nitrate; GLY, glycine; BSA, bovine serum albumin. Means and standard errors are shown.

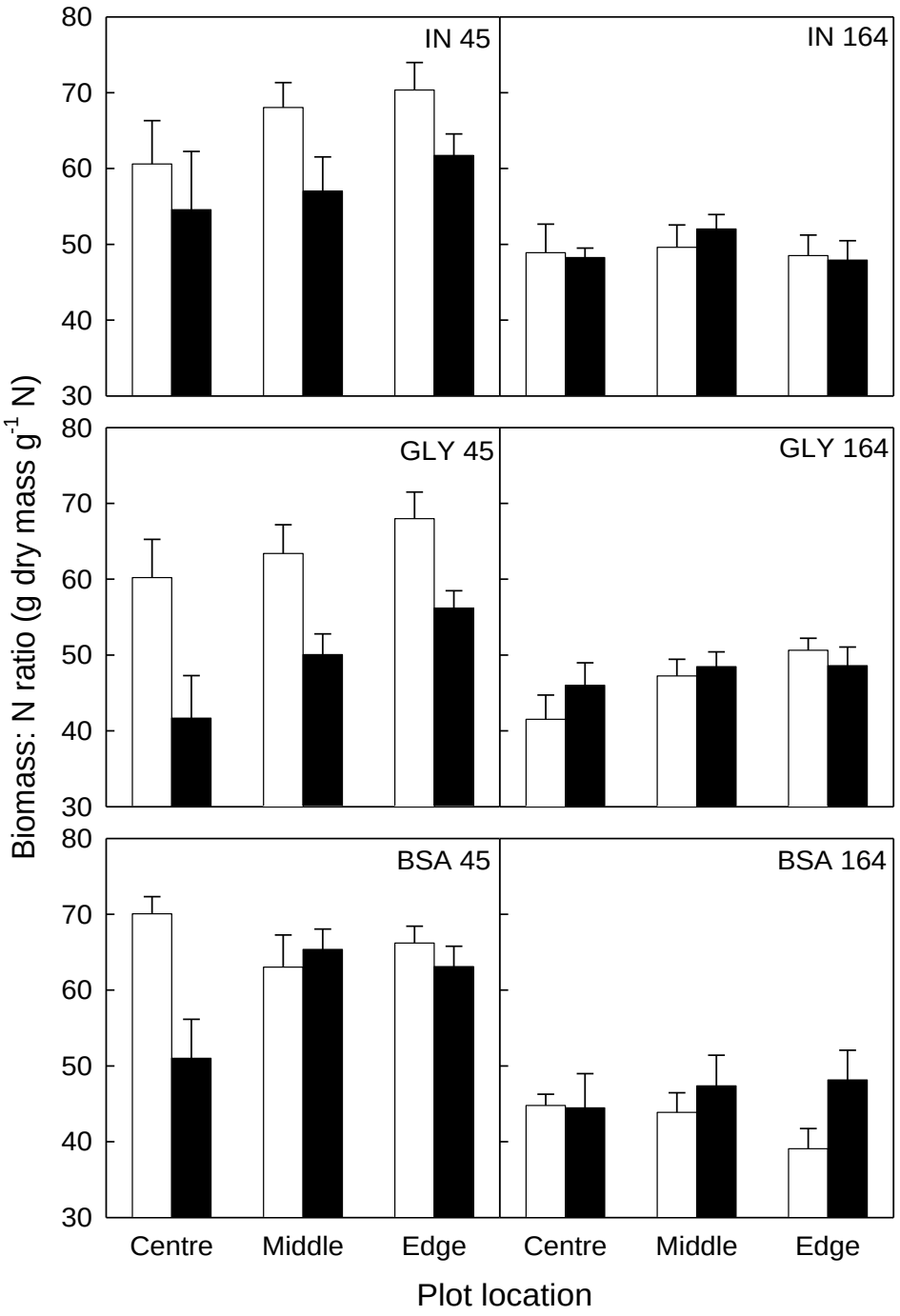

Figure 2-6 Effects of N form and N application pattern on the aboveground biomass: N ratio at different plot locations 45 days and 164 days after N addition. White bars and black bars indicate homogeneous and heterogeneous treatments respectively. N forms are given by: IN, ammonium nitrate; GLY, glycine; BSA, bovine serum albumin. Means and standard errors are shown.

2.3.3 Soil responses to N form and N application pattern within plots

As with plants, soil microbial biomass responses to N addition varied depending on time and within-plot location (Table 2-1). Significant treatment interactions were driven by a large stimulation of microbial C in the central zone of the BSA-Het treatment at the first harvest (+79 % compared to the central zone of BSA-Hom, Figure 2-7). Stimulation of microbial C observed in the central zone of the BSA-Het treatment persisted to the second harvest (Figure 2-7), leading to a significant Pattern x N form interaction at each harvest date ($F_{2,30}$ = 3.95, P < 0.05 and $F_{2,30}$ = 3.83, P < 0.05 for harvests 1 and 2 respectively). Overall, microbial responses to heterogeneous N were limited to a smaller spatial area (the central zone of the experimental plots, Figure 2-7) compared with plant responses. The smaller microbial biomass response to patchy BSA addition at the second harvest resulted in a significant four-way interaction term (Table 2-1).

Responses of dissolved organic carbon (DOC) to N treatments were qualitatively similar to soil microbial biomass responses (Table 2-1, Figure 2-8). Values for DOC were greatest in the central zone of the BSA-HET treatment at both harvests, but the BSA-induced stimulation in the HET treatment was smaller at the second harvest (Figure 2-8).

Unlike microbial biomass and DOC, soil inorganic N showed short-lived responses to N addition treatments. At the first harvest, heterogeneous N sources significantly increased soil inorganic N (Table 2-2); irrespective of N form, increases in soil inorganic N in the HET treatments varied depending on plot location, and were most pronounced across the central zone of the plots (Table 2-3). In contrast, experimental treatments had no effect on soil inorganic N in the 0-15 cm soil layer at the second harvest date (data not shown).

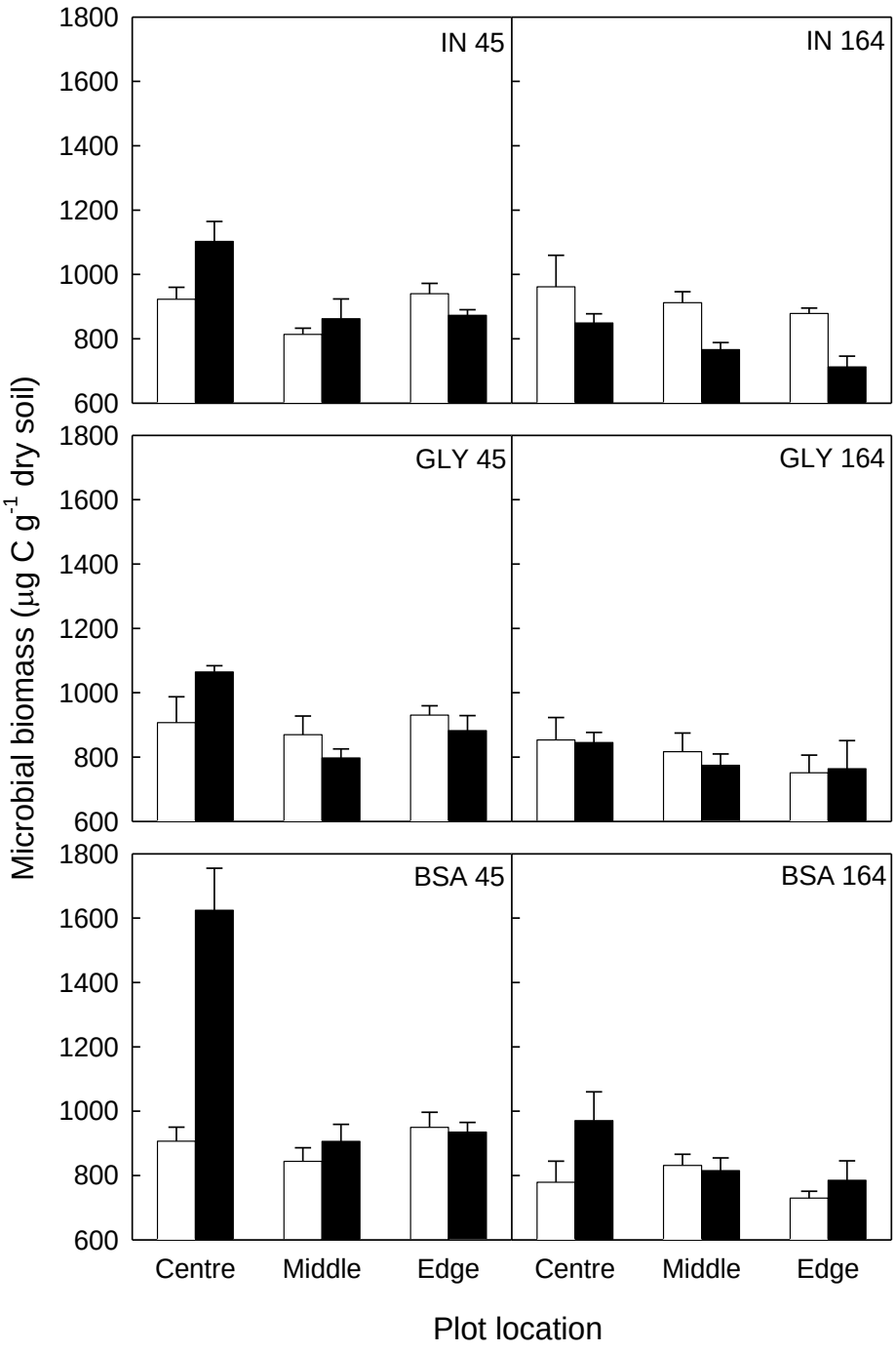

Figure 2-7 Responses of microbial biomass in the 0-15cm soil layer to N form and N application pattern at different plot locations 45 days and 164 days after N addition. White bars and black bars indicate homogeneous and heterogeneous treatments respectively. N forms are given by: IN, ammonium nitrate; GLY, glycine; BSA, bovine serum albumin. Means and standard errors are shown.

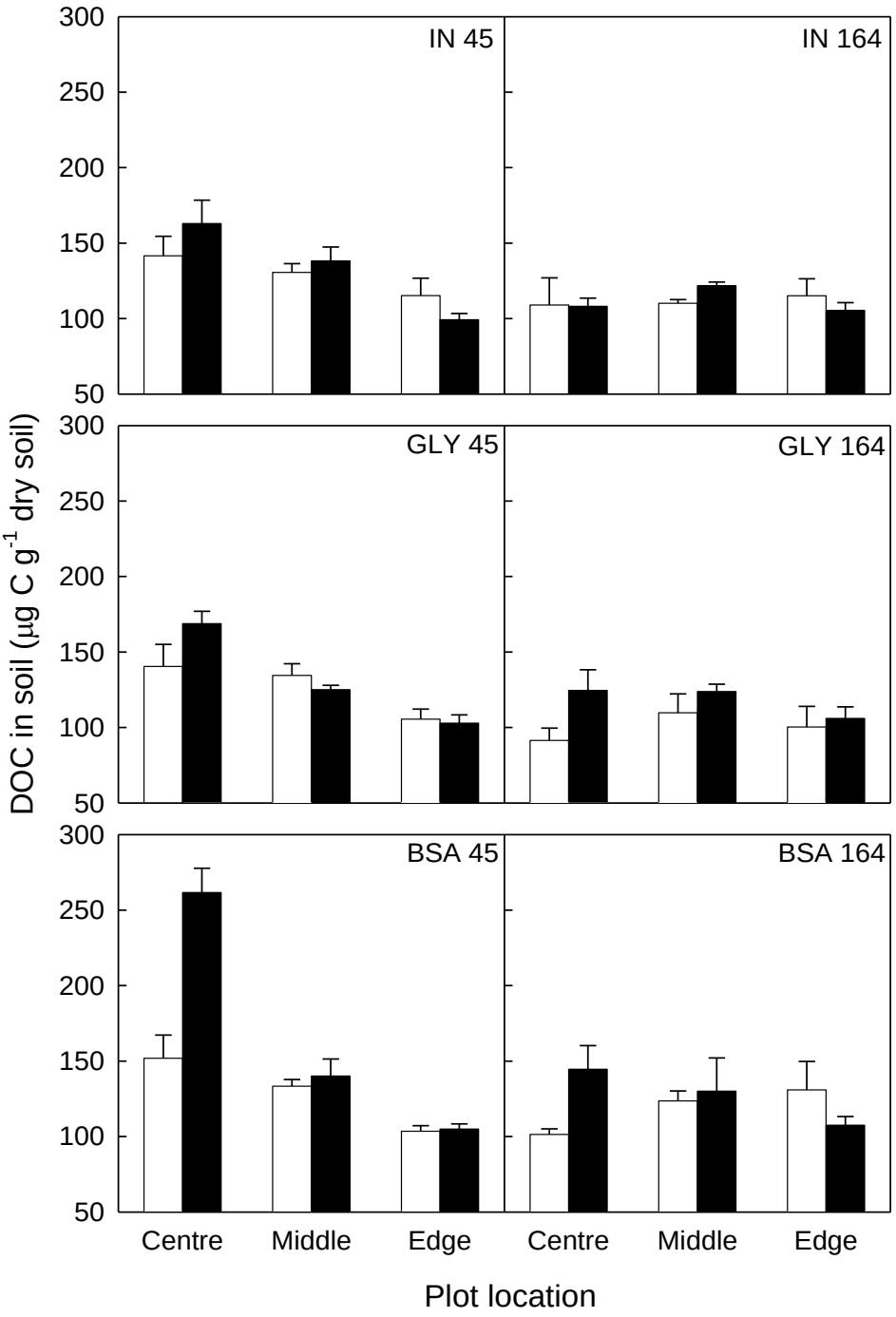

Figure 2-8 Responses of dissolved organic carbon (DOC) in the 0-15cm soil layer to N form and N application pattern at different plot locations 45 days and 164 days after N addition. White bars and black bars indicate homogeneous and heterogeneous treatments respectively. N forms are given by: IN, ammonium nitrate; GLY, glycine; BSA, bovine serum albumin. Means and standard errors are shown.

Table 2-3 Effects of N form, N application pattern on total soil inorganic N and soil NH_4^+: NO_3^- ratio in the 0-15cm soil layer within plots 45 days after N addition. Treatments are given by: HOM, homogeneous; HET, heterogeneous; IN, ammonium nitrate; GLY, glycine; BSA, bovine serum albumin. Mean and SE are shown (n = 6).

			HOM			HET		
Variable		Control	IN	GLY	BSA	IN	GLY	BSA
Total soil inorganic N (mg N g^{-1} dry soil)								
	Centre	4.1 ± 0.6	4.1 ± 0.6	3.1 ± 0.5	6.7 ± 2.0	22.4 ± 2.2	82.3 ± 17.3	15.8 ± 1.4
	Middle	4.2 ± 0.8	5.3 ± 0.7	4.9 ± 2.2	4.2 ± 0.5	4.3 ± 0.9	4.9 ± 0.9	4.0 ± 1.0
	Edge	3.8 ± 0.9	3.7 ± 0.5	2.5 ± 0.5	3.9 ± 0.4	2.8 ± 0.6	4.0 ± 1.4	2.8 ± 0.2
NH_4^+: NO_3^- ratio								
	Centre	0.32 ± 0.14	0.30 ± 0.14	0.37 ± 0.11	1.12 ± 0.90	3.09 ± 0.69	1.32 ± 0.58	4.64 ± 0.43
	Middle	0.06 ± 0.06	0.14 ± 0.08	0.38 ± 0.24	0.06 ± 0.05	0.10 ± 0.05	0.01 ± 0.01	0.05 ± 0.04
	Edge	0.10 ± 0.09	0.14 ± 0.04	0.12 ± 0.05	0.11 ± 0.04	0.05 ± 0.05	0.07 ± 0.07	0.08 ± 0.04

2.4 Discussion

Understanding plant and soil responses to spatial variation in N inputs is critical for the improved prediction of field-scale processes and ecosystem service provision from grazed grasslands (Hutchings et al. 2007). To date, however, field studies have rarely addressed the impacts of heterogeneous addition of different nutrient forms on plant and soil processes in multi-species grasslands. The present study addresses this knowledge gap by simultaneously examining the interactive effects of N form and N application pattern on above- and belowground grassland function in an *in situ* field experiment.

2.4.1 Patchy N inputs enhance community-level plant production in the short term

Based on results from micro-and mesocosm experiments using artificial species assemblages (Wijesinghe et al. 2005, Maestre and Reynolds 2007a, García-Palacios et al. 2011), we hypothesized that heterogeneous N inputs would increase plant production more than homogeneous inputs. Our results were consistent with this hypothesis in the short term (45 days after N addition); irrespective of N form, aboveground biomass production was 22% greater in heterogeneous compared to homogeneous treatments. Biomass increases due to patchy N inputs did not persist in the longer term (164 days after N addition), which may reflect greater N uptake and exports from the HET treatments at the first harvest, as well as greater N losses from the high N patches via N_2O emissions or leaching losses (Hutchings et al. 2007).

Enhanced plant production in heterogeneous conditions has previously been attributed to increases in root biomass and root foraging precision which enhance efficient N uptake (Wijesinghe et al. 2005). In the present study, heterogeneous N application was associated with a 23 % increase in belowground biomass recorded in the 0-15 cm soil layer. Increases in root biomass were particularly pronounced in the central zone of HET plots, consistent with root foraging in high N patches. In addition the HET treatments decreased aboveground biomass : N ratios, characteristic of plant responses to high N availability and an N dilution effect (Vázquez de Aldana and Berendse 1997). These observed changes in response to patchy inputs of nitrogen could affect ecosystem nutrient cycling via concurrent changes in litter inputs and root exudates which modify C inputs to the soil and influence N mineralization-immobilization dynamics (de Vries and Bardgett 2012). Thus, although plant responses to patchy inputs disappeared during the growing season, these transient increases in plant production may have carry-over effects on ecosystem function in following years (Vellinga et al. 2010, but see van der Hoek et al. 2004).

2.4.2 Complex organic N forms stimulate both plant and microbial biomass

Although plants are capable of rapid uptake of mineral N, it is generally thought that soil microbes are superior competitors for organic N in the short term (Kaye and Hart 1997, Hodge et al. 2000b). In line with this idea, we predicted that soil microbes would show stronger short-term responses to the addition of complex organic N than inorganic N, with the C in the organic compounds stimulating microbial growth. As expected, BSA-induced increases in microbial biomass were greater than stimulation by inorganic N at the first harvest. Surprisingly, aboveground plant biomass also responded to complex organic N, with equally strong short-term increases following both BSA and inorganic N addition.

Plant biomass may be stimulated by addition of complex organic N if the organic form is mineralized by soil microbes, providing plant-available N in an inorganic form. Incubation experiments have shown that BSA mineralization is slow compared to simple amino acids (Jones and Kielland 2012), but field conditions following N addition (high temperature, soil moisture; Figure 2-1) may have favoured faster mineralization rates of BSA in the soil. However, given the rapid plant height increases observed in all N form treatments, it seems unlikely that mineralization is the sole mechanism at work. BSA-induced increases in plant growth could also arise if plants take up the organic form directly. Increasing evidence suggests that organic N uptake may play a significant role in plant N nutrition (Bardgett et al. 2003, Näsholm et al. 2009) and recent work has shown that plants are able to take up BSA in laboratory conditions (Paungfoo-Lonhienne et al. 2008). As yet, direct plant uptake of proteins remains to be tested with ^{15}N labelling techniques in the field. Of course, similar plant growth responses to inorganic and organic N treatments could also be explained by increased leaching losses of NH_4NO_3 compared with BSA. High soil moisture and regular rain events during the first growth period following N addition are likely to have promoted transfers of mobile inorganic N toward the lower soil layers and increased risks of leaching losses as well as gaseous nitrous oxide losses (Luo et al. 2013). Further work should determine the relative importance of organic N uptake and inorganic N transformations/losses in plant production responses to different N forms.

2.4.3 Plant and soil responses to patchy N inputs differ in spatial scale

Patchy nutrient inputs have the potential to generate significant spatial variation in ecosystem processes via nutrient-induced changes in plant and soil properties inside nutrient patches and in their immediate surroundings (Orwin et al. 2009, Gillet et al. 2010). Given that inorganic N is more mobile in soil than complex organic N forms, we predicted that plant and soil responses to patchy inorganic N inputs would occur over a wider area compared to patchy

organic N inputs. Generally, our results did not support this prediction; N form did not affect the spatial scale at which plant and microbial biomass responses to patchy N inputs were expressed. These findings may indicate greater vertical leaching of soluble inorganic N compared with lateral transfers in our study, as tracer studies in soil have suggested that vertical movement of soluble N occurs at the expense of lateral movement (Williams and Haynes 1994, Olatuyi et al. 2012). Limited lateral diffusion in our study could also reflect the patch size of N inputs. According to Ficks's First Law of diffusion (Fick 1855, cited in Orwin et al. 2009), lateral diffusion out of high concentration patches should be greater in small patches with smaller horizontal distances over which concentrations vary. Work with artificial urine patches of different sizes supports the idea that large patches (similar in size to those used in the present study) retain inorganic N for a longer period of time due to lower lateral diffusion of N out of patches (Orwin et al. 2009).

In line with our final hypothesis, plant biomass responses to patchy N inputs were observed at a greater spatial scale than those of microbial biomass (centre+middle of plot *versus* centre alone for plants and soil microbes respectively). The observed divergence in spatial pattern of plant and microbial responses to patchy N inputs is consistent with root foraging behaviour which benefits plants adjacent to the nutrient patches, as well as those inside the patch (Robinson 1994). Our findings also confirm results from field studies which show that dung and urine patches may stimulate plant growth over areas considerably greater than the original size of the nutrient patch (Haynes and Williams 1993, Gillet et al. 2010). The large size of plants relative to microbes, coupled with spatially-selective root foraging in plants, underlies differences in the way that these two groups of organisms perceive and respond to patchy environments (Ettema and Wardle 2002). Limited evidence suggests that nutrient patch size and distribution in space, particularly inter-patch distances, may be critical for microbial activity and the outcomes of plant-microbial competition for N (Wang and Bakken 1997, Korsaeth et al. 2001, Loecke and Robertson 2009), but this remains to be tested under field conditions.

In addition to scale-dependent variation in plant and soil responses to patchy N inputs, our results pointed to temporal asynchrony in plant-soil responses. Patchy N inputs had no effect on plant biomass at the second harvest whereas small positive impacts of patchy BSA inputs on microbial biomass were still apparent at this time. Previous studies have demonstrated that single additions of organic amendments can have lasting effects on the soil, in part due to soil properties such as clay content which influence the longevity of microbial biomass responses to organic inputs (Wardle 1992, Bach et al. 2010, Kallenbach and Grandy 2011). It is therefore possible that the high clay content of soil in our study system promoted

the maintenance of high microbial biomass following patchy BSA addition. Direct stimulatory effects of BSA addition on microbial biomass may have been further enhanced by indirect, plant-mediated effects, with BSA-induced increases in plant growth increasing inputs of labile carbon substrates to the soil over a longer time-scale via root exudates or plant residues. Such fresh C inputs are known to stimulate microbial biomass and decomposition of recalcitrant soil organic matter, with effects that persist after the soil treatment (Kuzyakov et al. 2000, Fontaine et al. 2010). Thus, both the spatial and temporal asynchrony in plant and soil responses to patchy N inputs recorded here could have significant implications for microbial activity and plant-soil competition for N, with cascading effects on above-and belowground community structure via changes in soil N availability (Ettema and Wardle 2002; Dunn et al. 2006).

2.5 Conclusions

Spatial pattern of N inputs had significant effects on both aboveground plant production and soil properties in the short-term. Unlike plant biomass, microbial biomass and DOC showed significant pattern x N form interactions, driven by a strong positive response to patchy inputs of complex organic N. Patch-induced responses in microbial biomass occurred over a smaller spatial area compared with plant responses, but were longer-lasting. We suggest that such shifts in spatial and temporal patterns of plant and soil responses could modify the competitive balance between plants and soil biota, changing plant-soil interactions and nutrient cycling. Overall our data highlights the importance of spatial heterogeneity in nutrient inputs for grassland ecosystem properties, and indicates that N form may be a greater driver of microbial community function than of plant productivity. Additional work is needed to examine how such impacts of patchy nutrient inputs can be integrated into models of grassland ecosystem function.

Chapter Three

Plant Community Responses to Precipitation and Spatial Pattern of Nitrogen Supply in an Experimental Grassland Ecosystem

Chapter 2 generated the surprisingly result that N form had no significant effect on plant production in patchy conditions. One possibility is that the high water availability during the experiment may have buffered N form effects; this raises the question of the importance of interactions between water and N availability for mediating heterogeneity effects. To address this question, a mesocosm experiment was carried out in semi-controlled conditions to examine possible interactive effects of spatial N pattern and chronic changes in rainfall regimes on grassland structure and function. Use of a standard plant community minimized possible confounding effects of heterogeneous vegetation distribution in the field. For this study, designed by J. Bloor and P. Carrère, I was responsible for setting up and performing the experiment as well as carrying out all data analysis. I also carried out additional plant trait measurements and BIOLOG assays to determine treatment effects on the physiological profiles of the soil microbial community (Appendix 2).

