Quantifying the nitrogen demand of individual plants in heterogeneous canopies: A case study with crop and weed species

Laurène Perthame, Nathalie Colbach, Sophie Brunel-Muguet, Hugues Busset, Julianne Lilley, Annick Matejicek, Delphine Moreau

European Journal of Agronomy

Original research paper

Quantifying the nitrogen demand of individual plants in heterogeneous canopies. A case study with crop and weed species.

Laurène Perthame[a], Nathalie Colbach[a], Sophie Brunel-Muguet[b], Hugues Busset[a], Julianne M. Lilley[c], Annick Matejicek[a] and Delphine Moreau[a]*

[a] Agroécologie, AgroSup Dijon, INRAE, Univ. Bourgogne, Univ. Bourgogne Franche-Comté, F-21000, Dijon, France

[b] EVA, Normandie Univ, UNICAEN, INRAE, F-14000, Caen, France

[c] CSIRO Agriculture and Food, GPO Box 1700, Canberra, ACT 2601, Australia

*For correspondance.

UMR1347 Agroécologie

17 Rue Sully, BP 86510

21065 Dijon Cedex, France

E-mail delphine.moreau@inrae.fr

Abstract

Crop mixtures may decrease reliance on pesticides but reducing herbicide use might increase weeds. Individual-based crop models can provide management guidelines. In heterogeneous canopies, light availability of individual plants depends on their dominant or dominated position. Estimating nitrogen demand at the plant scale, independently of light environment, is a challenge for modelling. A relationship linking shoot nitrogen amount to leaf biomass (or leaf area) at optimal nitrogen nutrition was investigated to establish if it could allow estimating nitrogen demand at the plant scale independently of its light environment. Crop and weed species were grown in greenhouse under various nitrogen treatments and, for two species, under two light levels. At the plant scale, shoot nitrogen amount was proportional to leaf biomass (or leaf area) at optimal nitrogen nutrition at vegetative stage. At reproductive stage, the relationship was allometric. The effect of light on shoot nitrogen amount per leaf biomass was minor and greater when using leaf area. Using data from previous experiments showed that the relationship using leaf biomass was stable across diverse growing conditions. The relationship linking shoot nitrogen amount to leaf biomass at optimal nitrogen nutrition can be used to model nitrogen demand of individual plants in heterogeneous canopies.

Key words: Nitrogen demand; Individual plant; Heterogeneous canopy; Crop; Weed; Individual-based model

1. Introduction

With the advent of more environmentally friendly cropping systems, multispecies canopies will become more frequent. Indeed, crop mixtures are recommended to reduce the use of chemical inputs (Malezieux *et al.*, 2009; Altieri *et al.*, 2015; Duchene *et al.*, 2017; Barot *et al.*, 2017). Moreover, as herbicides constitute the most effective weed management technique (Munier-Jolain *et al.*, 2008), the decrease in herbicide use may result in a residual weed flora in arable fields.

Tools are needed to design and manage more sustainable agricultural systems including multispecies heterogeneous canopies (Gaudio *et al.*, 2019). Indeed, designing and managing such canopies is challenging because they are constituted of several species and show genetic, spatial (sowing patterns, weed patches) and temporal (multiple emergence flushes) heterogeneities which affect the environment experienced by individual plants (Colbach *et al.*, 2014). Most existing crop models and diagnosis tools have been developed for homogeneous canopies and are not adapted to heterogeneous situations.

Mechanistic individual-based crop models can provide management guidelines for multispecies canopies because they allow evaluating various cropping systems scenarios under multiple environments (Gaudio *et al.*, 2019). Indeed, they describe the processes underlying plant development