Recent work suggests that soil nutrient heterogeneity may modulate plant responses to global change drivers, but interactions between nitrogen (N) heterogeneity and changes in rainfall regime remain poorly understood. We used a model grassland system to investigate the interactive effects of N application pattern (homogeneous, heterogeneous) and precipitation magnitude manipulation during the growing season (control, +50% rainfall, -50% rainfall) on aboveground biomass and plant community dominance patterns. Our study resulted in four major findings: (1) patchy N addition increased within-plot variability in plant size structure at the species-level, but did not alter total aboveground biomass, (2) patchy N addition increased community dominance and caused a shift in the ranking of subordinate plant species, (3) unlike community-level biomass, plant species differed in their biomass response to the rainfall treatments, and (4) neither aboveground biomass nor community dominance showed significant interactions between N pattern and rainfall manipulation, suggesting that grassland responses to patchy N inputs are insensitive to water addition or rainfall reduction in our temperate study system. Overall, our results indicate that spatial pattern of N inputs has greater effects on species biomass variability and community dominance than on aboveground production. These short-term changes in plant community structure may have significant implications for longer-term patterns of vegetation dynamics and plant-soil feedbacks. Moreover, our results suggest that precipitation magnitude change during the growing season play a limited role on grassland responses to heterogeneous organic N inputs. We emphasize the need to also consider rainfall timing to better predict the effects of precipitation changes on heterogeneous ecosystem function.

This chapter has been submitted to *Oecologia* (Xi, Carrère and Bloor 2015) and has been accepted, pending revisions.

3.1 Introduction

Heterogeneous resource availability is a widespread phenomenon in natural and managed ecosystems, with significant ecological consequences (Caldwell and Pearcy 1994, Hutchings et al. 2003). Spatial heterogeneity in above- and belowground resources has the potential for significant effects on biotic interactions, plant community dynamics and ecosystem processes (Tilman 1988, Collins and Wein 1998, Lin et al. 2010). A number of recent studies suggest that plant responses to heterogeneous resource availability may be modulated by abiotic conditions associated with global change (Fridley et al. 2011, García-Palacios et al. 2012, Hartmann et al. 2013). To date however, knowledge of ecosystem responses to heterogeneous nutrient inputs in a changing environment remains limited.

Previous work under ambient climatic conditions has shown that individual plants often respond to heterogeneous nitrogen (N) inputs by adjusting root: shoot ratio and increasing root production or N uptake in nutrient-rich patches, resulting in improved plant growth (Campbell et al. 1991, Einsmann et al. 1999, Wijesinghe and Hutchings 1999). Increased plant growth in nutrient-rich compared to nutrient-poor patches can also modify population size structure and promote plant size variability in monocultures (Day et al. 2003a, b). Nevertheless, many studies indicate that plant responses to spatial heterogeneity in N inputs are species–specific, driven by differences in growth rates, plasticity and their ability to capture resources from nutrient patches (Farley and Fitter 1999, Wijesinghe et al. 2001, Hodge 2004). Interspecific variation in response to heterogeneous N inputs has the potential to affect community structure (species richness, relative abundance) and productivity via altered competitive interactions (Schwinning and Weiner 1998, Hutchings et al. 2003). Indeed, results from a growing number of studies suggest that soil nutrient heterogeneity promotes plant community biomass in multi-species grassland assemblages (Maestre et al. 2005, Wijesinghe et al. 2005, García-Palacios et al. 2011, Gazol et al. 2013). However, these experiments have found mixed impacts of nutrient heterogeneity on community structure, and the linkages between species- and community-level responses to nutrient heterogeneity remain unclear.

In contrast to the large body of work addressing impacts of nutrient heterogeneity on plant responses under standard environmental conditions, interactions between nutrient heterogeneity and abiotic factors have rarely been explored (García-Palacios et al. 2012). Among abiotic factors likely to influence plant responses to heterogeneous N inputs, precipitation-driven changes in soil water availability deserve special attention. As with N addition, soil water availability exerts significant impacts on plant productivity, community structure and plant-plant interactions in grassland ecosystems (Lauenroth and Sala 1992,

Harpole et al. 2007, Fiala et al. 2009, Gilgen and Buchmann 2009). Moreover, soil N availability is closely linked to water availability through water effects on: i) soil microbial activities and N mineralization, and ii) mobility and losses of inorganic N in soil solution (Fierer and Schimel 2002, Gordon et al. 2008, Borken and Matzner 2009). This is of particular interest in the context of climate change because rainfall regimes are expected to be modified by ongoing climate change, resulting in wetter or drier than average years, as well as an increased frequency of extreme rainfall events (IPCC 2007). Results from an elegant microcosm experiment suggest that aboveground biomass increases due to heterogeneous N availability may be greater in high- compared to low-water availability in low-diversity plant communities (Maestre and Reynolds 2007). However, studies addressing this subject are scarce, and interactions between N heterogeneity and water/ rainfall regimes have yet to be demonstrated for complex plant communities.

Here, we use an outdoor mesocosm experiment to examine interactive effects of N application pattern and contrasting rainfall regimes on species- and community-level responses in a grassland community of nine species. We chose managed grasslands as a model system because they experience both evenly-distributed and patchy inputs of N due to fertilizer management and grazer activity respectively. We focused on responses to homogeneous and patchy addition of organic N during one plant growing season, altering the growing season rainfall regime by experimentally increasing or decreasing precipitation by 50% of the long-term average. Four main hypotheses were addressed: (i) patchy N inputs promote species-level biomass variability; (ii) patchy N inputs increase species- and community-level biomass; (iii) patchy N inputs modify community dominance patterns; (iv) rainfall regimes modify plant biomass responses to patchy N inputs.

3.2 Materials and Methods

3.2.1 Experimental design

The mesocosm experiment was conducted outdoors at INRA-Clermont-Ferrand, France ($45^{o}47'$ N, $03^{o}05'$ E, 350 m a.s.l.). Mean annual temperature at this site is 12.4 °C and mean annual precipitation is 575 mm. The experiment comprised of two treatments in a factorial design: rainfall regime (control, wet, dry) and N spatial pattern (homogeneous, heterogeneous). Each of the six treatment combinations was replicated six times, resulting in a total of 36 mesocosms.

3.2.2 Mesocosm preparation

In September 2012, experimental mesocosms (stainless steel boxes, 50 cm x 50 cm x 40 cm; Figure 3-1) were filled with 100 L of topsoil (0.19 ± 0.003 % N, 2.01 ± 0.05 % C, pH_{H2O} = 5.92 ± 0.002) collected from a nearby grassland. Mesocosms were free-draining (81 drainage holes, 1.5 cm diameter). Prior to mesocosm-filling, soil was homogenized by mixing and large stones were removed by sieving (1.2 cm mesh size). A model grassland community was established in the mesocosms based on species assemblages commonly found in grazed, upland grasslands in central France (Hulin et al. 2011). The community comprised of six grass species (*Alopecurus pratensis*, *Dactylis glomerata*, *Festuca rubra*, *Lolium perenne*, *Poa pratensis*, *Trisetum flavescens*), two forb species (*Achillea millefolium*, *Taraxacum officinale*) and one legume (*Trifolium repens*). This level of species richness is consistent with that found in 50 cm × 50 cm quadrats in the field, i.e. quadrats equivalent to the size of the mesocosms in this study (J.M.G. Bloor, unpublished data). Vegetative tillers/ ramets of mature plants, obtained from five-year old grassland plots at the study site, were transplanted individually into plug trays filled with experimental soil in August 2012 and left to grow for four weeks under glasshouse conditions. One week prior to planting in the mesocosms, plug trays were moved outside to acclimatize.

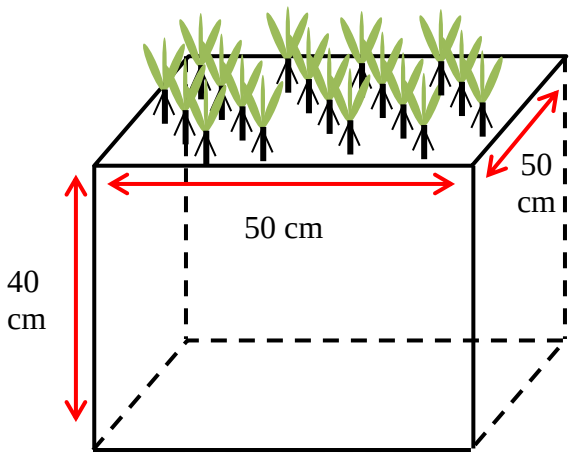

Figure 3-1 Size of mesocosm used in the experiment.

Individual plants of all nine focal species were transplanted into each mesocosm at the end of September 2012 to generate identical community composition in all mesocosms (total of 224 individuals/ m^2). A wire grid fastened to the top of mesocosms was used to generate a consistent planting grid pattern, but planting positions per species within the grid were random for each replicate and treatment combination. Planting densities were consistent with species abundance patterns observed in the field (Table 3-1), and initial community

dominance of each mesocosm was 0.13 (Simpson's dominance index based on numbers of individuals/ clonal fragments per species, Magurran 2004). Following planting, mesocosms were placed in specially-prepared holes in the ground (lined with a 0.3 m deep layer of volcanic gravel) such that mesocosms were flush with the soil surface. Four mesocosms were grouped together to form one experimental block (total of 9 blocks, average distance of 2 m between blocks), and blocks were assigned at random to one of the three rainfall treatments. Regular watering was carried out for two weeks to ensure successful plant establishment across mesocosms. Plants were then left to grow in natural conditions until application of experimental treatments in March 2013. No senescence was observed during the winter months, and a survey of mesocosms immediately prior to treatment application in March 2013 confirmed that community composition (species densities, richness) had not changed since initial planting.

Table 3-1 Planting density of species in the experimental mesocosms

Species	Functional group	Number of plants per mesocosm
Achillea millefolium	Forb	4
Alopecurus pratensis	Grass	6
Dactylis glomerata	Grass	12
Festuca rubra	Grass	10
Lolium perenne	Grass	10
Poa pratensis	Grass	5
Taraxacum officinale	Forb	2
Trifolium repens	Legume	4
Trisetum flavescens	Grass	3

3.2.3 Rainfall treatment establishment

Three rainfall treatments were established based on precipitation during the growing season: control (CON; average rainfall based on long-term weather records), rainfall addition (WET; +50% compared to control) and rainfall reduction (DRY; -50% compared to control). Rainfall at the experimental site was monitored and mesocosms in the control treatment were supplemented by irrigation on a weekly basis where necessary to generate matching patterns with the 30-year average monthly rainfall records (Meteo-France).

For the drought treatment, rainout shelters (Figure 3-2) were constructed with a sloping wooden frame covered by a clear polycarbonate screen (2m x 1.5m, light transmission

90%). Shelters were deployed over DRY blocks periodically (whenever rain was forecast) from 30 March until mid-June 2013, and placed at a minimum height of 0.6 m above the mesocosms to allow near-surface air exchange. Shelters only covered mesocosms during rainy weather conditions to minimize their effects on other environmental variables, and effectively excluded all rainfall from the DRY mesocosms. Rain shelters also served to collect rain for supplementary irrigation and were each equipped with gutters and a water butt (Figure 3-2). Target rainfall levels in the DRY treatment were achieved by low water addition (50 % of control) within 24 h of each rain event using a bespoke sprinkler irrigation system. Target rainfall levels in the WET treatment were achieved by supplementary watering (in addition to ambient rainfall) within 24 h of each rain event.

Figure 3-2 Rainfall shelter equipped with gutters and a water butt.

Control mesocosms received 150.2 mm rainfall during the experimental period, in line with the long-term average at the study site (30 y average of 156.2 mm for the same period). DRY mesocosms received 79.5 mm whereas WET mesocosms received 232.4 mm rainfall, corresponding to -47% and +55% of the control rainfall respectively. Soil moisture was monitored in all mesocosms at roughly weekly intervals throughout the experimental period using an SM200 probe coupled to a HH2 moisture meter (Delta-T Devices, Cambridge, England).

3.2.4 Nitrogen treatment establishment

Two N pattern treatments were established by applying Bovine serum albumen (BSA, a model protein) solution to mesocosms within each block on the 16th April; two mesocosms per block received homogeneous N inputs and the remaining two mesocosms per block

received patchy N inputs. We used BSA to simulate the complex organic N forms present in cow dung, and to avoid the rapid leaching losses associated with inorganic N forms (Xi et al. 2014). Each mesocosm received the equivalent of 50 kg N ha^{-1} combined with a simulated small rainfall event (3.2 mm). In the homogeneous treatments (HOM), 800 mL of dilute N solution was applied uniformly across the whole mesocosm (Figure 3-3). In the heterogeneous treatments (HET), 50 mL of concentrated N solution was applied to central 12.5 cm x 12.5 cm zone of each mesocosm, and 750 mL of distilled water was added to the remaining area (Figure 3-3). The N application rate in the central zone of the heterogeneous mesocosms (800 kg N ha^{-1}) was consistent with N loading from cattle excretion (Haynes and Williams 1993). Results from a previous field experiment have shown that i) grassland biomass shows strong short-term responses to the BSA application rates used here for both HOM and HET treatments; ii) root foraging and plant growth responses to patchy BSA inputs are restricted to plants in and immediately adjacent the high N patch; iii) soil responses to patchy BSA inputs are restricted to the high N patch (Xi et al. 2014).

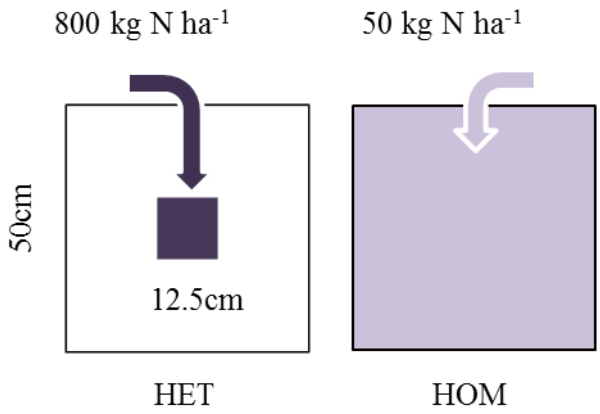

Figure 3-3 Spatial patterns in N inputs. HET, concentrated BSA solution applied to central 12.5x12.5 cm zone, water applied to the remainder of mesocosm; HET, dilute BSA solution uniformly applied to the whole mesocosm.

Non-destructive monitoring of vegetation height inside and outside the nutrient patches was used to check that HET treatments accurately simulated a heterogeneous application of BSA within the mesocosms. Irrespective of rainfall regime, HET mesocosms showed rapid height responses to patchy BSA addition with greater vegetation height (based on average height across all species) inside compared to outside nutrient patches (Figure 3-4). In contrast, vegetation height responses to N addition in the HOM treatment showed no spatial differences within mesocosms during the experimental period (Figure 3-4).

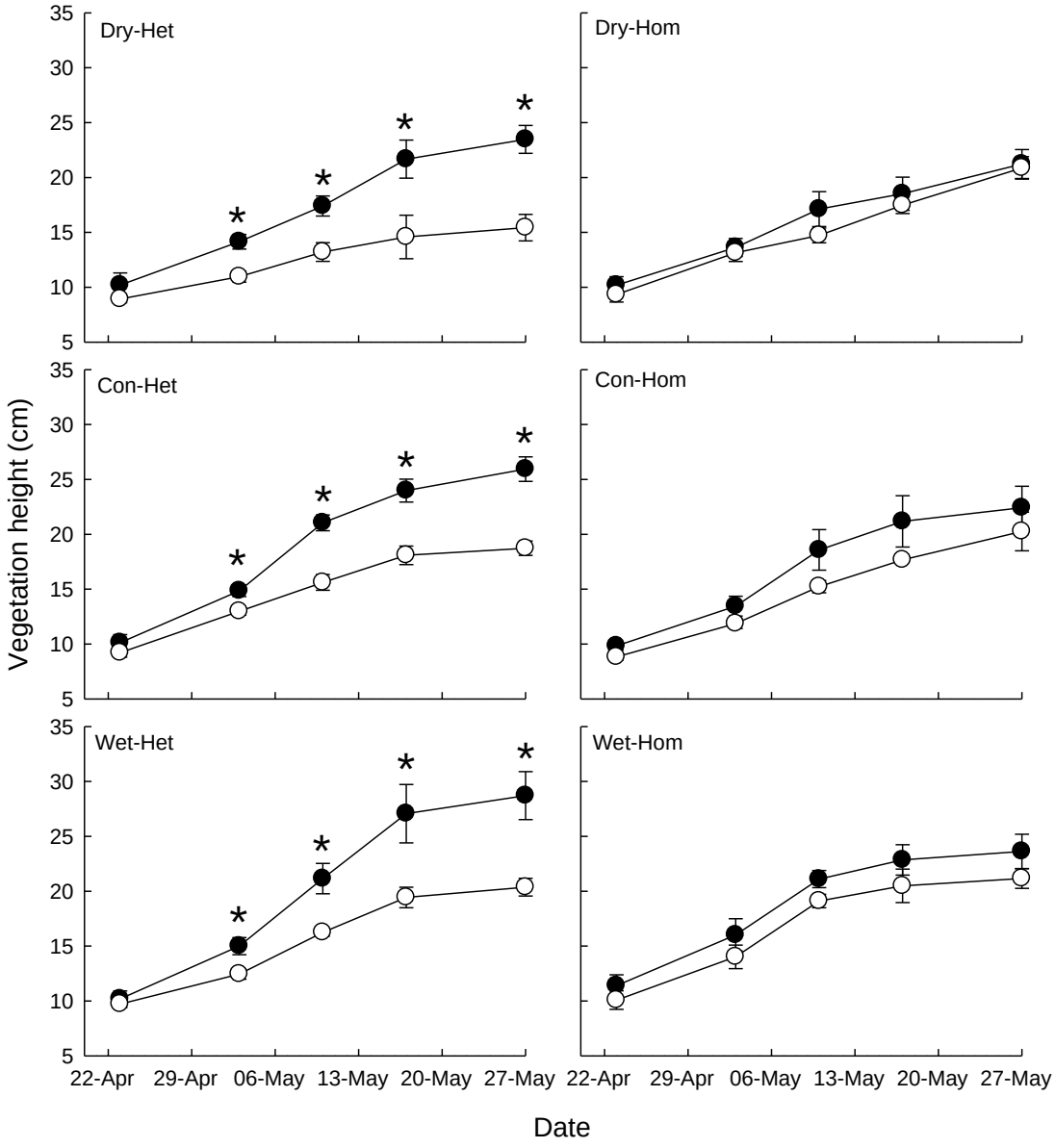

Figure 3-4 Mean vegetation height at different locations within mesocosms (centre, black circles; edge, white circles) during the experimental period. Rainfall treatments are given by: Con, control; Dry, rainfall reduction treatment; Wet, rainfall addition treatment. N pattern treatments are given by: Hom, homogeneous; Het, heterogeneous. Average height across all individuals per species was determined in the central zone versus the periphery. Means and standard errors are shown (n = 6); significant differences between plot locations per measuring date are indicated by an asterisk ($P < 0.05$).

3.2.5 Data collection and calculation

Plant biomass was harvested in mid-June, corresponding to peak biomass for vegetation at the study site. Clonal fragments (aggregation of ramets/tillers derived from a common parent ramet/ tiller) were cut to a height of 5 cm, sorted to species and oven- dried (60 ^{o}C, 48 hr) prior to weighing to determine dry mass. Species biomass variability was determined per mesocosm using the coefficient of variance (CV) of natural log-transformed clonal fragment

biomass to avoid possible confounding effects of inherent species size. CV was calculated for a log-normal distribution following Koopmans et al. (1964):

$$CV = \sqrt{e^{SD^2} - 1}$$

where SD is the standard deviation of clonal fragments per species and per mesocosm. Community dominance (a measure of diversity in terms of species equitability) was assessed using species biomass values and Simpson's dominance index. Simpson's dominance index was calculated as follows:

$$D = \sum P_i^2$$

where P_i is the relative abundance of species i in community and the sum of P_i is equal to 1 (Magurran 2004).

3.2.6 Statistical analysis

Community-level data were analysed using a mixed model procedure for split-plot two way ANOVA, with rainfall treatment as the fixed whole-plot factor, N treatment as the fixed sub-plot factor and block as the random factor (Quinn and Keough 2002). Species-level data were analysed using a mixed model procedure for split-plot three way ANOVA including species as an additional sub-plot factor. Due to high mortality in all treatments during the growing season, *Taraxacum* data could not be analysed at the species-level at final harvest. Soil moisture during the experimental period was analysed using a mixed model procedure and a repeated-measures, split-plot two-way ANOVA. Differences between treatments were determined using Tukey's HSD post-hoc tests.

Mixed model procedures were conducted using the 'nlme' package in R (R Development Core Team 2013). Remaining analyses were carried out using Statgraphics 4.1 (Statistical Graphics Corp., Rockville, Maryland, USA). Data were log- or arcsine-transformed to meet assumptions of variance homogeneity and residual normality where required.

3.3 Results

3.3.1 Soil moisture in experimental treatments

Significant effects of rainfall addition and reduction treatments on soil moisture were detected early in the experiment (Figure 3-5), and persisted throughout the experimental period (repeated measures ANOVA, $F_{2,6} = 351.11$, $P < 0.001$). In general, soil moisture content was lower in the DRY treatment and higher in the WET treatment compared to the control (mean soil moisture difference of -18.0% and +17.1% for DRY and WET respectively versus CON

during the experiment, Figure 3-5). Soil moisture showed no rainfall x N pattern interactions during the study period ($F_{2, 274}$ = 2.06, P = 0.13).

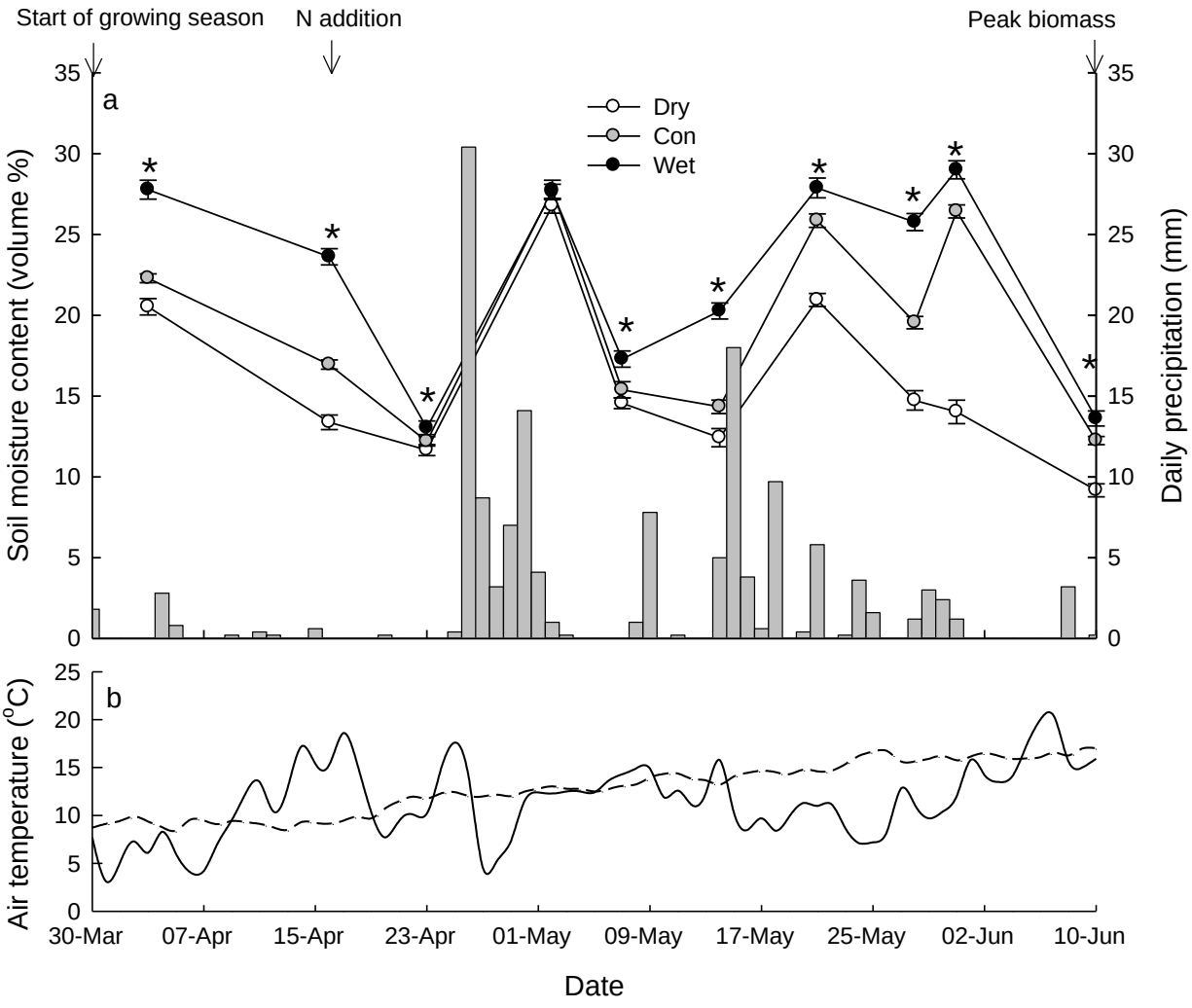

Figure 3-5 Daily temperature, precipitation and course of soil moisture content recorded over the experimental period. Rainfall treatments in (a) are given by: Con, control; Dry, rainfall reduction treatment; Wet, rainfall addition treatment. Means ± SE are shown for soil moisture per rainfall treatment (n = 12); significant treatment differences are indicated by asterisks (P < 0.05). Solid and dashed lines in (b) represent daily air temperature during the experimental period and the long-term average (30 y) for the same period respectively.

3.3.2 Community-level responses to rainfall and N pattern treatments at peak biomass

Aboveground biomass ranged from 415.1 to 552.7 g m^{-2} across treatments at peak biomass (Figure 3-6a). Aboveground biomass did not show a significant response to either rainfall treatment or spatial pattern in N inputs due to significant within-treatment variation (Table 3-2), but there was a tendency for greater aboveground biomass in the WET treatment (+20% on average compared to CON, Figure 3-6a).

Community dominance at peak biomass was significantly higher across treatments compared with initial planting patterns (mean Dominance Index of 0.21 and 0.13 respectively, paired T-test, $P < 0.001$). At peak biomass, community dominance was also significantly greater in HET compared to HOM treatments (Table 3-2, Figure 3-6b) driven by increased abundance of the dominant species. Community dominance showed no response to rainfall treatments or a significant rainfall x N pattern interaction (Table 3-2).

Table 3-2 Effects of interactive rainfall and nitrogen (N) spatial pattern treatments on whole-plot aboveground biomass and community dominance. *F* and *P* values derived from analysis of variance are shown with degrees of freedom (*df*).

		Aboveground biomass		Simpson's dominance index	
Effect	*df*	*F*	*P*	*F*	*P*
Rainfall	2, 6	1.87	0.234	1.21	0.363
N pattern	1, 22	0.660	0.425	4.43	0.047
Rainfall x N pattern	2, 22	0.433	0.654	0.154	0.858

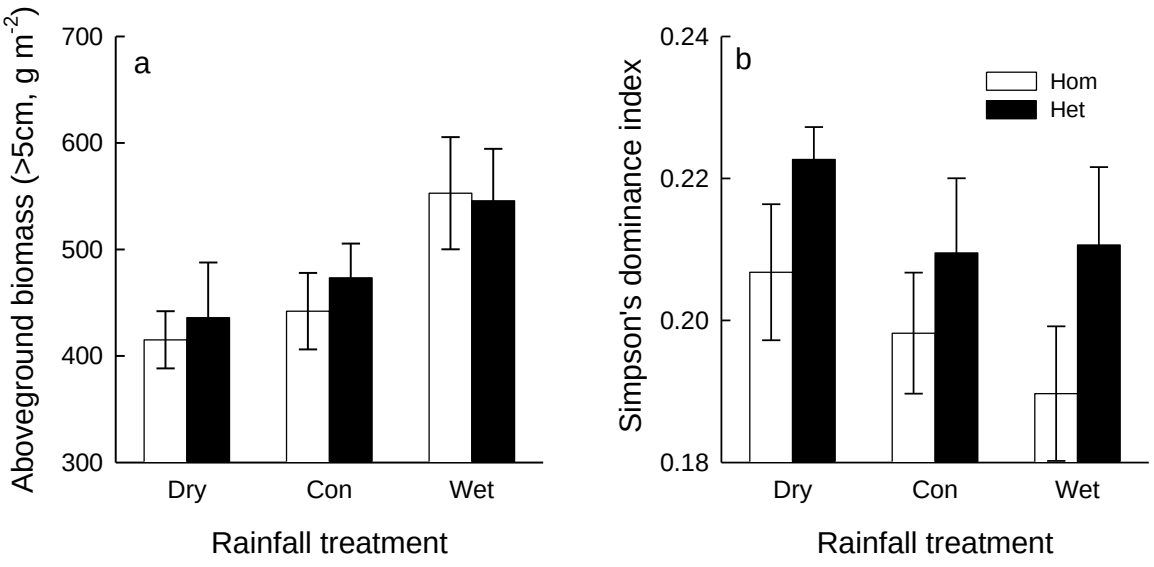

Figure 3-6 Effects of interactive rainfall and nitrogen spatial pattern treatments on (a) total aboveground biomass, and (b) community dominance. Nitrogen pattern treatments are given by: Hom, homogeneous; Het, heterogeneous. Rainfall treatments are given by: Con, control; Dry, rainfall reduction treatment; Wet, rainfall addition treatment. Means ± SE are shown (n = 6).

3.3.3 Species-level responses to rainfall and N pattern treatments at peak biomass

Lolium perenne and *Dactylis glomerata* dominated the plant communities across treatments, accounting for approximately 50% of the total biomass in all mesocosms (Figure 3-7). In general, species-level biomass showed stronger responses to rainfall treatment than to N pattern (Table 3-3), although small HET-induced shifts in the ranking of subordinate species were apparent. Biomass responses to rainfall treatment varied depending on species (significant Species x rainfall interaction, Table 3-3). Species could be broadly classed into three groups depending on their biomass response (Figure 3-7): increased biomass with increased rainfall (*Dactylis glomerata*, *Lolium perenne*, *Trisetum flavescens*, *Trifolium repens*), no clear biomass response to rainfall quantity (*Alopecurus pratensis*, *Festuca rubra*, *Poa pratensis*) and increased biomass in WET/DRY treatments (*Achillea millefolium*).

Unlike total biomass values, species-level biomass variability responded more strongly to N pattern than to rainfall treatment (Table 3-3). In general, HET mesocosms showed greater biomass variability than HOM mesocosms (Figure 3-8). Neither species biomass nor biomass variability showed any interaction between rainfall and N pattern treatment (Table 3-3).

Table 3-3 Effects of interactive rainfall and nitrogen (N) spatial pattern treatments on aboveground biomass and biomass variability across species. *F* and *P* values derived from analysis of variance are shown with degrees of freedom (*df*).

		Aboveground biomass		Biomass variability	
Effect	*df*	*F*	*P*	*F*	*P*
Rainfall	2, 6	4.14	0.074	2.63	0.151
N pattern	1, 215	0.127	0.722	4.51	0.045
Species (S)	7, 215	64.7	<0.001	12.7	<0.001
Rainfall x N pattern	2, 215	0.254	0.776	0.84	0.434
Rainfall x S	14, 215	2.81	<0.001	1.14	0.324
N pattern x S	7, 215	0.434	0.881	1.50	0.167
Rainfall x N pattern x S	14, 215	0.966	0.490	0.44	0.960

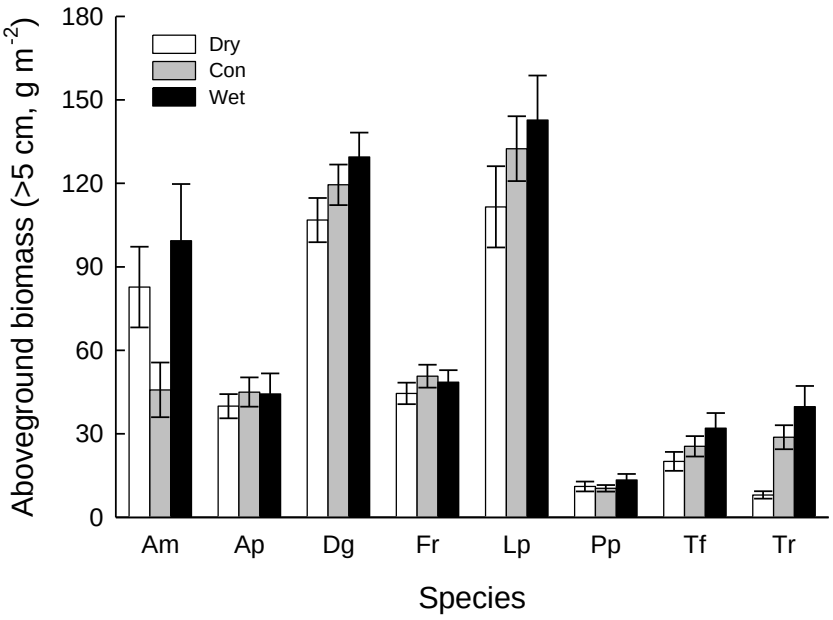

Figure 3-7 Interaction between plant species and rainfall regime on aboveground biomass. Rainfall treatments are pooled across nitrogen spatial pattern treatments and given by: Con, control; Dry, rainfall reduction treatment; Wet, rainfall addition treatment. Species codes are given by: *Achillea millefolium* (Am), *Alopecurus pratensis* (Ap), *Dactylis glomerata* (Dg), *Festuca rubra* (Fr), *Lolium perenne* (Lp), *Poa pratensis* (Pp), *Trisetum flavescens* (Tf), *Trifolium repens* (Tr). Means ± SE are shown (n = 6).

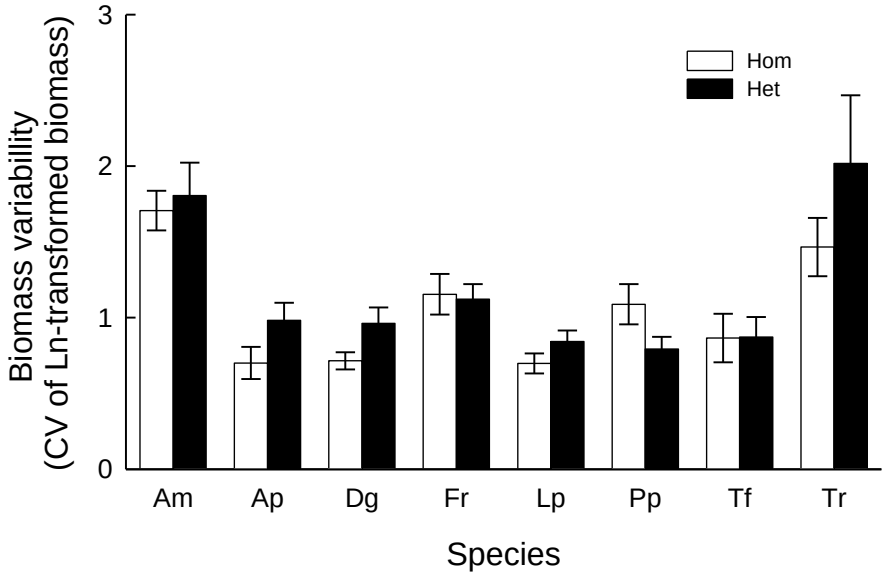

Figure 3-8 Interaction between plant species and nitrogen spatial pattern on aboveground biomass variability. Species codes are given by: *Achillea millefolium* (Am), *Alopecurus pratensis* (Ap), *Dactylis glomerata* (Dg), *Festuca rubra* (Fr), *Lolium perenne* (Lp), *Poa pratensis* (Pp), *Trisetum flavescens* (Tf), *Trifolium repens* (Tr). Nitrogen pattern treatments are pooled across rainfall treatment and given by: Hom, homogeneous; Het, heterogeneous. Means ± SE are shown (n = 6)

3.4 Discussion

Despite growing recognition that the spatial pattern of N plays an important role for plant and soil processes (Maestre and Reynolds 2006, Orwin et al. 2009, Xi et al. 2014), very few studies have examined the interactive effects of patchy nutrient additions and abiotic conditions on grassland community structure and function (García-Palacios et al. 2012). The present study provides valuable information to fill this knowledge gap by examining species- and community-level responses to N application pattern and rainfall regimes in a multi-species grassland experiment.

In line with our first hypothesis, we found greater biomass variability in HET treatments compared to HOM treatments at the species level. Plants inside N patches showed greater growth potential (Figure 3-4), consistent with spatially-explicit patterns of plant growth observed in heterogeneous conditions elsewhere (Day et al. 2003b, Xi et al. 2014). Initial patch-induced growth advantages for plants in N patches may be further enhanced by size-related differences in foraging success and asymmetric competition for both soil resources and light (Schwinning and Weiner 1998). Despite patch-induced changes in plant size and biomass variability, we found no significant effects of N spatial pattern on aboveground biomass at either the species or community level. This contrasts with findings from some mesocosm experiments (Maestre et al. 2005, Wijesinghe et al. 2005), but agrees with the idea that soil nutrient heterogeneity may increase size variation without necessarily affecting mean plant size, and hence aboveground biomass (Casper and Cahill 1996).

Lack of consistent heterogeneity effects on aboveground biomass across studies is perhaps unsurprising since responses to patchy N inputs reflect a complex interplay between patch size (relative to plant root systems) and patch contrast (difference in resource availability inside/outside the patch) (Hutchings et al. 2003). Comparisons across studies are difficult due to differences in methodology (N quantity added, nutrient inputs versus buried nutrient patches, slow-release NPK tablets versus plant litter or organic N solution used here). Interestingly, most previous studies which recorded positive biomass responses to N heterogeneity (e.g. Maestre et al. 2005, Wijesinghe et al. 2005) were carried out using low fertility, soil-sand mixes as a growing medium (rather than 100% soil used here); high patch contrast coupled with higher 'total N input: total soil volume' ratios per mesocosm (0.01 g N L^{-1} in the present study) could in part explain significant heterogeneity effects observed elsewhere. Whole-plot responses to patchy N inputs may also be conditioned by patch density and spatial distribution (Bloor and Pottier 2014). It is possible that the patch size used in the present study (1/16 of mesocosm area) was insufficient to drive shifts in whole-plot biomass. However, it is important to note that absence of aboveground biomass responses to N spatial

pattern may mask significant belowground responses; previous studies have found aboveground biomass to be less sensitive to nutrient patches compared with total biomass (root and shoot biomass combined) (Wijesinghe et al. 2005, Maestre and Reynolds 2007).

In theory, nutrient patch-driven shifts in size inequalities and plant competition should modify species' relative abundance and dominance patterns via changes in plant growth and mortality (Schwinning and Weiner 1998, Hutchings et al. 2003). Previous work with oldfield species in relatively simple mixtures (four species) has shown that patchy soil resources increase plant community dominance and appear to benefit species with greater foraging capacity (Rajaniemi 2011). Our study confirms this pattern for more complex, species-rich grassland communities and supports our third hypothesis; community dominance was greater in HET compared to HOM treatments. Observed increases in community dominance were driven by increased abundance of large-sized, fast-growing species (*Dactylis, Lolium*). This agrees with the idea that large plant size increases the foraging footprint of plants, enhancing species' ability to integrate across small-scale resource patches and dominate heterogeneous environments (Hutchings et al. 2003, Eilts et al. 2011). Moreover, species with fast growth rates are typically associated with an exploitative resource-use strategy and a suite of plant traits such as high root foraging precision which may promote competitive ability in heterogeneous environments (Kembel et al. 2008). These findings add to the growing body of evidence which suggests that small-scale resource heterogeneity has a negative effect on diversity (Eilts et al. 2011, Gazol et al. 2013 but see Williams and Houseman 2014).

Species abundance patterns dictate the distribution and variance of traits within plant communities, with significant implications for functional diversity and biogeochemical cycling (Hillebrand et al. 2008). In the present study we found that HET treatments induced small changes in the species ranking of subordinate species (*Alopecurus, Festuca, Trisetum, Trifolium*; mean species biomass change of 2.2 ± 0.43 % between HOM and HET) rather than shifts in the ranking of dominant species. Numerous studies suggest that the traits of dominant plant species drive ecosystem processes and plant-soil interactions (Grime et al. 1998, Mokany et al. 2008, Grigulis et al. 2013). However, recent work indicates that the influence of subordinate species on soil biota and associated soil processes may be disproportionate to their low biomass (Mariotte 2014). For example, work in grasslands has shown that increasing the cover of a single subordinate species (*Trifolium pratense*) from 0.4 % to 1.6 % significantly improved soil C and N storage (DeDeyn et al. 2011). The small shifts in abundance of subordinate species observed here may therefore result in larger-than-expected changes in belowground processes. Moreover, functional responses mediated by community dominance may be amplified or buffered by phenotypic plasticity and intraspecific trait

variability in patchy environments (Jung et al. 2014). Interestingly, leaf traits recorded for the two dominant species (*Dactylis* and *Lolium*) during the study indicated significant intraspecific variation in response to N patches ($P < 0.05$, T-test comparisons for specific leaf area and leaf dry matter content of plants inside/outside nutrient patches). Thus, the impacts of transient patchy N inputs on dominance patterns, subordinate species and plant trait variability observed here could translate into longer-lasting effects on both plant community dynamics via demographic storage effects (*sensu* Chesson and Warner 1981, Yang et al. 2008) and nutrient cycling via plant-soil feedbacks (Grigulis et al. 2013).

Experimental studies with uniform N addition treatments have shown that grassland productivity is often co-limited by water and N availability, and that N stimulation of biomass may be greater at high water availability (Harpole et al. 2007, St. Clair et al. 2009, but see Bloor and Bardgett 2012). Previous work also suggests that nutrient heterogeneity may interact with altered rainfall patterns to determine plant productivity (Maestre and Reynolds 2007). Given that soil water availability plays a key role for lateral and vertical N transfers in soil, we expected that biomass responses to HOM and HET treatments would converge in particularly dry or wet conditions, with greatest divergence between patchy and uniform treatments in ambient rainfall conditions. In very dry conditions, the magnitude of patch-induced plant growth increases should be small due to decreased rates of N mineralization and plant N uptake within N-rich patches (Bloor and Bardgett 2012). In very wet conditions, increased N losses from N-rich patches due to leaching or gaseous emissions should also constrain patch-induced plant growth increases and hence reduce the biomass difference between HOM and HET treatments. Our results did not support this prediction; neither species- nor community-level biomass (or species-level biomass variability) showed any significant N pattern x rainfall interactions, and community biomass was generally insensitive to altered rainfall regimes. These results suggest that rainfall regime during the growing season has little influence on grassland responses to N spatial pattern in our model grassland community.

Absence of interactions between N spatial pattern and rainfall regime could partly reflect idiosyncratic species' responses to rainfall treatment which confound biomass responsiveness to N. Different species' responses to N pattern and water addition observed in the present study are consistent with findings elsewhere (Maestre and Reynolds 2007), and are likely driven by interspecific variation in plant functional traits linked to water and N-use efficiency (St. Clair et al. 2009). Rainfall x N pattern interactions may also have been confounded by a number of high rainfall events during the study, promoting relatively high soil moisture content across treatments (Figure 3-5). It seems reasonable to suppose that more

extreme rainfall regimes with prolonged periods of drought could have stronger impacts on soil N cycling, and hence N patch dynamics, compared with the 50% 'chronic' rainfall reductions applied in the present study. Rainfall manipulation experiments have demonstrated that rainfall variability and numbers of dry days affect grassland carbon cycling (Knapp et al. 2002), and have the potential to modify plant responses to both water and N enrichment (Kong et al. 2013). The timing of rainfall/ drought events may also modulate grassland responses to resource availability due to concurrent seasonal variation in temperature and plant N demand (Hovenden et al. 2014). Additional work is needed to determine whether the results obtained here for 'chronic' rainfall manipulation can be generalized to extreme rainfall events in different seasons, and to examine the relative importance of inter- and intra-specific plant trait variation for ecosystem responses to patchy nutrients.

Chapter Four

Do Patch Attributes Mediate Effects of Heterogeneous Nitrogen Supply on Grassland Production? A Modelling Study

Results from Chapter 2 and 3 indicate significant impacts of spatial N heterogeneity on grassland structure and function at small spatial scales, but the extent to which these response patterns hold at the larger-scale remain unclear. Investigating large-scale responses to heterogeneity is difficult to do experimentally for logistical reasons (e.g. large surfaces needed, difficulty of sampling at the large scale, time-consuming). During my thesis I had the opportunity to work with a spatially-explicit model of grassland production (recently developed by J. Bloor and Raphaël Martin). This chapter therefore investigates the importance of patch attributes and N timing in mediating larger-scale heterogeneity effects on grassland production using modelling simulations. To carry out this work, I first needed to test the internal stability of this model. I contributed to the experimental design and the elaboration of hypotheses for this study, and was responsible for running the subsequent model simulations and analyzing the data.

Patchy nutrient inputs can be characterized by patch attributes such as patch size and the nutrient contrast between patch and background soil. Timing of nutrient inputs may modify impacts of patchy nutrient inputs due to different plant phenological stages during the growing season. However, the importance of patch attributes and N timing in mediating heterogeneity effects remains unclear. This study investigated interactive effects of patch contrast and patch size, as well as patch size and timing of patchy N inputs on grassland production using two modelling experiments. Total N inputs were kept constant across simulations, to avoid possible confounding effects of N quantity. Results showed that grassland production at peak biomass showed a positive response to patch size but decreased in high- versus low-patch contrast conditions. Negative responses of production to increasing patch contrast appeared to be driven by patch-driven shifts in local-scale plant competition. Patch size did not interact with patch contrast on grassland production. Moreover, N timing did not modify impacts of patchy N inputs on grassland production. The additive effects of patch attributes observed here suggest that studies focusing on single patch attributes are useful for predicting ecosystem function of complex nutrient patch dynamics in natural conditions.

4.1 Introduction

Heterogeneous nutrient inputs are a key feature of grazed grassland ecosystems due to the presence of animal returns (Haynes and Williams 1993, Williams et al. 2000). Such patchy inputs are known to have significant ecological consequences for plants at individual, population, and community levels (Campbell et al. 1991, Day et al. 2003a, Wijesinghe et al., 2005). However, previous studies have found conflicting results on the direction and magnitude of plant responses to heterogeneous nutrient inputs, possibly reflecting the different patch attributes used in these experiments (Huber-Sannwald and Jackson 2001, Hutchings et al. 2003, Hodge 2004). Determining the importance of different patch attributes on plant responses to heterogeneous nutrient inputs is a key step for understanding field-scale grassland function.

Patchy nutrient inputs can be characterized by patch size, patch shape and spatial arrangement (Peters et al. 2006), as well as by their nutrient content and composition which determines the nutrient contrast between patch and background soil (hereafter referred as patch contrast; Wijesinghe and Hutchings 1999). Each of these factors has the potential to modify larger-scale grassland function via shifts in nutrient fluxes across space, as well as subsequent plant nutrient uptake and plant growth. For example, soluble nutrients tend to show greater lateral diffusion out of small compared with large nutrient patches, promoting plant nutrient uptake by surrounding vegetation (Orwin et al. 2009). Moreover, work with annual plants grown in mesocosms indicates both patch size and nutrient contrast can influence individual plant responses to spatial nutrient heterogeneity, with greater root precision and more allocation to belowground biomass and consequently higher whole-plant biomass in large-sized and high-contrast patches compared to small-sized and low contrast patches (Wijesinghe and Hutchings 1997, 1999).

Shifts in plant growth and biomass allocation pattern induced by heterogeneous nutrient inputs may have cascading effects on plant-plant interactions and competition (Hutchings et al. 2003, Schenk 2006). According to a predictive framework proposed by Hutchings et al. (2003), impacts of patchy nutrients on plant-plant interactions are determined by a complex interplay between plant size (and hence plant perception of environmental heterogeneity), nutrient patch size and patch contrast. Small, high-contrast patches are expected to promote intense competition throughout the plant community, whereas large, high-contrast patches are expected to increase plant competition primarily at patch boundaries. Patch boundaries are of particular interest as edge effects and patch ‘perimeter-to-area’ ratios do not scale uniformly with patch size, and are thought to influence species interactions in

diverse systems (Cantrell et al. 2005). To date, however, interactions between patch size and contrast on field-scale grassland production remain unclear.

In addition to being spatially variable, animal returns represent a transient and temporally unpredictable supply of resources (Yang et al. 2008). Nutrient dynamics within such patches, and their subsequent impacts on ecosystem function, may vary depending on the timing of resource inputs due to abiotic factors such as freezing, thawing and rainfall which influence nutrient transfers and biotic soil processes (Bilbrough and Caldwell 1995, James and Richards 2005, Schimel et al. 2007). The capacity of plants to respond to nutrient enrichment also differs depending on the phenological stages of plant growth during the growing season (Pregitzer et al. 1993, Bilbrough and Caldwell 1997). Greater plant responses to patchy nutrients are expected to occur in early vegetative growth stages when plant growth rate and nutrient demand are higher compared to reproductive growth stages (James and Richards 2007). Previous work indicates that some grass species respond to nutrient patches by higher root proliferation and foraging ability when nutrient patches are supplied in early growing season compared to inputs later in the growing season (Eissenstat and Caldwell 1988, Larigauderie and Richards 1994). High potential growth rates during early vegetative growth may also result in intense plant competition for soil nutrients (Bilbrough and Caldwell 1997, James and Richards 2007). However, the impacts of nutrient patch timing on grassland production have rarely been tested.

Here, we use a spatially-explicit grassland model to examine impacts of patch size, patch contrast and patch timing on production of grassland plots subjected to heterogeneous N. We use a temperate grassland community dominated by fast-growing species as a model system and simulate patchy inorganic nitrogen inputs during the growing season. Specifically we hypothesize that, for the same total amount of nutrients applied: (i) field-level grassland production increases with larger nutrient patch sizes due to smaller patch perimeter/ area ratios and hence lower occurrence of intense competitive effects at patch boundaries; (ii) high contrast N patches reduce field-level grassland production i.e. lower biomass in high- versus low-contrast patch treatments; (iii) timing of patchy N inputs modifies grassland production, due to shifts in potential plant growth rates and intensity of plant competition; (iv) timing of patchy N inputs mediates patch size effects on field-level grassland production due to phenology-driven shifts in competitive effects at patch boundaries.

4.2 Methods

4.2.1 Model description

CNSPAT is a spatially-explicit model predicting the dynamics of grassland production based on a non-spatial model developed by Jouven et al. (2006). The field is simulated as a series of $0.1m^2$ cells that are equivalent to the size of a grazing animal feeding station (Figure 4-1; WallisDeVries et al. 1998). Each cell is assumed to be homogeneous in terms of vegetation type and soil characteristics. Simple competition rules allow interaction between adjacent cells. The model is designed to respond to various management regimes (fertilizing, mowing, grazing; Figure 4-2). Inputs include environmental variables (temperature, rainfall, and photosynthetically active radiation), basic soil characteristics (soil water-holding capacity, nitrogen availability index) and a simple vegetation description based on average functional attributes (phenology, leaf lifespan, specific leaf area and leaf proportion in green vegetative biomass).

The vegetation in each cell is divided into four compartments, representing sward structural components (Jouven et al. 2006): green vegetative biomass, dry vegetative biomass, green reproductive biomass and dry reproductive biomass. Each compartment is described by standing aboveground biomass (kg dry mass ha^{-1}). Total growth rate (GRO, kg dry mass ha^{-1} day^{-1}) is calculated daily according to the equation:

$$GRO = PGRO \times ENV \times SEA \times (1 - CS)$$

where PGRO is the potential growth rate of plants obtained in optimum conditions (kg dry mass ha^{-1} day^{-1}), ENV is environmental or abiotic limitation of growth, SEA is the seasonal effect on growth, and CS is the degree of competition or biotic limitation of growth (competitive suppression). Functions for PGRO, ENV and SEA are described by Jouven et al. (2006).

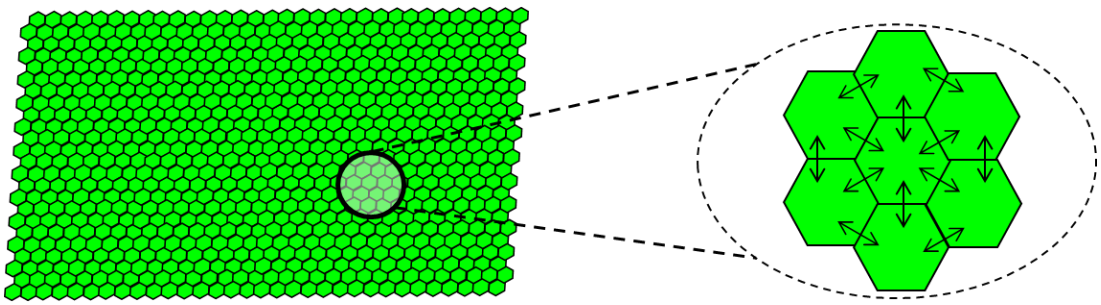

Figure 4-1 Representation of field plots in the CNSPAT model. Arrows show competition between adjacent cells.

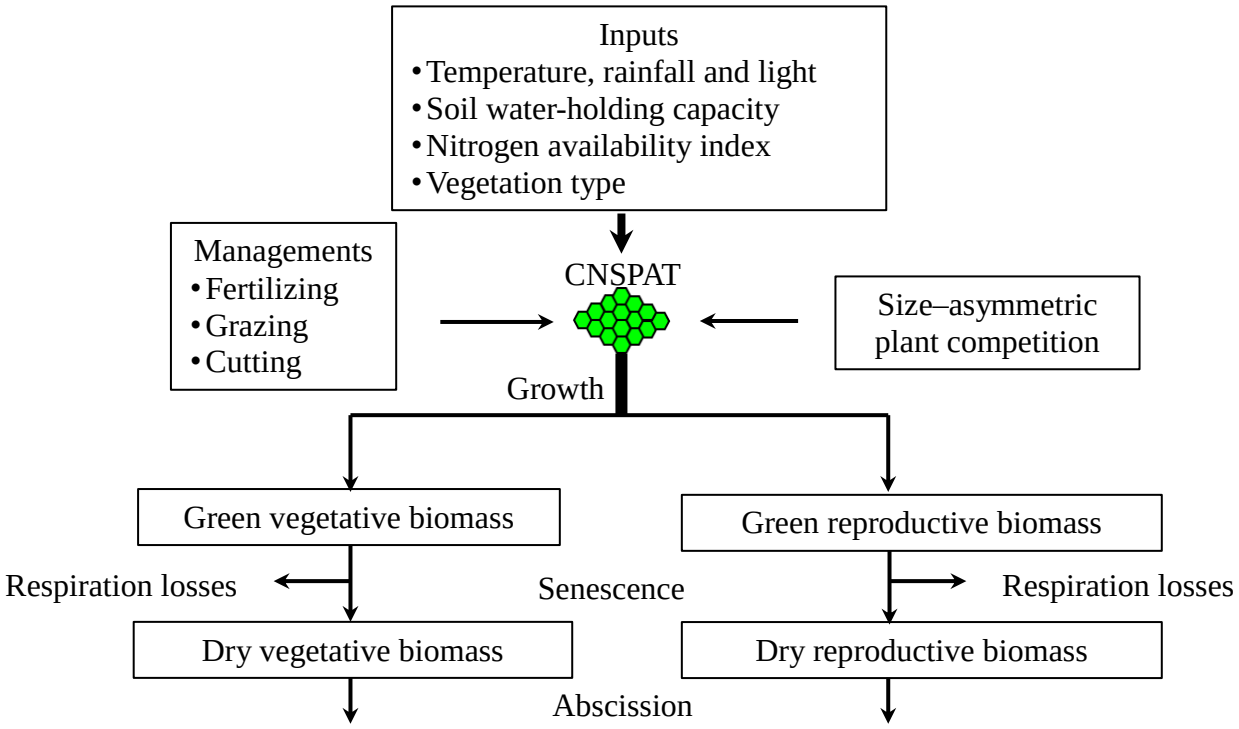

Figure 4-2 Flow diagram of the CNSPAT model. Inputs and outputs are represented by boxes. Management regimes influence grassland production by defoliation or modification of nitrogen nutrition index.

The model simulates competition between adjacent plants by spatial competition rules, and assumes that competition is size asymmetric i.e. larger plants obtain a disproportionate amount of resources and suppress the growth of their smaller neighbours (Schwinning and Weiner 1998). The competition factor CS (an index of competitive suppression experienced by plants within cells) is a function of the ratio of target cell biomass to the average biomass of neighbour cells:

$$CS = min(1 - min\left(\frac{B_i}{\frac{\sum_1^n B_j}{n}}, 1\right)^{\theta}, 0.9)$$

where B_i is aboveground biomass of target cell i, B_j is aboveground biomass of neighbour cell j, n is equal to the number of neighbour cells, and θ refers to the degree of asymmetric competition (Schwinning and Weiner 1998). A recent review of experimental studies indicates that the average competition suppression experienced by grass species is 0.3 for mixed communities, and the maximum suppression experienced by grasses is 0.9 (Kiær et al. 2013). We calibrated θ using the CNSPAT model to obtain an average CS value of 0.3, comparing simulations with varying initial levels of biomass in neighbouring cells and varying degrees of asymmetric competition. For model simulations, θ is fixed at 1.3 in order to minimize the errors associated with this parameter estimation, and CS varies with dynamics of plant growth. When target cell biomass is greater than the average biomass of neighbour cells, competition suppression is equal to 0, implying that growth of vegetation in the target cell is not significantly reduced by the presence of neighbouring cells. When target cell biomass is less than the average biomass of neighbour cells, growth of vegetation in the target cell is reduced by neighbouring cells.

4.2.2 Model simulations

All simulations were run using an experimental plot of 10 m^2 (100 cells) and a fast-growing, temperate grass community characteristic of fertile sites (classified by Cruz et al. (2002) as 'type A' grasses based on functional traits: fast growth rate, high specific leaf area and early reproductive growth). The environmental data used for simulations was obtained from long-term weather records in an upland grazed grassland in central France (Laqueuille, 45°38'N, 02°44'E, 1040m a.s.l.); in the present study, we used nine years of climate data (2004 and 2006-2013, Figure 4-3) with complete records for temperature, rainfall and photosynthetically active radiation as experimental replicates. Mean annual temperature at this site is 7.5°C, and mean annual precipitation is 1200 mm.

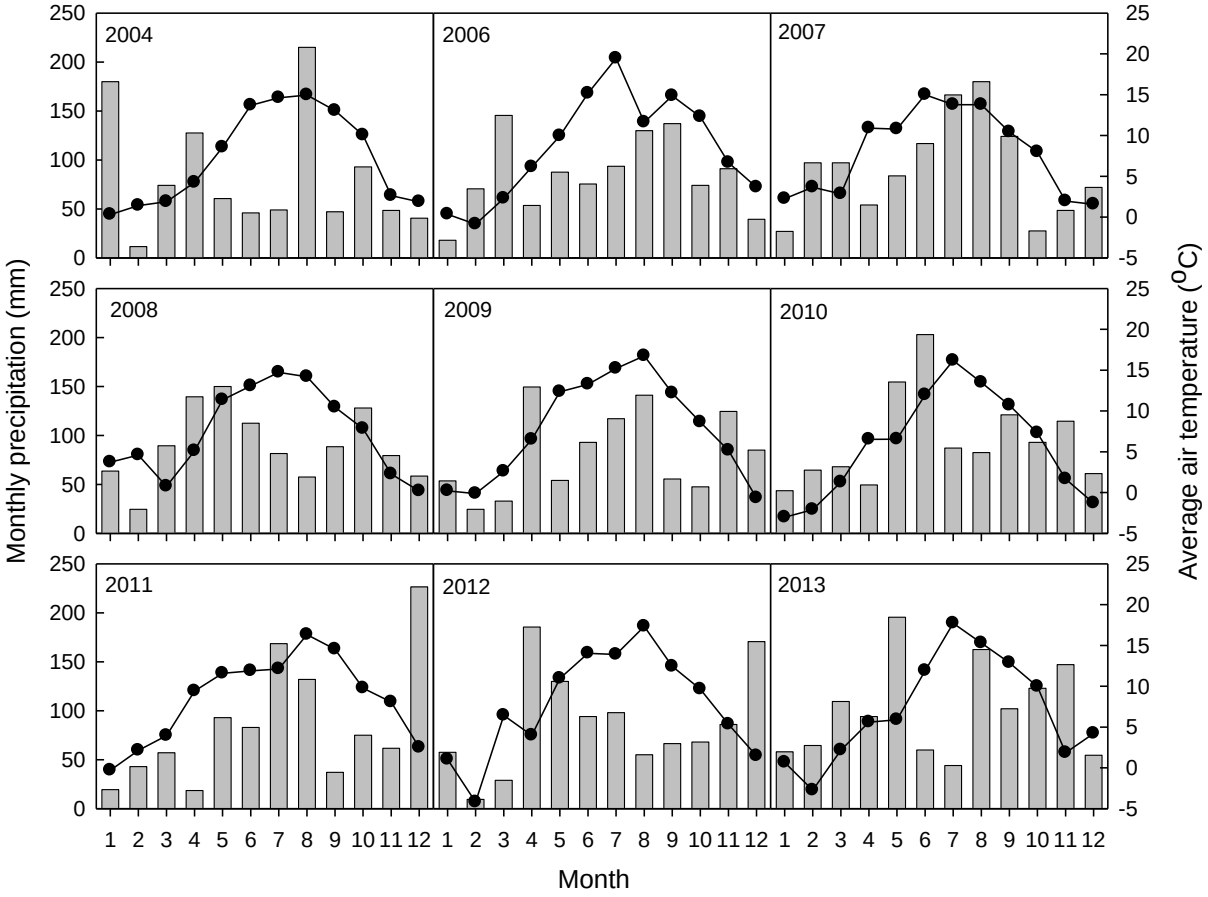

Figure 4-3 Monthly precipitation and mean monthly air temperature recorded over 9 years at the Laqueuille grassland site.

Patchy nutrient inputs in model simulations were generated by an increase in soil nitrogen availability index (NI) for specific cells on a particular date, using a fixed and uniform spatial pattern to avoid possible confounding effects of patch overlap. The levels of NI used in simulations are within the range for temperate grasslands, with 0.6 and 1.0 corresponding to limiting and unlimiting N status respectively (Farruggia et al. 2004). Homogeneous N additions (i.e. NI increases across all cells) were used as controls where appropriate. In the present study, nutrients were assumed to be readily available to be taken up by plants under moist soil conditions; a lag of seven days before significant N effects can be observed on plant growth was incorporated in the model (based on field observations of N effects, Xi et al. 2014).

All simulations were run from the start of the year (January 1st) until peak biomass (June 29th), when total aboveground biomass was harvested. Field-level peak biomass and biomass variability at harvest were determined for each model run using cell averages (kg ha^{-1}) and coefficients of variance (CV).

Experiment 1: Interactive effects of patch contrast and patch size on grassland production

Experiment 1 crossed two patch contrast treatments with four patch size treatments. The two patch contrast treatments were 'High' (NI of 1 inside patches versus 0.6 outside patches) and 'Low' (NI of 0.74 and 0.65 inside and outside patches respectively). Total, field-level NI was equal for all treatments. Patch size treatments were generated with one, two, four and 16 cells in varying configurations (Figure 4-4). All patch size treatments comprised a total of 16 cells within the experimental field i.e. 16% of the surface area, consistent with the proportion of the field surface affected by animal returns in a grazed field (Orwin et al. 2009). The 'size 1' treatment is consistent with the size of individual dung patches created by cattle, whereas 'size 2' and 'size 4' represent the size range of bovine urine patches (Haynes and Williams 1993). Patchy N additions were applied on 15th April (day 105), corresponding to the start of the growing season at the Laqueuille site. Additional model simulations were conducted to examine grassland production responses to soil nitrogen availability using homogeneous NI throughout the growing season (NI levels of 0.6, 0.65, 0.74 and 1.0).

Experiment 2: Interactive effects of patch size and timing of patchy N inputs on grassland production

Experiment 2 crossed two patch size treatments with two dates of N input. The patch size treatments were 'Small' ('size 1' patches as in experiment 1) and 'Large' ('size 16' patch as in Experiment 1). The two timings of N input were 'Early' (15th April, corresponding to the average start of growing season at the Laqueuille grassland site) and 'Late' (20th May, consistent with the onset of reproductive growth at the site). The initial NI in each cell was 0.6, and NI inside patches was increased to 1.0 following N addition. Total NI at whole-plot scale was the same for all treatments. Additional model simulations were conducted to examine grassland production responses to timing of homogeneous N addition ('Early' versus 'Late').

4.2.3 Statistical analysis

Analysis of covariance (ANCOVA; Quinn and Keough 2002) was performed to test effects of patch contrast and size on field-level peak biomass and biomass variability in Experiment 1, using patch contrast as a categorical independent variable and patch size as a continuous covariate. The relationship between patch size and peak biomass was determined using linear regression. Analysis of variance (ANOVA) was conducted to examine effects of patch size and N input timing on field-level production and biomass variability in Experiment 2. Differences between treatments were determined using Tukey's HSD post-hoc tests. All

analyses were conducted using Statgraphics 4.1 (Statistical Graphics Corp., Rockville, Maryland, USA). Data were log- or arcsine-transformed to meet assumptions of variance homogeneity and residual normality where required.

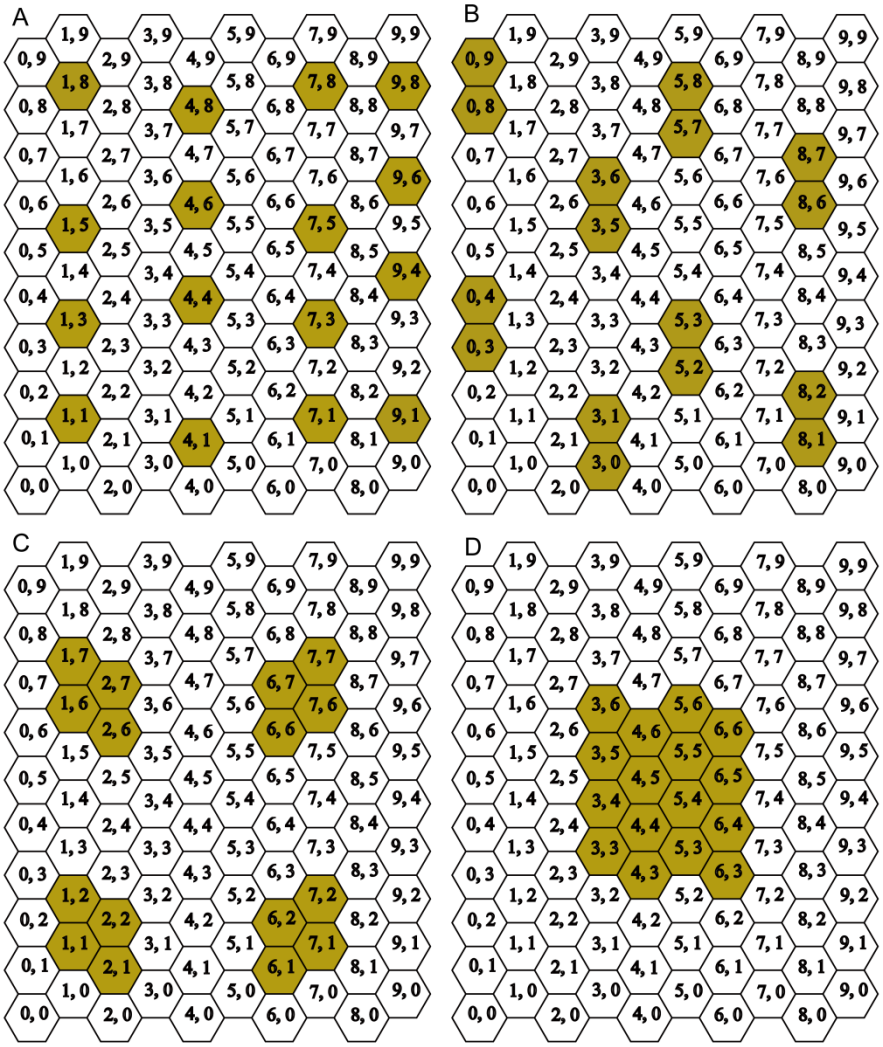

Figure 4-4 Schematic diagram of patch configurations in the plot for (A) 'size 1', (B) 'size 2', (C) 'size 4' and (D) 'size 16'. Coordinates for each cell are shown.

4.3 Results

4.3.1 Effects of patch contrast and size on grassland production

Tests with homogeneous nitrogen inputs showed that peak biomass ranged from 3978.0 to 7035.3 kg dry mass ha^{-1} depending on nitrogen availability (Figure 4-5). Peak biomass showed a significant positive response to soil nitrogen availability (one-way ANOVA, $F_{3, 32} = 15.9$, $P < 0.001$).

Tests with patchy N inputs showed that patch contrast had a significant effect on field-level peak biomass (Table 4-1), with greater plant production in the low contrast treatments

compared to the high contrast treatments (Figure 4-6a). Irrespective of patch contrast, peak biomass showed a significant positive response to patch size (Table 4-1, Figure 4-6a). Biomass responses to patch contrast did not interact with patch size (Table 4-1).

Table 4-1 Effects of patch contrast, patch size and their interaction on simulated peak biomass and biomass variability. *F* values derived from analysis of covariance are shown with degrees of freedom (*df*); significant effects ($P < 0.05$) are given in bold type.

Effect	*df*	*F* values	
		Peak biomass	Biomass variability
Patch contrast	1, 68	**16.73**	**488.78**
Patch size	1, 68	**4.12**	**11.53**
Patch contrast × Size	1, 68	1.54	**7.19**

In general, biomass variability (recorded at peak biomass) was significantly greater in the high contrast treatments compared to the low contrast treatments (Table 4-1, Figure 4-6b). However, effects of patch contrast on biomass variability differed depending on patch size (significant Patch contrast x size interaction, Table 4-1). In the low contrast treatments, biomass variability at peak biomass decreased with patch size, whereas in the high contrast treatments the patch size-induced reductions in biomass variability were greater (significantly steeper slope, Figure 4-6b).

Within-field peak biomass responses to patch contrast varied with field location (significant Patch contrast x Location interaction, Table 4-2). Peak biomass inside N patches was greater in the high contrast treatments compared to the low contrast treatments, whereas the inverse pattern for peak biomass outside patches (Figure 4-7). Effects of patch size on within-field peak biomass differed depending on location (significant Patch size x Location interaction, Table 4-2). Patch size treatments had no effect on peak biomass inside patches, while there was a significant tendency of higher biomass outside patches for increased patch sizes (Figure 4-7).

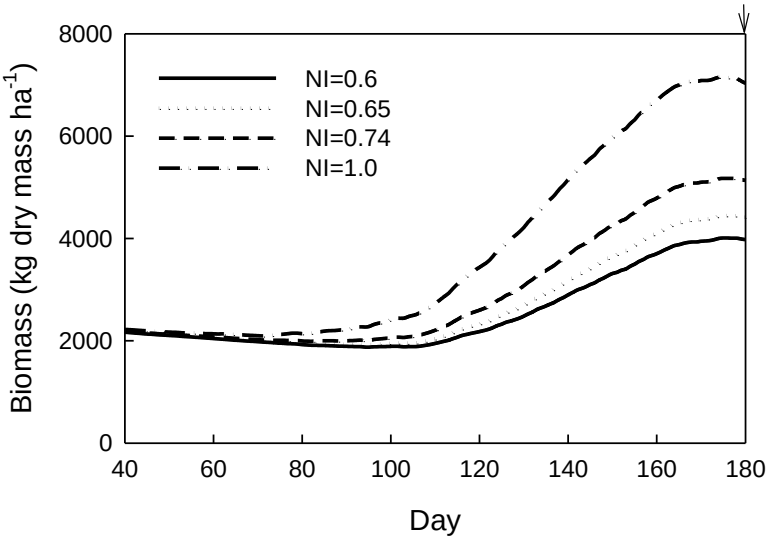

Figure 4-5 Simulated biomass responses to contrasting levels of homogeneous soil nitrogen availability (nitrogen availability index, NI). Harvested peak biomass (the end of June) is indicated by an arrow. Average responses to 9 years of climate data are shown.

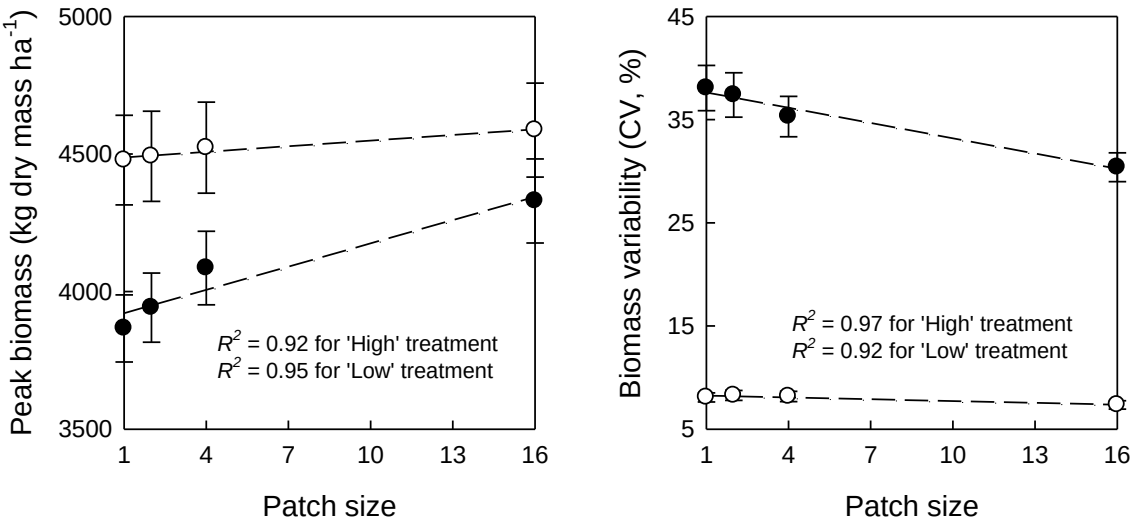

Figure 4-6 Effects of patch contrast and size on (a) simulated peak biomass and (b) biomass variability. Significant regressions between patch size and peak biomass/ variability ($P < 0.05$) are shown using dashed lines. Black and white circles indicate high- and low-contrast patch treatments respectively. Means ± standard errors are shown (n = 9).

Table 4-2 Effects of patch contrast, patch size, location (inside versus outside N patches) and all interactions on within-field plant production at simulated peak biomass. *F* values derived from split-plot three-way ANOVA (with patch contrast and size as the whole-plot factors and location as the within-plot factor) are shown with degrees of freedom (*df*); significant effects ($P < 0.05$) are given in bold type.

Effect	*df*	*F* values
Patch contrast	1, 64	1.90
Patch size	3, 64	0.46
Location	1, 64	**3279.12**
Patch contrast × Size	3, 64	0.39
Patch contrast × Location	1, 64	**1235.50**
Patch size × Location	3, 64	**10.13**
Patch contrast × Size × Location	3, 64	1.81

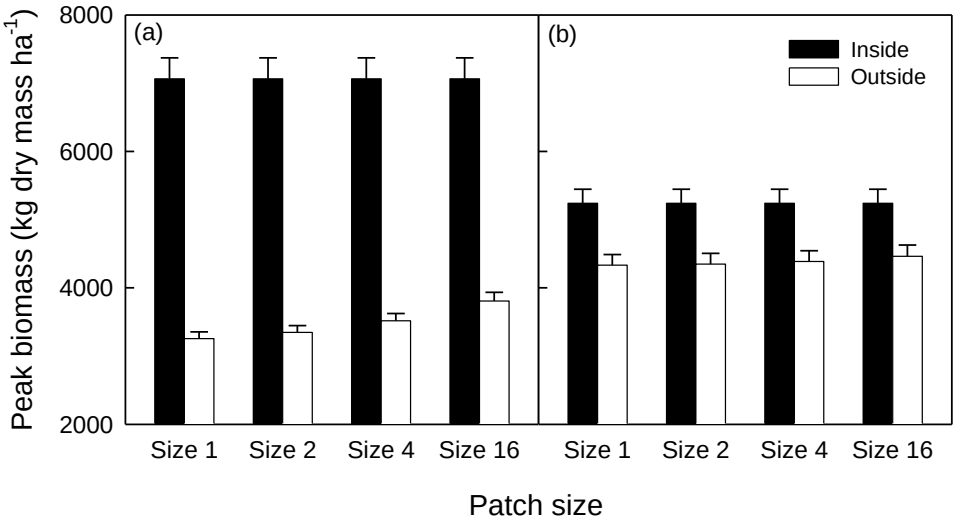

Figure 4-7 Simulated peak biomass at different locations (inside and outside N patches) in the patch size treatments when (a) high patch contrast was applied, and (b) low patch contrast was applied. Means and standard errors are shown (n = 9).

4.3.2 Effects of patch size and timing of N input on grassland production

Tests with homogeneous N addition indicated field-level responses to timing of N input (paired T-test, t = 8.80, P < 0.001), with greater peak biomass for early compared to later nitrogen addition (Figure 4-8). In contrast, field-level peak biomass did not show any response to N timing when N was added in patchy pattern (Table 4-3, Figure 4-9a). Patch size had a marginally significant effect on peak biomass (Table 4-3), driven by contrasting responses between large and small patches in the 'Early' treatment (Figure 4-9a).

Unlike field-level peak biomass, biomass variability was significantly greater in the 'Early' compared to 'Late' N addition treatments (Table 4-3, Figure 4-9b). Biomass variability at peak biomass also responded significantly to patch size (Table 4-3), with stronger responses in small patches than in large patches (Figure 4-9b). Neither peak biomass nor biomass variability showed any significant timing x patch size interactions (Table 4-3).

Effects of timing of N input on within-field peak biomass varied depending on location (significant Timing x Location interaction, Table 4-4). Although peak biomass inside patches was significantly smaller in the 'Late' compared to 'Early' N addition treatments, the 'Late' N addition induced greater peak biomass outside patches (Figure 4-10). Within-field peak biomass responded to Patch size x Location interaction in the same way as experiment 1 suggested (Table 4-4, Figure 4-10).

Table 4-3 Effects of timing of N input, patch size and their interaction on simulated peak biomass and biomass variability. *F* values derived from analysis of variance are shown with degrees of freedom (*df*); significant effects (P < 0.05) are given in bold type.

Effect	*df*	*F* values	
		Peak biomass	Biomass variability
Timing	1, 32	0.53	**102.41**
Patch size	1, 32	3.67*	**4.45**
Timing × Patch size	1, 32	1.43	2.23

*, 0.05 < P < 0.1

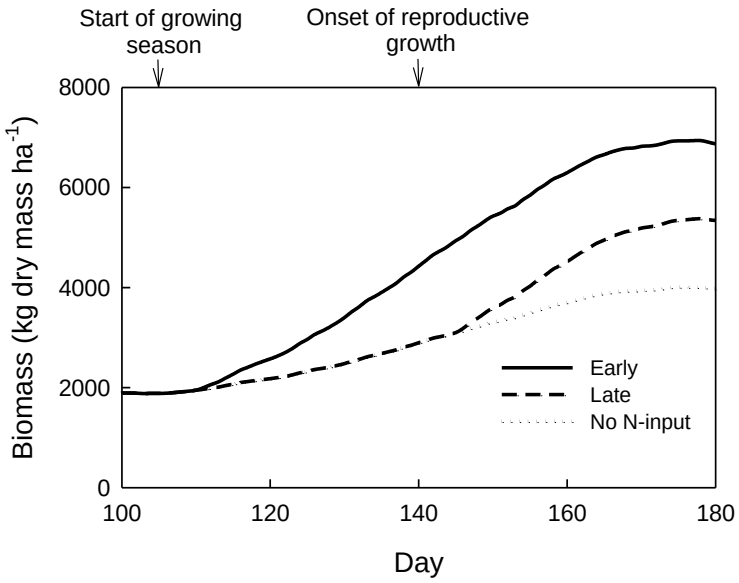

Figure 4-8 Simulated plant responses to timings of homogeneous N input. The start of the growing season (day 105, mid-April) and the onset of reproductive growth (day 140, the end of May) are indicated by arrows. Timings of N input are given by: 'Early', the start of growing season; 'Late', the onset of reproductive growth. Plant growth in the absence of N addition is used as a baseline. Average responses to 9 years of climate data are shown.

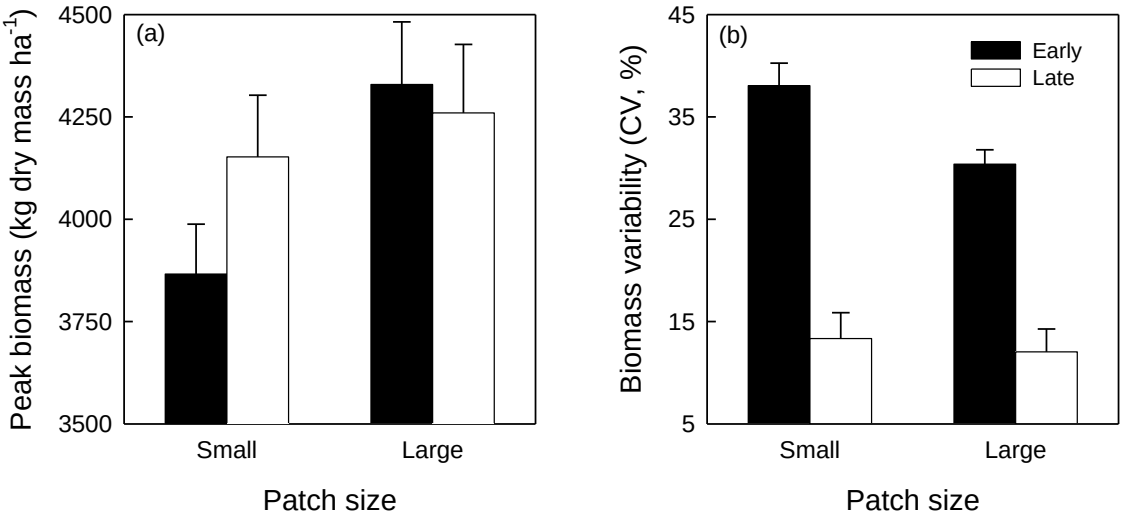

Figure 4-9 Effects of patch size and timing of N input on (a) simulated peak biomass and (b) biomass variability. Timings of N inputs are given by: 'Early', the start of growing season; 'Late', the onset of reproductive growth. Means and standard errors are shown (n = 9).

Table 4-4 Effects of timing of N input, patch size, location (inside versus outside N patches) and all interactions on within-field plant production at simulated peak biomass. *F* values derived from split-plot three-way ANOVA (with patch size and timing as the whole-plot factors and location as the within-plot factor) are shown with degrees of freedom (*df*); significant effects ($P < 0.05$) are given in bold type.

Effect	*df*	*F* values
Timing	1, 32	3.10*
Patch size	1, 32	1.14
Location	1, 32	**546.69**
Timing × Patch size	1, 32	0.50
Timing × Location	1, 32	**86.16**
Patch size × Location	1, 32	**4.87**
Timing × Patch size × Location	1, 32	2.15

*, $0.05 < P < 0.1$

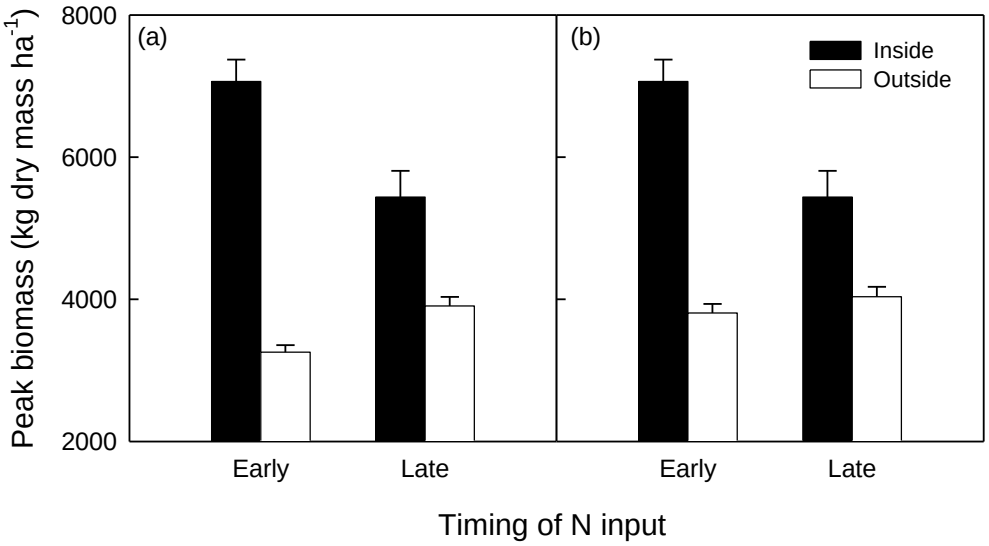

Figure 4-10 Simulated peak biomass at different locations (inside and outside N patches) for small (a) and large (b) patch sizes. Timings of N input are given by: 'Early', the start of growing season; 'Late', the onset of reproductive growth. Means and standard errors are shown (n = 9).

4.4 Discussion

The importance of large-scale patch properties in heterogeneous landscapes has long been recognised (Peters et al. 2006), but surprisingly few studies have examined impacts of patch attributes on ecological processes at the plot or community level (Hodge 2004). In line with Hutchings et al. (2003), we predicted that patch size and patch contrast would mediate effects of heterogeneous nutrient inputs on large-scale grassland production. Our model outputs showed that plot-level grassland production showed a positive response to nutrient patch size for the same overall resource supply, but decreased in high- compared to low-contrast conditions between patch and non-patch areas. These results are consistent with previous work on patch size and contrast at small spatial scales (patch size < 0.1 m^2, Wijesinghe and Hutchings 1997, 1999, Hutchings and Wijesinghe 2008, but see Lamb et al. 2004). To our knowledge, only one study has tested the effects of patch size at larger spatial scales under field conditions (patch size = 0.1 - 0.4 m^2, Orwin et al. 2009). These authors found no significant effects of patch size on plot-level aboveground grassland production despite a positive effect of patch size on plant growth in patches, a result attributed to patch size-related differences in nutrient diffusion.

In the present study, the increase in plot-level production observed in larger patch treatments appeared to be driven by smaller boundary effects (fewer cells experiencing intense plant competition). Boundary effects can be estimated using patch 'perimeter-to-area' ratios (6, 4, 2.5, 1.1 for patches of size '1', '2', '4' and '16' respectively), and decrease as patch size increases. Given that boundary effects suppress plant growth adjacent to nutrient patches via competition, large boundary effects may offset patch-induced increases in plant growth. Conversely, small boundary effects associated with large patches may result in greater plant growth adjacent to nutrient patches in large- compared to small-patch treatments. Within-plot biomass responses observed here support this idea. Our results also showed that plot-level biomass variability decreased as patch size increased, consistent with previous findings that patchy nutrient inputs increase plant size inequalities (Day et al. 2003a, Maestre et al. 2006).

Theoretical work suggests that the degree of contrast between nutrient patches and background soil may influence the rate of plant N uptake, and that high-contrast patches may represent a 'higher value' resource promoting plant growth (Kotliar and Wiens 1990). Indeed, work with clonal plants and 25 x 25cm nutrient patches found a significant yield increase in high- compared to low-contrast treatments (Wijesinghe and Hutchings 1999). However, high contrast between patches and background soil is also expected to increase local competition between plants at patch boundaries (Hutchings et al. 2003). Consequently, plot-level

responses to patch contrast reflect a trade-off between increased plant growth within nutrient patches and decreased plant growth adjacent to patch boundaries. In the present study, plant growth was higher within patches but lower adjacent to these patches in high- compared to low-contrast treatments. The increased plant growth inequality in high-contrast treatments resulted in higher plot-level biomass variability at peak biomass. Lower plot-level grassland biomass in high-contrast treatments almost certainly reflects lower plant growth outside nutrient patches, as the area outside nutrient patches was large relative to the overall area of nutrient patches in the present study (84% versus 16% of the plot area).

Unlike previous work with clonal plants (Wijesinghe and Hutchings 1999), we found no significant interactions between patch size and patch contrast on plot-level plant biomass. Nevertheless, patch size-induced reductions in biomass variability were greater in the high contrast treatments resulting in significant patch size x patch contrast interactions. Local-scale, interactive effects of patch size and contrast on plant biomass may arise due to foraging and morphological plasticity which modify nutrient acquisition and determine plant ability to match fluctuations in environmental conditions (Wijesinghe and Hutchings 1999). Adaptive plant responses are not integrated in the CNSPAT model, so it is perhaps unsurprising that patch size x patch contrast interactions are underestimated in our study. However, our results confirm that interactions between patch attributes could modify plant size inequalities and hence plant-plant interactions at patch boundaries, with implications for longer-term plant community dynamics (Schwinning and Weiner 1998).

Plant responses to sporadic nutrient supply are known to depend on timing of inputs and plant capacity to utilize resource pulses (Bilbrough and Caldwell 1997). Experimental work with grass monocultures has shown that N addition triggers greater plant growth when applied in the early growth stage compared to the late growth stage (Bilbrough and Caldwell 1997). Model simulations using homogeneous N addition confirm this response pattern, with greater peak biomass obtained for N inputs at the start of the growing season compared to the reproductive growth stage. However, contrary to expectations, plot-level grassland production did not show a significant response to timing of patchy N inputs. Higher plant biomass within nutrient patches in the ‘early’ treatments was offset by lower growth outside these patches, resulting in no difference in plot-level biomass between ‘early’ and ‘late’ N treatments. Our results suggest that patch-induced shifts in plant size inequalities, coupled with increased competition between fast-growing grasses during the vegetative growth stage (James and Richards 2007), may buffer field-level production responses to timing of patchy N inputs.

Given that timing of N addition can modify plant-plant competition for N (James and Richards 2007), we predicted that timing of N inputs would alter plant competition at patch

boundaries, and hence effects of patch size on grassland production. Our model outputs did not support this hypothesis; neither plot-level grassland production nor biomass variability showed any significant interaction between timing of N inputs and patch size. Of course nutrient patches vary in terms of their persistence and decline over time, governed by within-patch soil processes and interactions with surrounding matrix (Peters et al. 2006). Including patch dynamics and basic soil processes (e.g. nutrient diffusion) in the CNSPAT model would provide additional insight into the impacts of spatiotemporal heterogeneity on large-scale grassland function.

4.5 Conclusions

Overall our results indicate that patch size and contrast mediate effects of heterogeneous nitrogen inputs on grassland production, for a fixed amount of total N. Plot-level production was positively correlated with patch size but showed a negative response to increasing patch contrast as a result of patch-driven shifts in local-scale competition, specifically at patch boundaries. Unlike homogeneous N addition, responses to patchy N addition were unaffected by timing of N inputs during the growing season. This highlights the importance of including spatial heterogeneity in field-scale models of grassland function for accurate predictions of large-scale ecosystem responses to fluctuating environmental conditions. Interestingly, we found no significant interactions between patch size and contrast, or between patch size and N timing, on plot-level production. This suggests that effects of patch attributes on grassland production may be additive, and that studies focusing on single patch attributes may provide valuable information on ecosystem function in absence of other patch attribute treatments. Additional work is needed to examine whether modelling results observed here can be generalized across grassland ecosystems with varying botanical composition and climates.

Chapter Five

General Discussion

Impacts of spatial nutrient heterogeneity/ patchy nutrient inputs on plant function have faced increasing attention in recent years. These studies have focused on different ecological levels and have used a range of methods, making comparisons among studies difficult. Indeed, most studies have worked with different patch scales, nutrient complexities and concentrations in patch, and been conducted under different environmental conditions (see Hodge et al. 2000b, Maestre and Reynolds 2006, Hutchings and Wijesinghe 2008, Orwin et al. 2009 for some examples). To date, the relative importance of patch attributes and abiotic environment properties on heterogeneity effects in grasslands is poorly understood. My thesis has taken nutrient heterogeneity research a critical step forward by examining the roles of patch attributes and rainfall regime using experimental and modelling approaches. This chapter draws on all of the results obtained to address three overarching questions: i) Does spatial pattern of N inputs matter for plot-level grassland structure and function? ii) How do patch attributes influence heterogeneity effects on grassland production? iii) Does rainfall regulate N heterogeneity effects on grassland structure and function? General study limitations are discussed and suggestions for further work are presented.

5.1 Does spatial pattern of N inputs matter for plot-level grassland structure and function?

Results in the thesis demonstrate positive plant production responses to heterogeneous N at the small scale (i.e. within plots), as well as an increase in biomass variability across plots. Nevertheless, comparisons of homogeneous and heterogeneous treatments in the experimental chapters suggest that whole-plot plant production does not significantly respond to spatial pattern of N inputs. This result was consistent for different plant community composition and soil types (see Chapters 2 and 3), and was also found for different N forms on a given soil type (Chapter 2). In contrast, community structure at the whole-plot level responded significantly to spatial N pattern, with higher community dominance in heterogeneous N conditions due to enhanced relative abundance of fast-growing species (Chapter 3). These findings suggest that for the patch area examined (6.25% of plot area, applied as a single patch), spatial pattern of N inputs does not matter for grassland production in the short-term, but it has implications for community structure. Shifts in community structure and plant size inequalities, coupled with altered plant-soil interactions, may have significant implications for plant-plant interactions (Hillebrand et al. 2008), functional diversity according to the biomass ratio hypothesis (Grime 1998), and biogeochemical cycling in grassland systems (Wardle et al. 2004, Grigulis et al. 2013).

These findings confirm the idea that impacts of nutrient heterogeneity may not be detected at the plot-level when patch scale is small relative to the grain size (Wiens 1989, Li and Reynolds 1995), although in the experimental chapters here, patch scale cannot be dissociated from patch proportion. Choice of grain size is therefore critical to the outcome of heterogeneity effects. When the patch proportion is increased relative to the plot size (16% of plot area, Chapter 4), modelling results indicate a significant heterogeneity effect on grassland production. This result suggests that N heterogeneity may have greater impacts in intensively- versus extensively-grazed fields (more dung patches associated with higher stocking rate), but emphasizes the difficulty of generalizing across heterogeneity studies. Interestingly, field-level modelling results showed that timing of N inputs could mediate grassland production responses to patchy N, driven by differences in plant growth rate and N demand during the growing season. Thus, timing of N inputs (driven by management practices) could have important implications for grazed grassland function.

5.2 How do patch attributes influence heterogeneity effects on plant production?

Results from this thesis provide insights into the importance of N form, patch size and patch contrast for grassland production. Experimental results indicated that impacts of patchy N inputs on both small-scale (within-plot) and plot-level production were consistent for the three N forms examined (Chapter 2, plot-level data not shown). However, N form had significant effects on microbial biomass, with greater magnitude and longer-lasting, small-scale responses to complex organic N patches compared to inorganic N. Results from modelling simulations (Chapter 4) demonstrate that plot-level grassland production responds negatively to patch contrast, with lower plant production in the high contrast treatments compared to the low contrast treatments. In addition, plot-level production showed a positive response to nutrient patch size. Patch size and patch contrast did not interact on grassland production, suggesting that effects of patch attributes may be additive

These results demonstrate that not all patch attributes are equally important in mediating heterogeneity effects on grassland function; based on present results, the influence of patch attributes on plot-level plant production can be ranked patch contrast > patch size > N form for fast-growing grassland communities. The strong responses to patch contrast observed here are consistent with previous experimental studies (Wijesinghe and Hutchings 1997, 1999), and were most likely driven by shifts in plant-plant competition at patch boundaries in response to N quantity. This supports the idea that the plant competition is more intense at high productivity where plant biomass is greater (Grime 1973). The absence of N form effects on plant production observed here may indicate that N form has smaller effects

on plant competition than on plant-soil interactions. Indeed, work on plant N uptake in the field suggests that plasticity in the uptake of different forms of N may be a mechanism by which co-occurring plants reduce competition for N (Miller et al. 2007). Nevertheless, patch-induced spatial and temporal asynchrony in plant and microbial responses to N form could have longer term implications for grassland function (Wardle et al. 2004, Dunn et al. 2006, de Vries and Bardgett 2012).

5.3 Does rainfall regulate N heterogeneity effects on grassland structure and function?

Contrary to expectations, grassland responses to patchy N inputs were not modified by chronic changes in rainfall quantity (Chapter 3). Absence of significant N pattern x rainfall interactions at the whole-plot, community-level may have been partly driven by idiosyncratic species' responses to rainfall treatments. Although these results do not match previous findings (Maestre and Reynolds 2007a), the lack of data available in the literature makes it difficult to determine whether N pattern x water interactions are a common phenomenon in grassland ecosystems. It is also possible that a clearer response pattern would be obtained using inorganic N; both the present work and the study by Maestre and Reynolds manipulated organic nutrients.

Lack of community responses to patchy, variable conditions may reflect high ecosystem stability against environmental fluctuations due to high diversity in plant species or functional groups (Johnson et al. 1996, Bloor and Bardgett 2012). Ecosystem stability may also be mediated by plant trait variability (Jung et al. 2014) or plant-soil interactions (Bardgett et al. 2013). Thus, local-scale responses to patchy N inputs observed in terms of microbial biomass (Chapter 2) and plant traits (Chapter 3) may contribute to buffering temperate grasslands responses in a changing environment.

5.4 Synthesis

Overall, the results of this thesis have underlined the importance of patchy inputs and patch attributes for determining the outcome of heterogeneity effects in the short term. Magnitude of N heterogeneity effects vary depending on the variables considered (production versus community structure); when nutrient patches cover a small proportion of the plot area, community structure is more sensitive to patchy N inputs than production. Biotic interactions (plant-plant, plant-microbe) appear to play a relatively greater role than abiotic factors (chronic rainfall changes) for heterogeneity effects.

Based on previously published work and the results obtained in this thesis, a conceptual model is proposed to connect spatial nutrient heterogeneity with ecosystem

structure and function (Figure 5-1). Heterogeneous N inputs alter plant and soil community properties via both direct and indirect effects, mediated by characteristics of the patchy inputs (spatial distribution i.e. random, uniform or aggregated; patch attributes e.g. patch size, concentration), existing attributes of the plant community and local environmental/ climatic conditions. As a consequence, heterogeneous nutrient inputs may have cascading effects on nutrient cycling over space and plant-soil feedbacks. This model highlights the importance of considering both plant and soil components in spatial heterogeneity studies.

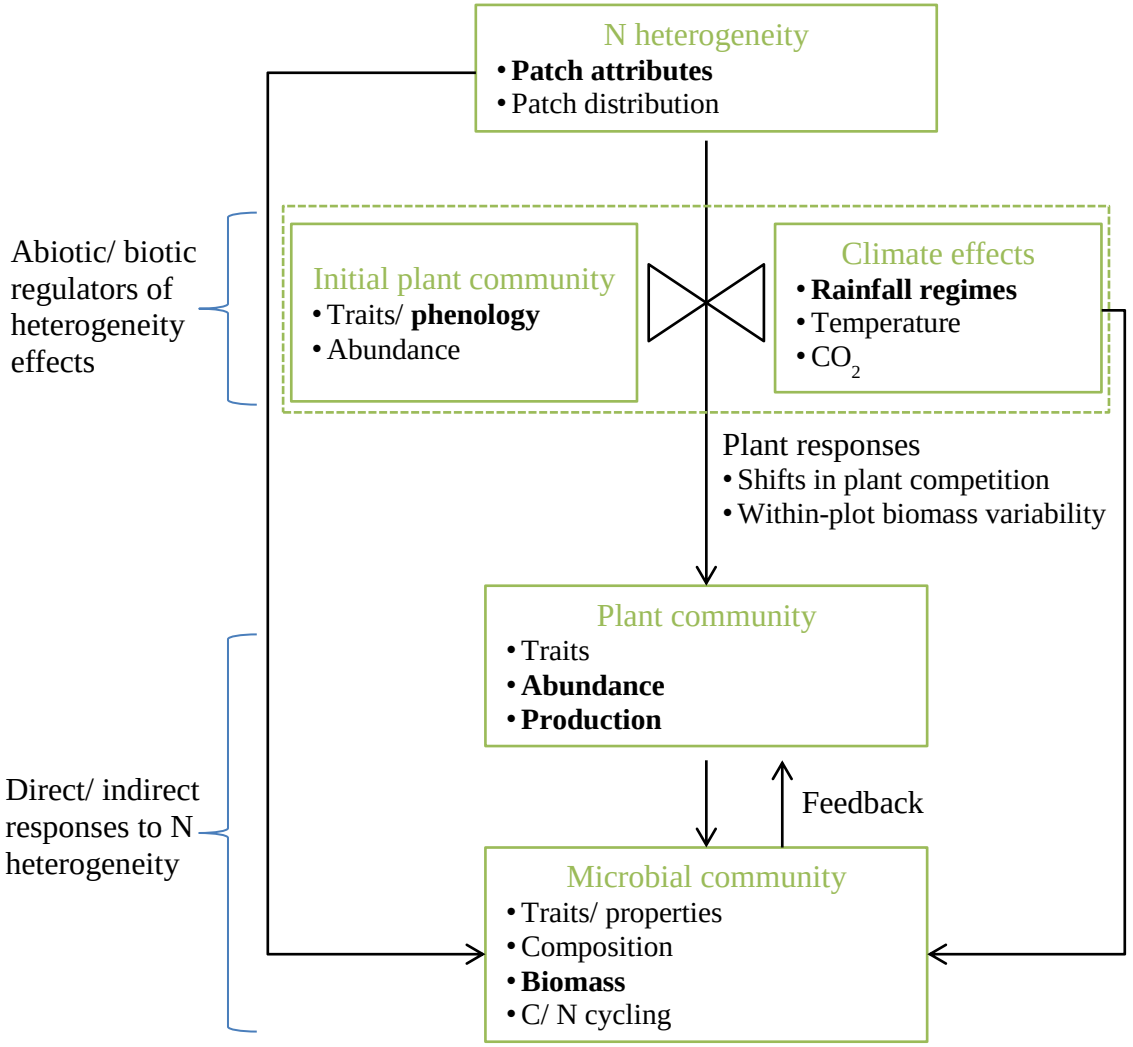

Figure 5-1 Model proposed to describe plant and soil responses to soil N heterogeneity and climate factors (adapted from Lavorel and Garnier 2002, García-Palacios et al. 2012). Topics addressed in this thesis are given in bold type.

Local- (within plot) and plot-scale response patterns recorded in this thesis for relatively small-scale N patches (relative to the plot area) are summarized in Figure 5-2. Chronic change in rainfall quantity has little influence on heterogeneity effects, whereas N timing/ plant phenological stage modifies impacts of spatial N pattern. Plant production strongly depends on patch contrast and patch size, but shows no interaction between these two factors. N form does not affect plant responses to patchy N inputs, but significantly changes microbial responses at the local scale. The extent to which these response patterns mask shifts in plant and microbial traits remains to be determined.

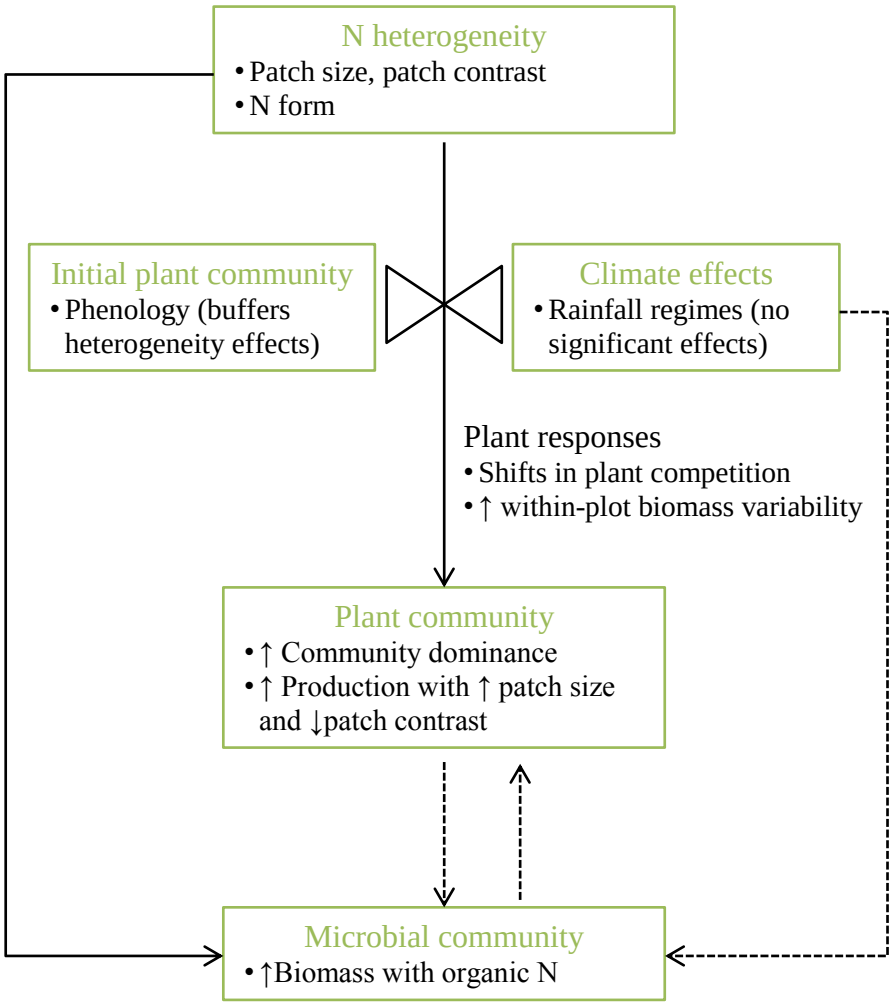

Figure 5-2 Plant and soil responses to N heterogeneity and rainfall regime in this thesis. Dash lines represent processes that were not explicitly examined in the thesis.

5.5 Study limitations and directions for future work

This thesis has provided novel information on coupled plant/soil responses to spatial N heterogeneity in temperate grassland systems, and examined plant community structure and function at a variety of spatial scales by a combination of experimental and modeling approaches. Areas of further research and experimental limitations relevant to particular chapters have been highlighted in those chapters. Here general issues relevant to the whole thesis are considered.

Firstly, the experimental studies conducted in this thesis were relatively short-term, providing no information on lagged or carry-over effects due to experimental treatments. It seems reasonable to assume that carry-over effects following a single, transient N input are of lower magnitude than those following more regular N inputs. Nevertheless the importance of plant-soil feedbacks in patchy environments needs to be assessed in longer-term studies. Use of ^{15}N labelling techniques (to trace N fluxes) and PLFA analysis (to identify bacteria: fungi ratios in the microbial community) would be valuable additions to study plant-soil interactions. Longer term experiments might also reveal shifts in species richness, rather than just relative abundance. Secondly, this thesis focused on fast-growing grassland communities on moderately fertile soil as a model system. This model system is interesting as the constituent species are expected to respond relatively rapidly (and therefore be sensitive) to N inputs. However, further work with communities dominated by conservative plant species is required to test the generality of the novel results and trends described here.

In the mesocosm experiment (Chapter Three), *Achillea millefolium* appeared to benefit from the enclosed boxes and show far greater dominance than under natural conditions. It would be interesting to repeat the study without *Achillea*, but with other forbs (such as *Plantago lanceolata*) to see whether this modified community responses to rainfall. Work with the spatially-explicit model (Chapter Four) was limited to aboveground-responses since soil processes are lacking in this model. Future model work should include belowground processes such as soil water dynamics and nutrient diffusion that allow N patch dynamics in space and time. Model predictions need to be validated with field data. Finally, this thesis has specifically addressed the impacts of N heterogeneity. Of course, in natural systems, patchy nutrient inputs may involve other components (for example, carbon and phosphorus in cattle dung), and the results obtained here for N alone may be modified when N is in combination with other nutrients.

The results obtained in this thesis raise some interesting follow-up questions which require further investigation. Results showed that spatial N pattern influences community dominance and plant traits in the short-term. We also found evidence for shifts in plant-plant

interactions in response to N inputs of different patch sizes, which has implications for longer-term community dynamics and species richness. The responses of these three components of diversity (functional diversity, community dominance, community richness) to patchy nutrients are expected to vary depending on patch size, and differ over time (Hutchings et al. 2003). This idea could be tested by using model plant communities and a range of patch size treatments in a planted, field experiment. It would be interesting to compare trait responses of fast- and slow-growing species, as well as dominant versus subordinates. With small patches, community richness may decrease in a longer term due to competitive exclusion of slow-growing species whereas with large nutrient patches, community dominance should decrease and community richness should increase over time due to shifts in plant competition. Results in the thesis demonstrate that spatial pattern in N inputs promote asynchronous plant and soil responses. However, variation in the duration and frequency of nutrient pulse at a given location may be equally, if not more important than spatial pattern (Yang et al. 2008, Venail et al. 2011). To date, little is known about the interactions between spatial N heterogeneity and temporal variation (patch frequency) on aboveground and belowground processes of grassland systems. This topic could be examined experimentally by manipulating N spatial pattern, along with duration and frequency of N inputs. Isotopic (^{13}C-^{15}N) labelling would provide valuable insights into the spatial and temporal partitioning of nutrients between plant and microbial components. .

Results here suggest that spatial N heterogeneity does not interact with chronic changes in rainfall quantity to affect grassland structure and function, but impacts of extreme rainfall/ drought events remain unknown. Given that extreme drought can dramatically change soil water fluxes, and may thereby affect soil N transformations and mobility, it seems reasonable to expect water-N heterogeneity interactions with extreme rainfall scenarios. However, timing of extreme events may mediate interactions between water and patchy N inputs, due to differences in plant phenology (and hence patterns of water/N use) (DeBoeck et al. 2011). It would be interesting to test interactive effects of spatial N heterogeneity and extreme drought events by crossing N spatial pattern treatments with rainfall treatments (spring versus summer drought events). Experimental results could be compared with model outputs using the spatially-explicit model described earlier (Chapter 4) and ‘extreme climate’ weather data.

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Nitrogen form and spatial pattern promote asynchrony in plant and soil responses to nitrogen inputs in a temperate grassland

Nianxun Xi, Pascal Carrère, Juliette M.G. Bloor*

INRA, UR0874 Grassland Ecosystem Research Unit, 5 Chemin de Beaulieu, F-63100 Clermont-Ferrand, France

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ABSTRACT

Homogeneous and patchy inputs of inorganic and organic nitrogen (N) are common in grazed grasslands, but little is known about the interactions between spatial pattern and form of N inputs for plant and soil processes. Understanding coupled plant and soil responses to heterogeneous N inputs is a critical step towards the improved prediction of field-scale ecosystem function. We compared impacts of uniform and patchy N addition on plant and soil properties using three N forms of increasing complexity (inorganic N; a simple amino acid, glycine; a complex protein, BSA) in an *in situ* grassland experiment. One month after N addition, patchy N treatments increased plant production but decreased biomass produced per gram nitrogen (a proxy of N use efficiency) compared with uniform N treatments. Contrary to expectations, plant production showed limited differences among N form treatments. However, microbial biomass and dissolved organic carbon showed significant N form × pattern interactions, with strongest responses to patchy inputs of complex organic N. Irrespective of N form, plant responses to patchy N inputs occurred over a larger spatial area than soil microbe responses, consistent with optimal foraging by plant roots. Unlike plants, microbial responses to patchy N inputs were still observed after six months. Overall, our results indicate that patchy inputs of N promote the uncoupling of plant and soil properties, with greatest differences observed for complex organic N inputs. The spatial and temporal asynchrony between plant production and microbial biomass observed may have significant implications for the competitive balance of plants and soil microbes in space, as well as for plant–soil feedbacks involved with the regulation of biogeochemical cycling.

1. Introduction

Nitrogen (N) inputs play a key role for primary productivity, plant community composition, soil microbial diversity and plant–soil interactions in grassland ecosystems (Tilman, 1987; Bardgett et al., 1999; Hodge et al., 2000a). However, a growing body of evidence suggests that grassland responses to N addition may depend not only on the quantity and form of N (organic and inorganic), but also on the spatial pattern of N (Hutchings et al., 2003; Maestre and Reynolds, 2006; Orwin et al., 2009). This is of particular interest for managed grasslands where both evenly-distributed and patchy inputs of N are common due to fertilizer management and grazer activity respectively.

During grazing, up to 90% of ingested N from herbage is returned to the pasture in dung and urine patches which cover a small area but have high concentrations of N (equivalent to application rates of around 1000 kg N ha^{-1} for dung patches, Haynes and Williams, 1993). Such spatial variation in the quantity of soil N has the potential to influence plant processes from the individual to the community level (Hutchings et al., 2003; García-Palacios et al., 2012). For example, root systems of individual plants may respond to local increases in soil N by the production of new lateral roots in N patches, increased growth of existing roots or plasticity in nutrient uptake kinetics, enhancing efficient capture of N (Campbell et al., 1991; Robinson, 1994; Hodge, 2006). Root foraging responses and improved nutrient capture by plants in heterogeneous N conditions may also modify plant population structure and enhance yield (Day et al., 2003a). In theory, species-differences in N foraging efficiency and/or N complementarity should enhance community-level production in heterogeneous conditions (Wijesinghe et al., 2005; Kahmen et al., 2006). In practice, evidence from field experiments on natural plant communities is lacking.

* Corresponding author. INRA-UREP, Grassland Ecosystem Research Unit, 5 Chemin de Beaulieu, F-63100 Clermont-Ferrand, France. Tel.: +33 473 62 44 25; fax: +33 473 62 44 57.
E-mail addresses: nian-xun.xi@clermont.inra.fr (N. Xi), pascal.carrere@clermont.inra.fr (P. Carrère), juliette.bloor@clermont.inra.fr (J.M.G. Bloor).

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In contrast to the large number of studies addressing plant responses to patchy increases in soil N, very few studies have considered the impacts of soil heterogeneity on the plant–soil system as a whole (Day et al., 2003b; Maestre and Reynolds, 2006; but see Orwin et al., 2009). Soil N availability is a driver for microbial processes such as nitrification and denitrification in terrestrial ecosystems (Booth et al., 2005; Barnard et al., 2006), and recent work indicates that N inputs may also modify microbial community structure (Allison et al., 2008; Ramirez et al., 2012). In the absence of plants, soil respiration and microbial biomass have been shown to decrease under N addition (Ramirez et al., 2012). However, in the presence of plants, responses of microbial biomass to soil N enrichment are mixed (Hodge et al., 2000b; Allison et al., 2008; Treseder, 2008). Recent advances indicate that interactions at the above–belowground interface may provide important feedbacks regulating ecosystem processes and ecosystem N retention (de Vries and Bardgett, 2012). To date though, the effects of patchy increases in soil N on coupled plant–soil responses remain poorly understood.

Aside from modifying the quantity of soil N distributed across space, large herbivores alter the relative abundance of N forms in the soil via their animal returns (Augustine and Frank, 2001). The majority of N in urine is present as urea, which is rapidly hydrolysed to inorganic N (NH_4^+), whereas the bulk of N in large herbivore feces is in organic form (Haynes and Williams, 1993). Previous studies have shown that both plant species and microbial communities are capable of direct uptake of inorganic and organic N forms (Bardgett et al., 2003; Weigelt et al., 2005; Harrison et al., 2008; Näsholm et al., 2009). Work from pot and field experiments also suggests that plant and soil responses to homogeneous nutrient inputs may vary depending on whether inorganic or organic N is added (Dunn et al., 2006; Harrison et al., 2008). However, interactions between N form and spatial pattern are largely unknown. Ecosystem responses to heterogeneous N inputs could be modified by N form due to differences in diffusion, mineralization rates or availability for uptake between different N compounds (Hodge et al., 2000a; Jan et al., 2009). In addition, shifts in plant–soil competition for N over time (Hodge et al., 2000b) could promote variation in short- and long-term responses to patchy inorganic and organic inputs.

Here, we investigated plant and soil responses to N form and N application pattern using a factorial *in situ*, grassland experiment. We focus on impacts of uniform or patchy N addition on the soil–plant system in the short and longer term (one and six months respectively), using N forms of increasing complexity (inorganic N, simple amino acid, complex protein) to represent the range of N inputs that commonly occur in grasslands (Jones et al., 2004). We addressed four main hypotheses: (i) patchy N addition will enhance community-level plant production due to increased root biomass and nutrient-use efficiency; (ii) plant biomass will respond more strongly to inorganic compared with organic N forms, whereas carbon-limited microbial biomass will respond more strongly to organic N; (iii) plant and soil responses to patchy N addition will occur over a wider area within inorganic N plots compared to organic N plots, due to the higher mobility of inorganic N in soil; (iv) plant biomass responses to patchy N addition will occur over a wider area within plots compared to microbial biomass responses, due to plant root distribution and root foraging.

2. Material and methods

2.1. Study site

The study was conducted in a five-year old sown grassland located at INRA-Clermont-Ferrand, France (45°47′ N, 03°05′ E, 350 m a.s.l.). The climate is temperate with a mean annual precipitation of 575 mm and a mean annual temperature of 12.4 °C. The plant community is dominated by the grass *Dactylis glomerata*. Additional grass species include *Lolium perenne* and *Festuca rubra*. Other species include legumes (*Trifolium repens*, *Lotus corniculatus*) and forbs (*Taraxacum officinale*, *Achillea millefolium*). The soil type is silty clay loam (2.55% C, 0.23% N), with a pH_{H2O} of 7.8. Prior to this experiment, the site was mown three times a year and not fertilized.

2.2. Experimental design

In order to investigate the interactive effects of N form and N application pattern on plants and soil microbes, two spatial pattern treatments (homogeneous, HOM; heterogeneous, HET) were crossed with three N form treatments. The three N forms were ammonium nitrate (NH_4NO_3, inorganic N, hereafter abbreviated as IN), glycine ($C_2H_5NO_2$, a simple amino acid, abbreviated as GLY), and bovine serum albumen (a model protein, abbreviated as BSA). These represent the range of N forms present in the soil soluble N pool of temperate grasslands; NH_4NO_3 is commonly applied in fertilized grasslands, GLY is the dominant amino acid in hydrolysed cow urine (Bathurst, 1952), and BSA was used to represent complex organic N forms with high molecular weight present in cow dung. BSA was used rather than cow dung itself to avoid possible confounding effects of other nutrients present in dung (e.g. phosphorous). In addition, a control treatment without N addition was established (total of seven N treatments × 6 replicates = 42 plots).

In April 2012, 95 cm × 95 cm experimental plots were established across the study site. The botanical composition of all plots was determined using the point quadrat method with 25 points recorded per plot. Principal components analysis was used to identify two classes of plots according to the relative abundance of grasses, legumes and forbs (data not shown). Plots were then assigned to experimental treatments such that each treatment included equal numbers of each vegetation class chosen at random (this ensured no significant difference between treatments in the relative abundance of species at the start of the experiment). Vegetation was cut to 5 cm on 23rd April, in line with local cutting practices. Immediately prior to N application, measurements of vegetation height indicated no significant difference between experimental plots (one-way ANOVA, $P > 0.05$).

All N treatments were established on 11th May 2012; N application was in liquid form, combined with a simulated small rainfall event (4 mm). In the homogeneous N treatments, dilute N solution was applied across the whole plot. In the heterogeneous N treatments, concentrated N solution was applied to the central 25 × 25 cm patch of each plot (similar in size to cattle dung or urine patches) with distilled water alone applied to remainder of the plot. Total N and water addition were equal for all plots (N application equivalent to 50 kg N ha^{-1}, consistent with values of urea application and amino acid concentrations in grassland soil). Nitrogen loading in the central patch of the heterogeneous treatments (800 kg N ha^{-1}) was consistent with N from cattle excretion (Haynes and Williams, 1993). The control plots without N addition received water alone.

Plant communities were left to grow under natural rainfall conditions. Measurements of vegetation height were carried out at roughly ten-day intervals throughout the experimental period in the centre and 'edge' zone within all experimental plots (see description of within-plot zones below).

2.3. Harvests and analyses

Plants and soil were harvested at peak biomass (45 days after N addition) and again at the end of October (164 days after N

addition), following autumn regrowth (Fig. S1). In the second harvest, only 27 out of 42 plots were considered (3–4 replicates per treatment) due to significant rodent damage that occurred in some plots during September. At each harvest, experimental plots were cut to a height of 5 cm. Plant biomass data from a 10 cm-wide strip around the perimeter of each plot was discarded to avoid edge effects. The remaining 75 × 75 cm zone of each plot which was divided into three sub-zones: the central 25 × 25 cm patch ('centre'), and two concentric 12.5 cm wide strips extending out from the centre ('middle' and 'edge' respectively, Fig. S2). Plant samples from each sub-zone were dried (60 °C, 48 h) prior to weighing to determine dry mass. Dried aboveground plant material was ground and analysed for total N content using an elemental combustion analyzer (Flash EA 1112 CNS analyzer, ThermoFinnigan, Milan, Italy). Aboveground N concentrations were used to assess the ratio of aboveground biomass to N content (g dry mass g N^{-1}). Biomass: N ratios can be used as proxy of nitrogen use efficiency (NUE, e.g. Fargione and Tilman, 2006; Roscher et al., 2008), although the mean residence time of N in the plant also influences NUE (Berendse and Aerts, 1987).

Two soil cores were taken in each of the three sub-zones (2.5 cm diameter, 15 cm deep); soil cores were pooled per sub-zone and per plot, returned to the laboratory and maintained at <5 °C prior to analysis. All soil samples were sieved at 2 mm and the remaining below-ground plant biomass, coarse soil and organic debris were washed to extract and separate roots and rhizomes. Root samples were dried (60 °C, 48 h) prior to weighing to determine dry mass. Microbial biomass C was measured on 5 g subsamples of the sieved soil using the chloroform fumigation–incubation method (Brookes et al., 1985). Soil samples were extracted with 25 ml of 0.5 M K_2SO_4 solution, microbial C being calculated as the difference in total C extracted in fumigated and unfumigated soils (k_C = 0.35 as the adjustment factor, Sparling et al., 1990). Non-fumigated extracts were used as an estimate of dissolved organic C (K_2SO_4-extractable DOC). Soil mineral N (NH_4^+ and NO_3^-) was extracted by shaking 5 g of freshly sieved soil with 25 ml 1 M KCl on an orbital shaker for 1 h. The KCl extracts were filtered through Whatman glass microfibre filters and analysed by colorimetric measurements and auto-analyser procedures (Bran & Luebbe AutoAnalyser 3, Hamburg, Germany). Additional 5 g soil samples were oven-dried (105 °C, 24 h) to determine soil moisture content.

2.4. Statistical analysis

Comparisons of vegetation height at different within-plot locations (centre, edge) were assessed with one-way ANOVA at each measurement date during the study period. Harvest data were analysed using a mixed model procedure and a repeated measures, split-plot design (Quinn and Keough, 2002). Plots were considered as a random factor, with N form and N application pattern as fixed whole-plot factors, and within-plot sampling location (centre, middle and edge) and harvesting dates as fixed sub-plot factors. Differences between treatments were determined with Tukey's HSD post-hoc tests. Since data for the second harvest was only available for a subset of plots due to rodent damage, we checked that this would not introduce bias into the results; paired *T*-tests indicated no significant difference between the full and partial dataset for the first harvest ($P > 0.05$) so we assume that the partial dataset at the second harvest provides a good representation of treatment effects. We also checked that discarding the biomass from the perimeter strip did not introduce artefacts in the analysis of aboveground biomass data; data from the first harvest indicated no significant differences between the within-plot biomass of 'edge' and 'border' zones on an area-basis in all N treatments (paired *T*-test, $P > 0.1$), and split-plot ANOVA for the biomass at first harvest gave qualitatively the same results with/without the perimeter biomass included. We assume this holds true for the second harvest where border data was not available.

Analysis of harvest data was conducted using the 'nlme' package in R (R Development Core Team, 2013). Remaining analyses were conducted using Statgraphics 4.1 (Statistical Graphics Corp., Rockville, Maryland, USA); data were log-transformed to meet assumptions of homogeneity of variance and normality if necessary.

3. Results

3.1. Vegetation height dynamics

Vegetation height showed a strong and rapid response to N addition irrespective of N form, but within-plot responses (expressed as a difference in absolute vegetation height between N-fertilized and control plots) varied depending on the spatial pattern of N application (HOM *versus* HET, Fig. 1). In HOM treatments, vegetation height responded to N addition in the same way across the experimental plots (centre/edge) whereas in HET treatments, height responses were only observed in the centre of plots (Fig. 1). Significant height responses to homogeneous N addition did not persist after the first harvest. In contrast, significant height responses to heterogeneous N addition were observed in the centre of plots after the first harvest (Fig. 1B). These responses were most pronounced in response to organic N addition (GLY, BSA).

3.2. Plant biomass responses to N form and N application pattern within plots

Plant biomass responses to N addition varied with both time and plot location (Table 1, Fig. 2). At the first harvest (45 days after N addition), nitrogen application pattern had a significant overall effect on plant biomass ($F_{1,30} = 7.98$, $P < 0.05$), with increased plant biomass in heterogeneous N plots (Fig. 2). However, production increases in the HET treatments varied depending on plot location, and were most pronounced across the centre and middle zones of the plot (Pattern × Location interaction, $F_{2,60} = 17.74$, $P < 0.05$, Fig. 2). This pattern was mirrored by belowground biomass in the 0–15 cm soil layer (Table S1, Fig. S3). Moreover, aboveground plant biomass showed a significant Form × Location interaction at the first harvest ($F_{4,60} = 3.31$, $P < 0.05$), driven by strong responses to BSA-Het across the centre and middle zones of the plot (Fig. 2).

Unlike aboveground biomass, the aboveground biomass: N ratio responded to both N form and N pattern at the first harvest ($F_{2,30} = 3.95$, $P < 0.05$ and $F_{1,30} = 23.65$, $P < 0.05$ respectively). Biomass: N was greater in IN/BSA treatments compared to GLY, and had greater values in HOM compared to HET treatments (Fig. 3). Biomass: N responses to N treatments did not vary depending on within-plot sampling location.

At the second harvest (164 days after N addition), both aboveground biomass and biomass: N were significantly lower than at the first harvest (Fig. 2). Neither aboveground biomass nor biomass: N showed any response to either N form or pattern of N application, leading to significant Treatment × Time interactions (Table 1). Furthermore, experimental treatments had no effect on root biomass in the 0–15 cm soil layer (data not shown).

3.3. Soil responses to N form and N application pattern within plots

As with plants, soil microbial biomass responses to N addition varied depending on time and within-plot location (Table 1). Significant treatment interactions were driven by a large stimulation

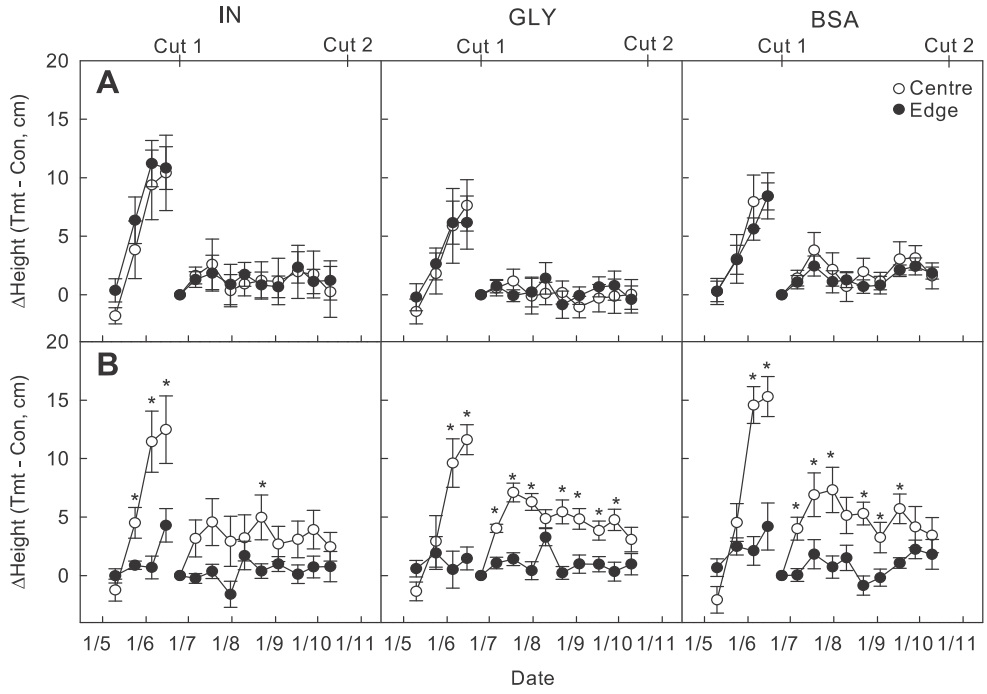

Fig. 1. The difference in mean vegetation height between N-fertilized and control plots at different plot locations (centre/edge) during the experimental period for homogeneous (A) and heterogeneous (B) nitrogen applications. Nitrogen forms are given by: IN, ammonium nitrate; GLY, glycine; BSA, bovine serum albumin. Means and standard errors are shown ($n = 6$); significant differences between plot locations per sampling date are indicated by an asterisk ($P < 0.05$).

of microbial C in the central zone of the BSA-Het treatment at the first harvest (+79% compared to the central zone of BSA-Hom, Fig. 4). Stimulation of microbial C observed in the central zone of the BSA-Het treatment persisted to the second harvest (Fig. 4), leading to a significant Pattern × N form interaction at each harvest date ($F_{2,30} = 3.95$, $P < 0.05$ and $F_{2,30} = 3.83$, $P < 0.05$ for harvests 1 and 2 respectively). Overall, microbial responses to heterogeneous N were limited to a smaller spatial area (the central zone of the experimental plots, Fig. 4) compared with plant responses. The smaller microbial biomass response to patchy BSA addition at the second harvest resulted in a significant four-way interaction term (Table 1).

Table 1
Effects of N form, N application pattern, within-plot location, harvesting time and all interactions on aboveground (>5 cm) and belowground (0–15 cm) variables. *F* values derived from analysis of variance are shown with degrees of freedom (*df*); significant effects ($P < 0.05$) are given in bold type.

Effect	*df*	*F* values			
		Aboveground biomass	Biomass: N	Microbial biomass C	DOC[a]
N form (*F*)	2, 30	0.96	2.06	2.44	**4.70**
N pattern (*P*)	1, 30	2.72	**15.45**	3.67	**6.62**
Within-plot location (*L*)	2, 111	**16.50**	**6.09**	**39.40**	**48.74**
Time (*T*)	1, 111	**172.32**	**89.91**	**60.53**	**46.53**
F × *P*	2, 30	1.17	1.43	**4.89**	3.05
F × *L*	4, 111	2.06	0.96	**4.01**	**3.90**
P × *L*	2, 111	**11.90**	1.51	**24.23**	**15.24**
F × *T*	2, 111	0.34	**4.07**	**5.62**	0.94
P × *T*	1, 111	**4.79**	**17.43**	**14.55**	1.95
L × *T*	2, 111	**11.36**	2.97	**23.33**	**41.86**
F × *P* × *L*	4, 111	0.73	1.65	**5.70**	**4.15**
F × *P* × *T*	2, 111	0.83	0.42	1.12	2.70
F × *L* × *T*	4, 111	**4.13**	0.04	**3.64**	**3.91**
P × *L* × *T*	2, 111	**13.53**	0.55	**21.12**	**3.74**
F × *P* × *L* × *T*	4, 111	**2.99**	1.00	**2.71**	0.54

[a] DOC, Dissolved organic carbon.

Responses of dissolved organic carbon (DOC) to N treatments were qualitatively similar to soil microbial biomass responses (Table 1, Fig. 5). Values for DOC were greatest in the central zone of the BSA-HET treatment at both harvests, but the BSA-induced stimulation in the HET treatment was smaller at the second harvest (Fig. 5).

Unlike microbial biomass and DOC, soil inorganic N showed short-lived responses to N addition treatments. At the first harvest, heterogeneous N sources significantly increased soil inorganic N (Table S1); irrespective of N form, increases in soil inorganic N in the HET treatments varied depending on plot location, and were most pronounced across the central zone of the plots (Table S2). In contrast, experimental treatments had no effect on soil inorganic N in the 0–15 cm soil layer at the second harvest date (data not shown).

4. Discussion

Understanding plant and soil responses to spatial variation in N inputs is critical for the improved prediction of field-scale processes and ecosystem service provision from grazed grasslands (Hutchings et al., 2007). To date, however, field studies have rarely addressed the impacts of heterogeneous addition of different nutrient forms on plant and soil processes in multi-species grasslands. The present study addresses this knowledge gap by simultaneously examining the interactive effects of N form and N application pattern on above- and belowground grassland function in an *in situ* field experiment.

4.1. Patchy N inputs enhance community-level plant production in the short term

Based on results from micro-and mesocosm experiments using artificial species assemblages (Wijesinghe et al., 2005; Maestre and Reynolds, 2007; García-Palacios et al., 2011), we hypothesized that

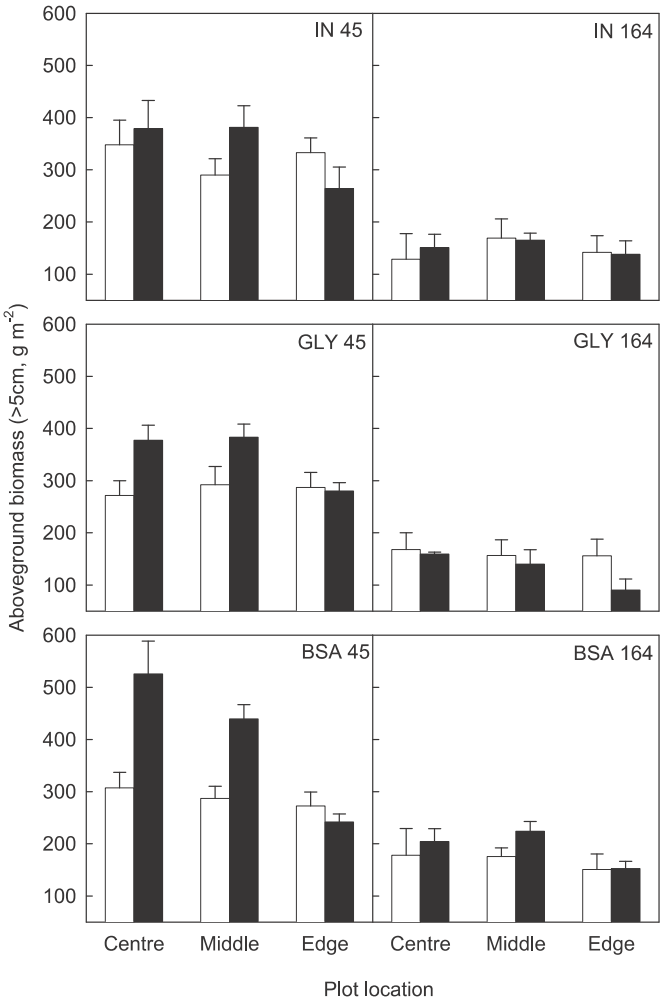

Fig. 2. Effects of N form and N application pattern on aboveground biomass at different plot locations 45 days and 164 days after N addition. White bars and black bars indicate homogeneous and heterogeneous treatments respectively. N forms are given by: IN, ammonium nitrate; GLY, glycine; BSA, bovine serum albumin. Means and standard errors are shown.

heterogeneous N inputs would increase plant production more than homogeneous inputs. Our results were consistent with this hypothesis in the short term (45 days after N addition); irrespective of N form, aboveground biomass production was 22% greater in heterogeneous compared to homogeneous treatments. Biomass increases due to patchy N inputs did not persist in the longer term (164 days after N addition), which may reflect greater N uptake and exports from the HET treatments at the first harvest, as well as greater N losses from the high N patches via N_2O emissions or leaching losses (Hutchings et al., 2007).

Enhanced plant production in heterogeneous conditions has previously been attributed to increases in root biomass and root foraging precision which enhance efficient N uptake (Wijesinghe et al., 2005). In the present study, heterogeneous N application was associated with a 23% increase in belowground biomass recorded in the 0–15 cm soil layer. Increases in root biomass were particularly pronounced in the central zone of HET plots, consistent with root foraging in high N patches. In addition the HET treatments decreased aboveground biomass : N ratios, characteristic of plant responses to high N availability and an N dilution effect (Vázquez de Aldana and Berendse, 1997). These observed changes in response to patchy inputs of nitrogen could affect ecosystem nutrient cycling via concurrent changes in litter inputs and root exudates which modify C inputs to the soil and influence N mineralization-immobilization dynamics (de Vries and Bardgett, 2012). Thus, although plant responses to patchy inputs disappeared during the growing season, these transient increases in plant production may have carry-over effects on ecosystem function in following years (Vellinga et al., 2010, but see van der Hoek et al., 2004).

4.2. Complex organic N forms stimulate both plant and microbial biomass

Although plants are capable of rapid uptake of mineral N, it is generally thought that soil microbes are superior competitors for organic N in the short term (Kaye and Hart, 1997; Hodge et al., 2000b). In line with this idea, we predicted that soil microbes would show stronger short-term responses to the addition of complex organic N than inorganic N, with the C in the organic compounds stimulating microbial growth. As expected, BSA-induced increases in microbial biomass were greater than stimulation by inorganic N at the first harvest. Surprisingly, aboveground plant biomass also responded to complex organic N, with equally strong short-term increases following both BSA and inorganic N addition.

Plant biomass may be stimulated by addition of complex organic N if the organic form is mineralized by soil microbes, providing plant-available N in an inorganic form. Incubation experiments have shown that BSA mineralization is slow compared to simple amino acids (Jones and Kielland, 2012), but field conditions following N addition (high temperature, soil moisture; Fig. S1) may have favoured faster mineralization rates of BSA in the soil. However, given the rapid plant height increases observed in all N form treatments, it seems unlikely that mineralization is the sole mechanism at work. BSA-induced increases in plant growth could also arise if plants take up the organic form directly. Increasing evidence suggests that organic N uptake may play a significant role in plant N nutrition (Bardgett et al., 2003; Näsholm et al., 2009) and recent work has shown that plants are able to take up BSA in laboratory conditions (Paungfoo-Lonhienne et al., 2008). As yet, direct plant uptake of proteins remains to be tested with ^{15}N labelling techniques in the field. Of course, similar plant growth responses to inorganic and organic N treatments could also be explained by increased leaching losses of NH_4NO_3 compared with BSA. High soil moisture and regular rain events during the first growth period following N addition are likely to have promoted transfers of mobile inorganic N toward the lower soil layers and increased risks of leaching losses as well as gaseous nitrous oxide losses (Luo et al., 2013). Further work should determine the relative importance of organic N uptake and inorganic N transformations/losses in plant production responses to different N forms.

4.3. Plant and soil responses to patchy N inputs differ in spatial scale

Patchy nutrient inputs have the potential to generate significant spatial variation in ecosystem processes via nutrient-induced changes in plant and soil properties inside nutrient patches and in their immediate surroundings (Orwin et al., 2009; Gillet et al., 2010). Given that inorganic N is more mobile in soil than complex organic N forms, we predicted that plant and soil responses to patchy inorganic N inputs would occur over a wider area compared to patchy organic N inputs. Generally, our results did not support this prediction; N form did not affect the spatial scale at which plant and microbial biomass responses to patchy N inputs were expressed. These findings may indicate greater vertical leaching of soluble inorganic N compared with lateral transfers in our study, as

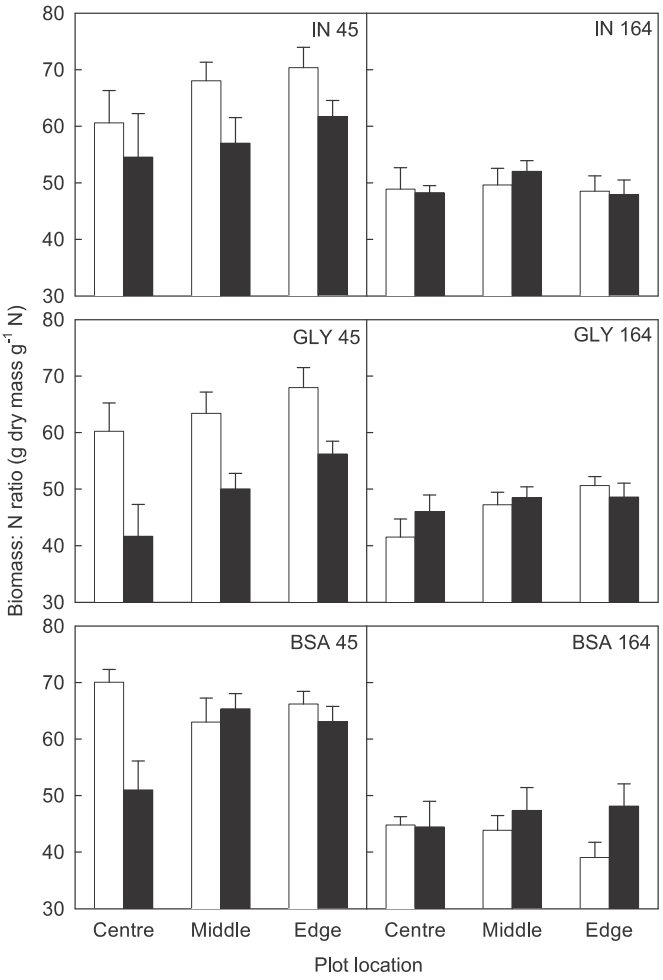

Fig. 3. Effects of N form and N application pattern on the aboveground biomass: N ratio at different plot locations 45 days and 164 days after N addition. White bars and black bars indicate homogeneous and heterogeneous treatments respectively. N forms are given by: IN, ammonium nitrate; GLY, glycine; BSA, bovine serum albumin. Means and standard errors are shown.

tracer studies in soil have suggested that vertical movement of soluble N occurs at the expense of lateral movement (Williams and Haynes, 1994; Olatuyi et al., 2012). Limited lateral diffusion in our study could also reflect the patch size of N inputs. According to Ficks's First Law of diffusion (Fick 1855, cited in Orwin et al., 2009), lateral diffusion out of high concentration patches should be greater in small patches with smaller horizontal distances over which concentrations vary. Work with artificial urine patches of different sizes supports the idea that large patches (similar in size to those used in the present study) retain inorganic N for a longer period of time due to lower lateral diffusion of N out of patches (Orwin et al., 2009).

In line with our final hypothesis, plant biomass responses to patchy N inputs were observed at a greater spatial scale than those of microbial biomass (centre + middle of plot *versus* centre alone for plants and soil microbes respectively). The observed divergence in spatial pattern of plant and microbial responses to patchy N inputs is consistent with root foraging behaviour which benefits plants adjacent to the nutrient patches, as well as those inside the patch (Robinson, 1994). Our findings also confirm results from field studies which show that dung and urine patches may stimulate plant growth over areas considerably greater than the original size of the nutrient patch (Haynes and Williams, 1993; Gillet et al., 2010). The large size of plants relative to microbes, coupled with spatially-selective root foraging in plants, underlies differences in the way that these two groups of organisms perceive and respond to patchy environments (Ettema and Wardle, 2002). Limited evidence suggests that nutrient patch size and distribution in space, particularly inter-patch distances, may be critical for microbial activity and the outcomes of plant-microbial competition for N (Wang and Bakken, 1997; Korsaeth et al., 2001; Loecke and Robertson, 2009), but this remains to be tested under field conditions.

In addition to scale-dependent variation in plant and soil responses to patchy N inputs, our results pointed to temporal asynchrony in plant–soil responses. Patchy N inputs had no effect on plant biomass at the second harvest whereas small positive impacts of patchy BSA inputs on microbial biomass were still apparent at this time. Previous studies have demonstrated that single additions of organic amendments can have lasting effects on the soil, in part due to soil properties such as clay content which influence the longevity of microbial biomass responses to organic inputs (Wardle, 1992; Bach et al., 2010; Kallenbach and Grandy, 2011). It is therefore possible that the high clay content of soil in our study system promoted the maintenance of high microbial biomass following patchy BSA addition. Direct stimulatory effects of BSA addition on microbial biomass may have been

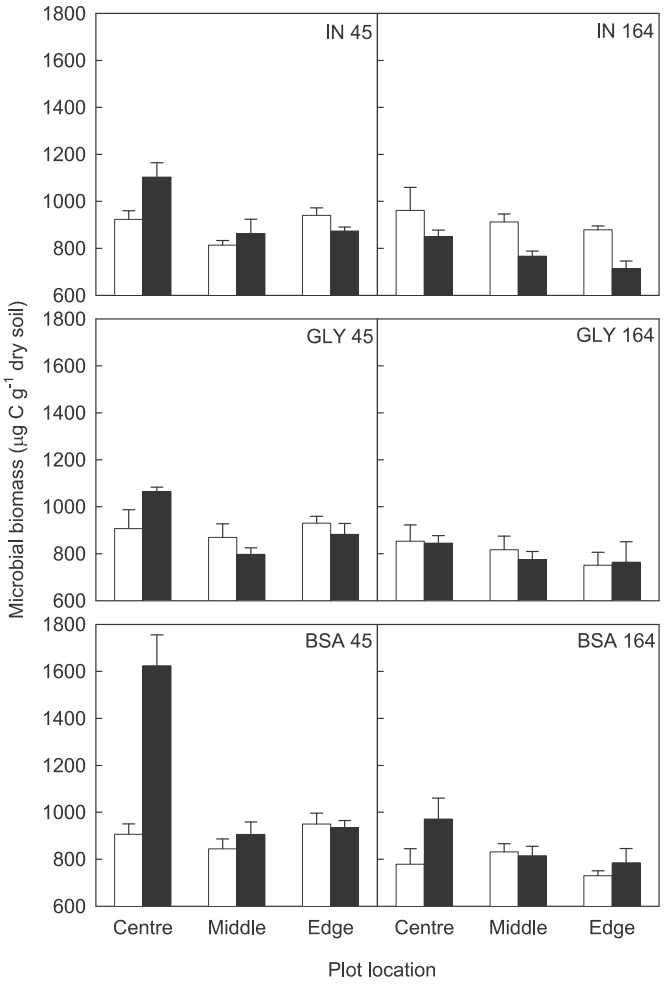

Fig. 4. Responses of microbial biomass in the 0–15 cm soil layer to N form and N application pattern at different plot locations 45 days and 164 days after N addition. White bars and black bars indicate homogeneous and heterogeneous treatments respectively. N forms are given by: IN, ammonium nitrate; GLY, glycine; BSA, bovine serum albumin. Means and standard errors are shown.

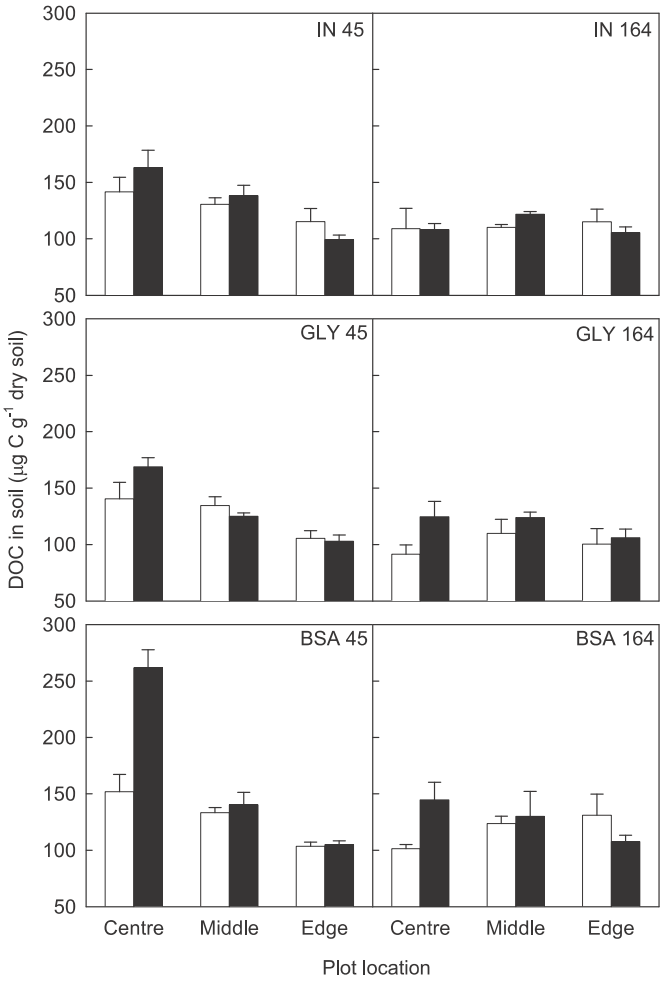

Fig. 5. Responses of dissolved organic carbon (DOC) in the 0–15 cm soil layer to N form and N application pattern at different plot locations 45 days and 164 days after N addition. White bars and black bars indicate homogeneous and heterogeneous treatments respectively. N forms are given by: IN, ammonium nitrate; GLY, glycine; BSA, bovine serum albumin. Means and standard errors are shown.

further enhanced by indirect, plant-mediated effects, with BSA-induced increases in plant growth increasing inputs of labile carbon substrates to the soil over a longer time-scale via root exudates or plant residues. Such fresh C inputs are known to stimulate microbial biomass and decomposition of recalcitrant soil organic matter, with effects that persist after the soil treatment (Kuzyakov et al., 2000; Fontaine et al., 2010). Thus, both the spatial and temporal asynchrony in plant and soil responses to patchy N inputs recorded here could have significant implications for microbial activity and plant–soil competition for N, with cascading effects on above- and belowground community structure via changes in soil N availability (Ettema and Wardle, 2002; Dunn et al., 2006).

5. Conclusions

Spatial pattern of N inputs had significant effects on both aboveground plant production and soil properties in the short-term. Unlike plant biomass, microbial biomass and DOC showed significant pattern × N form interactions, driven by a strong positive response to patchy inputs of complex organic N. Patch-induced responses in microbial biomass occurred over a smaller spatial area compared with plant responses, but were longer-lasting. We suggest that such shifts in spatial and temporal patterns of plant and soil responses could modify the competitive balance between plants and soil biota, changing plant–soil interactions and nutrient cycling. Overall our data highlights the importance of spatial heterogeneity in nutrient inputs for grassland ecosystem properties, and indicates that N form may be a greater driver of microbial community function than of plant productivity. Additional work is needed to examine how such impacts of patchy nutrient inputs can be integrated into models of grassland ecosystem function.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.01.008.

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Appendix Two

Microbial responses to interactive N spatial pattern and rainfall treatments

Nitrogen inputs can have important impacts on microbial activities and community composition either directly, by shits in soil nutrient content and C: N ratio (Hodge et al. 2000, Allison et al. 2008, Ramirez et al. 2012), or indirectly by plant-mediated litter inputs (Bardgett and Wardle 2003, Wardle et al. 2004). Moreover, soil water availability and soil drying or wetting associated with fluctuating rainfall regimes has the potential to modify microbial metabolism pathways and community composition due to different inherent resistance and acclimation abilities of microbial groups (Fierer et al. 2003, Schimel et al. 2007). Here we investigated interactive effects of spatial N pattern and rainfall regimes on microbial functional diversity using community-level physiological profiling (CLPP) (Garland and Mills 1991) and the mesocosm experiment described in Chapter 3. The CLPP approach has been effective in characterizing pattern of microbial carbon source utilization and can distinguish spatial and temporal changes in microbial communities (Zak et al. 1994, Garland 1997).

Methods:

Experimental treatments are described in Chapter 3. In mid-June, immediately following plant harvest at peak biomass of vegetation, two soil cores (1.8cm x 10cm deep) were taken in the centre and edge of each mesocosm; these soil cores were pooled to provide a representative sample of the microbial populations present at the mesocosm level. Two samples per treatment were combined to obtain three replicate, composite samples per treatment combination. Soil cores were returned to the laboratory and maintained at $< 5\ ^{o}C$ prior to analysis. All soil samples were sieved at 2 mm and subsamples of sieved soil was used to determine microbial functional diversity under each treatment using the Biolog technique (Zak et al. 1994).

Community-level physiological profiles were constructed using Biolog Ecoplates (Biolog Inc., Hayward, CA, USA) containing 31 single carbon substrates and a control in addition to a tetrazolium dye. Substrates can be classed into 6 chemical guilds (Table 1). Each substrate or water control is replicated 3 times per EcoPlate, resulting in a total of 96 wells. In brief, 150 µl aliquots of a 0.5% (wt/vol) soil suspension were inoculated to each well of the EcoPlates using a multi-channel automatic pipette under sterile conditions in a laminar flow hood. EcoPlates were incubated at $25^{o}C$ in the dark for eight days. Colour development of

each well was determined daily by a series of optical density reading (OD at 590 nm) during incubation using a BioTek plate reader (BioTek Inc., USA) associated with Gen5 data analysis software. Raw data were averaged for each substrate or water control after each reading, and optical density for substrates was corrected by subtracting OD in control wells.

Calculations and statistical analysis

Average well colour development (AWCD) was determined using the mean of corrected OD values for all 31 substrates (Garland and Mills 1991). Significant substrate utilization by microbes was defined as corrected OD above 0.25 (Garland 1996). OD values for substrate utilization were used to calculate substrate richness, substrate evenness and substrate diversity of microbial communities for each time point following Zak et al. (1994). Substrate richness (*S*) was simply the number of substrates utilized by microbial community. Substrate diversity (*H*) and evenness (*E*) were calculated using the following equations respectively:

$$H = -\sum_{i=1}^{S} P_i \cdot lnP_i$$

$$E = H/lnS$$

where P_i refers to the ratio of substrate *i* utilization to all substrate utilizations.

Biolog data were analysed using a spit-plot two-way ANOVA (Quinn and Keough 2002), with blocks as a random factor, rainfall treatments as a fixed whole-plot factor, and N treatments as a fixed sun-plot factor. Differences between treatments were determined with Tukey's HSD post-hoc tests. Principal components analysis (PCA) was conducted to compare utilization of substrate guilds by microbial communities under different treatments. Data were log-transformed to meet assumptions of variance homogeneity and residual normality if required. All analyses were done using Statgraphics 4.1 (Statistical Graphics Corp., Rockville, Maryland, USA).

Table 1 Carbon substrates in Biolog EcoPlates, divided into the substrate guilds suggested by Preston-Mafham et al. (2002).

Chemical guild	C substrate	Chemical formula
Amine	Phenylethylamine	$C_8H_{11}N$
	Putrescine	$C_4H_{12}N_2$
Amino acid	Glycyl-L-glutamic acid	$C_7H_{12}N_2O_5$
	L-arginine	$C_6H_{14}N_4O_2$
	L-asparagine	$C_4H_8N_2O_3$

	L-phenyl alanine	$C_9H_{11}NO_2$
	L-serine	$C_3H_7NO_3$
	L-threonine	$C_4H_9NO_3$
Carbohydrate	D-cellobiose	$C_{12}H_{22}O_{11}$
	D-mannitol	$C_6H_{14}O_6$
	D-xylose	$C_5H_{10}O_5$
	i-erythritol	$C_4H_{10}O_4$
	N-acetyl-D-glucosamine	$C_8H_{15}NO_6$
	ß-methyl-D-glucoside	$C_7H_{14}O_6$
	α-D-lactose	$C_{12}H_{22}O_{11}$
Carboxylic acid	2-hydroxy benzoic acid	$C_7H_6O_3$
	4-hydroxy benzoic acid	$C_7H_6O_3$
	D-galactonic acid γ-lactone	$C_6H_{10}O_6$
	D-galacturonic acid	$C_6H_{10}O_7$
	D-glucosaminic acid	$C_6H_{13}NO_6$
	D-malic acid	$C_4H_6O_5$
	Itaconic acid	$C_5H_6O_4$
	α-ketobutyric acid	$C_4H_6O_3$
	γ-hydroxybutyric acid	$C_4H_8O_3$
Miscellaneous	D,L-α-glycerol phosphate	$C_3H_9O_6P$
	Glucose-1-phosphate	$C_6H_{13}O_9P$
	Pyruvic acid methyl ester	$C_4H_6O_3$
Polymer	Glycogen	$(C_6H_{10}O_5)n$
	α-cyclodextrin	$C_{36}H_{60}O_{30}$
	Tween 40	×
	Tween 80	×

Results:

Microbial substrate use and AWCD increased over time, reaching a plateau after six days of incubation (data not shown). Response patterns of substrate use to treatments were qualitatively similar throughout the first week of incubation, and therefore only results for 48h of incubation are shown here.

Spatial pattern of N inputs had a significant effect on microbial substrate utilization over the incubation period, with greater AWCD for Het treatments compared to Hom

treatments at 48h (Table 2). AWCD showed no significant response to rainfall or any rainfall x N pattern interaction (Table 2). This response pattern was mirrored by diversity and richness of substrate use (Table 2, Figure 1). In contrast, substrate evenness showed a marginal N pattern x rainfall interaction (Table 2, Figure 1). Patchy N had no effect on evenness in control and wet conditions, but appeared to reduce eveness in the dry treatment.

Interestingly, PCA analysis provided some evidence for groups based on microbial patterns of carbon substrate use in response to N pattern and rainfall treatment (Figure 2). The two principle components accounted for 72.5% and 13.4% of the overall variation respectively. Axis one was positively correlated with microbial carbohydrate use whereas axis two was negatively correlated with microbial amine use. Homogeneous treatments were characterised by low utilization of carbohydrate compared to Het treatments. Within Het treatments, average/above-average rainfall treatments were associated with increased amine use, whereas dry conditions were associated with lower utilization of amine (Figure 2).

Results observed here suggest that patchy environments promote microbial functional diversity (assessed by microbial substrate utilization). Moreover, microbial community structure in patchy N conditions may vary depending on rainfall regime/ soil water availability. These indirect measurements of microbial community structure need to be confirmed with more direct measurements such as PLFA and molecular fingerprinting methods.

Table 2 Effects of interactive rainfall and nitrogen spatial pattern treatments on average well colour development (590 nm) of EcoPlates, substrate diversity, substrate richness and substrate evenness at 48h. *F* values derived from analysis of variance are shown with degrees of freedom (*df*): significant effects ($P < 0.05$) are given in bold type.

		F values			
Effect	*df*	AWCD (590 nm)	Substrate diversity	Substrate richness	Substrate evenness
Rainfall	2, 6	0.35	0.72	0.37	0.44
N pattern	1, 6	**16.21**	**16.97**	**17.05**	0.11
Rainfall x N pattern	2, 6	0.41	2.51	1.06	4.24*

*, $0.05 < P < 0.1$

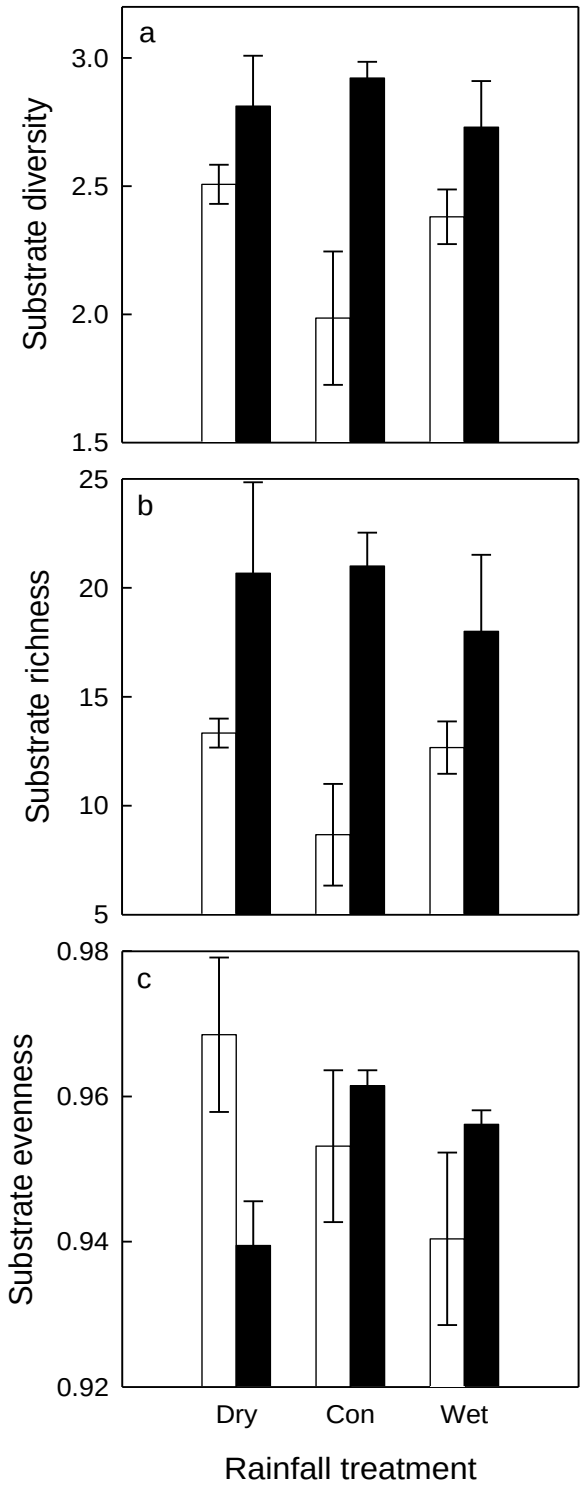

Figure 1 Effects of interactive rainfall and nitrogen spatial pattern treatments on (a) diversity, (b) richness and (c) evenness of substrate utilization for grassland microbial communities. Nitrogen pattern treatments are given by: Hom, homogeneous; Het, heterogeneous. Rainfall treatments are given by: Con, control; Dry, rainfall reduction treatment; Wet, rainfall addition treatment. Means ± SE are shown (n = 3) after 48 h of incubation.

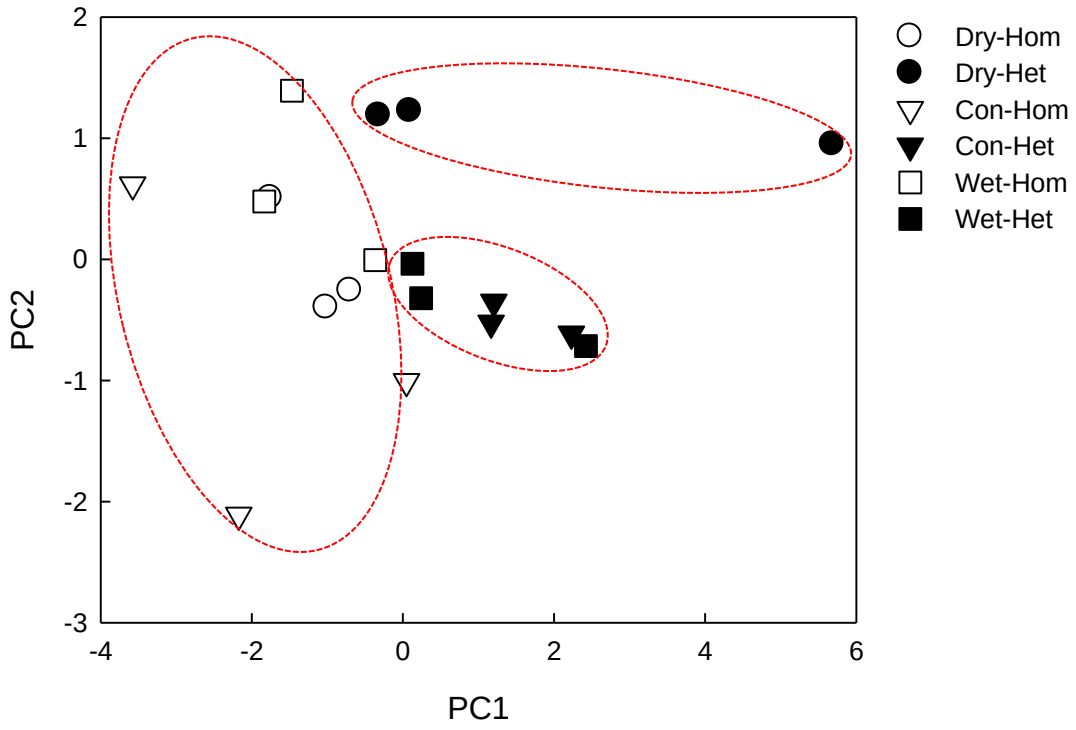

Figure 2 Plot of the first two components from a PCA ofmicrobial carbon utilization strategies in response to spatial N pattern and rainfall regime. Treatment abbreviations are as in Fig.1. The first two components account for 85.9% of the variation among treatments.

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PLANT PRODUCTION AND SOIL MICROBIAL BIOMASS RESPOND DIFFERENTLY TO N FORMS AND N SPATIAL PATTERN IN A SHORT-TERM GRASSLAND EXPERIMENT

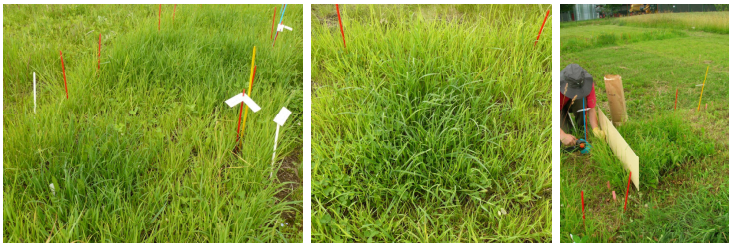
Nianxun Xi, Pascal Carrère, Juliette Bloor; INRA, UR874, Grassland Ecosystem Research Unit, 5 Chemin de Beaulieu, 63039 Clermont-Ferrand , France

INTRODUCTION

Homogeneous and patchy inputs of inorganic and organic N are common in grazed grasslands. Different forms may vary in terms of diffusion, mineralization rate and plant/ microbial uptake[1,2] but little is known about the interactions between form of N inputs and N spatial pattern for plant and soil components in grassland ecosystems. We examined this topic in a field experiment in central France.

HYPOTHESES

- Plants show greater short-term responses to inorganic N compared to complex organic N addition.
- Soil microbes show strong short-term responses to complex organic N addition.
- Responses to patchy inorganic N are expressed over a larger area than those of organic N due to higher diffusion rates.



EXPERIMENTAL APPROACH

In mid-May 2012, three N forms (inorganic N, NH_4NO_3; amino acid, glycine; protein, bovine serum albumin) were added to replicated 1m^2 plots in a sown grassland dominated by *Dactylis glomerata*. N addition was either homogeneous or patchy (Fig. 1) and total N addition was equivalent to 50kg/ha in all cases.

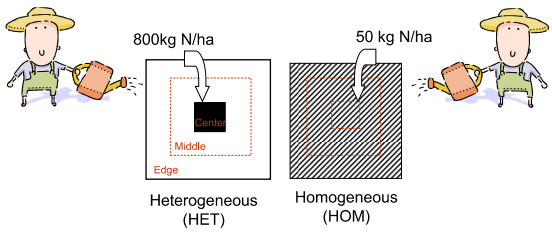


Figure 1. Spatial pattern treatments for N application (Homogeneous, dilute N solution applied across whole plot; Heterogeneous, concentrated N solution applied to central 25x25cm patch, H_2O applied to remainder of plot). Total N and H_2O addition were equal for all plots. Dashed red lines indicate sampling locations within plots at final harvest.

Vegetation was harvested at peak biomass (end June), taking samples from three locations within the plots: center, middle, edge (Fig. 1). Soil samples (0-10cm) were also taken in each zone to determine soil microbial biomass.

RESULTS

Plant production was generally greater in heterogeneous compared to homogeneous N treatments (Table 1, Fig. 2). Production increases in the HET treatments were found across a large area of the plot (centre+middle). Plant biomass responses to N addition did not vary significantly depending on N form, though there was a tendancy for stronger responses to BSA-Het (Fig. 2).

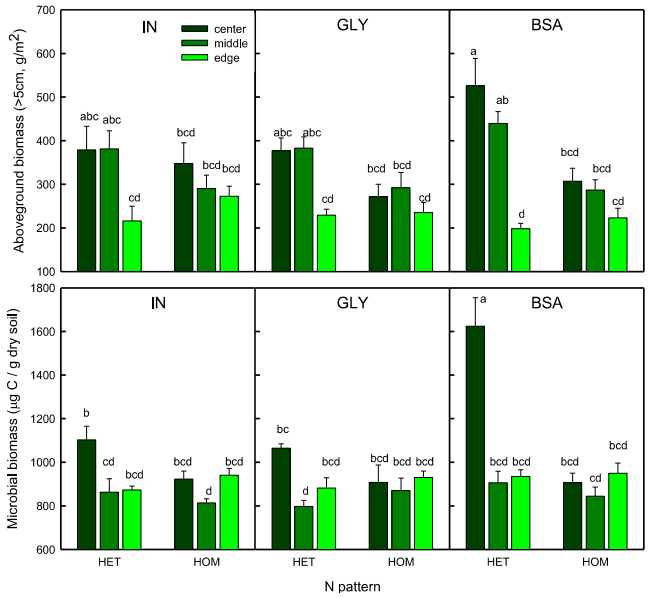


Figure 2. Effects of N form and N pattern on aboveground plant biomass and soil microbial biomass (0-10cm soil layer) recorded at different locations in experimental plots 40 days after N addition. Treatments are given by: IN, NH_4NO_3, GLY, Glycine; BSA, bovine serum albumin. Mean and SE are shown.

Table 1. P values for split plot 3-way ANOVA.

Factor	Aboveground biomass	Soil microbial biomass C
N form	0.503	0.026 *
N pattern	0.005 **	0.006 **
Position	<0.001 ***	<0.001 ***
N form x N pattern	0.238	0.030 *
N form x Position	0.022 *	0.011 *
N pattern x Position	<0.001 ***	<0.001 ***
N form x N pattern x Position	0.154	0.002 **

Soil microbial biomass showed significant treatment interactions, driven by a large stimulation of microbial C in the central zone of the BSA-Het treatment (Table 1, Fig. 2). Generally, microbial responses to heterogeneous N were limited to a smaller spatial area (the central zone of the experimental plots, Fig. 2) compared with plant responses.

CONCLUSIONS

Contrary to expectations, plant production did not vary with N form, possibly reflecting increased leaching losses of mobile, inorganic N. Microbial biomass C showed significant N form/ pattern interactions, with strongest responses to patchy inputs of complex organic N (protein). Plant responses to patchy N inputs occurred over a larger area than soil microbial responses, suggesting optimal foraging by roots. Patchy N inputs may therefore modify the competitive balance between plants and soil microbes in space.

References: [1]Näsholm et al. (2009). New Phytologist 182:31-48; [2]Harrison et al (2008) Soil Biology & Biochemistry 40: 228-237

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GRASSLAND RESPONSES TO RAINFALL REGIME AND HETEROGENEOUS RESOURCE INPUTS

NIANXUN XI, PASCAL CARRERE, JULIETTE M.G. BLOOR

Introduction

Soil nitrogen (N) heterogeneity is ubiquitous in natural ecosystems, with consequences for plant size variability and community structure[1]. Recent work suggests that plant responses to heterogeneous N inputs may be modulated by abiotic factors such as rainfall[2]. We used an outdoor mesocosm experiment and a model grassland community test interactive effects of spatial pattern in N inputs and rainfall quantity on aboveground production, community dominance and species-level biomass variability.

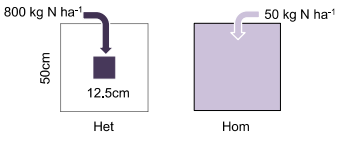


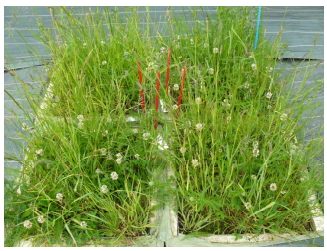
Fig. 1 Spatial patterns in N inputs. Het, concentrated N (bovine serum albumen, a model protein) solution applied to central 12.5x12.5 cm zone, water applied to the remainder of mesocosm; Hom, dilute N solution uniformly applied to the whole mesocosm. The total N and water were constant for all mesocosms.

Methods

We established 36 identical mesocosms in September 2012. We applied experimental treatments during the 2013 growing season, manipulating spatial N pattern (patchy versus uniform inputs; Fig. 1) and rainfall regime (ambient; Dry, -50% rainfall; Wet, +50% rainfall) using rain-screens and supplemental watering. Clonal fragments were cut at peak biomass, sorted to species and weighed to determine dry mass.

Results

Community aboveground biomass did not respond to either rainfall treatment or N pattern treatment (Fig 2a). Community dominance was significantly greater in Het compared to Hom treatments (Fig. 2b; $F_{1,22}$ =4.43, P<0.05). Species-level biomass responses to rainfall treatments varied depending on species (Fig. 3; $F_{1,215}$= 4.51, P<0.05). In general, patchy N inputs promoted species-level biomass variability (Fig. 4; $F_{14,215}$= 2.81, P<0.001).



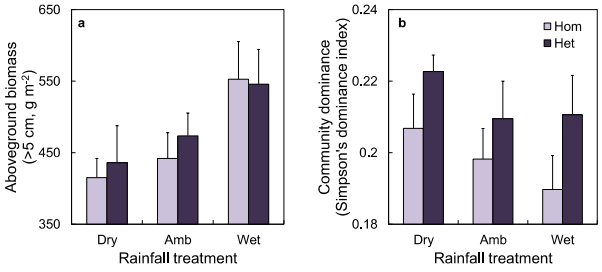


Fig. 2 Effects of rainfall regime and N spatial pattern on (a) community aboveground biomass and (b) community dominance. Community dominance was determined using Simpson's dominance index. Mean and SE are shown (n=6).

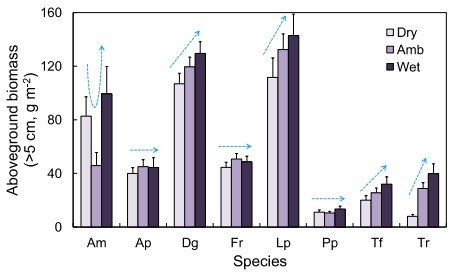


Fig. 3 Interaction between plant species and rainfall regime on aboveground biomass. Plant species are given by: *Achillea millefolium* (Am), *Alopecurus pratensis* (Ap), *Dactylis glomerata* (Dg), *Festuca rubra* (Fr), *Lolium perenne* (Lp), *Poa pratensis* (Pp), *Trisetum flavescens* (Tf), *Trifolium repens* (Tr). Arrows indicate directions of species responses to rainfall treatments. Mean and SE are shown (n=6).

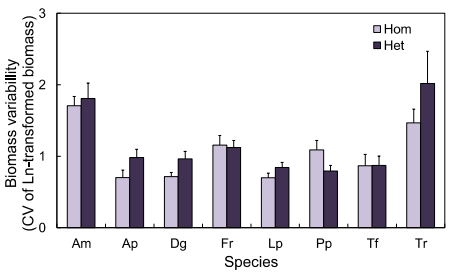


Fig. 4 Interaction between plant species and N spatial pattern on aboveground biomass variability. Plant species codes are given in Fig. 3. Mean and SE are shown (n=6).

Conclusions

- Community-level biomass responses to patchy N mirror species-level biomass responses.
- Community dominance is more sensitive to N spatial pattern than aboveground production.
- Rainfall quantity plays a limited role for grassland responses to patchy N inputs.

References: *1 Hutchings et al. 2003, Ecology 84:2322-2334; 2 García-Palacios et al. 2012, Journal of Ecology 100:1303-1314.*

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Abstract

Grasslands provide a variety of important ecological and economic services worldwide. Improved understanding of grassland structure and function is necessary for the development of sustainable management and maintaining the provision of multiple ecosystem services in a changing environment. However, predicting grassland structure and function is a challenge because grasslands are dynamic, heterogeneous systems. In grazed grasslands, large herbivore activities promote heterogeneity in soil nutrients via excretion, but the effects of patchy nutrient inputs and soil spatial heterogeneity on grassland structure and function remain unclear.

This thesis addresses effects of spatial heterogeneity in soil nitrogen (N) for grassland ecosystem structure and function, with particular emphasis on community responses. A combination of experimental and modelling approaches are used to study impacts of a number of different patch attributes (N form, patch size, patch contrast), as well as possible interactions with rainfall regime and timing of N inputs. We find that patchy N inputs enhance within-plot plant production and biomass variability irrespective of N form, but do not modify whole-plot plant production in the short term. Nevertheless, patchy organic N promotes spatial and temporal asynchrony in plant-soil responses, with implications for longer-term grassland function. Unlike plant production, community structure responds significantly to patchy N inputs, with increased community dominance and a shift in the rank of subordinate species. Contrary to expectations, rainfall quantity does not modify heterogeneity effects on either plant production or community structure. Modelling work shows that heterogeneity effects on field-scale production vary depending on patch size and patch contrast. For a fixed total N input, field-scale grassland production responds positively to patch size, but decreases in high- versus low-patch contrast conditions. Patch size does not interact with patch contrast or timing of N inputs on grassland production. Overall, our results highlight the importance of N heterogeneity for plant and soil processes at different spatial scales, and demonstrate that heterogeneity effects vary depending on patch attributes. Biotic interactions (competition) appear to play a relatively greater role than abiotic factors (chronic rainfall changes) for heterogeneity effects. Impacts of N heterogeneity on plant and soil processes may have significant implications on plant-soil feedbacks involved with the regulation of biogeochemical cycling, and provide useful information for the development of efficient N management strategies.

Key words: Climate change, Community structure, Grassland modelling, Nitrogen, Patch attributes, Production, Plant-soil interactions, Soil Microorganisms, Spatial scale, Spatial variability, Temperate grassland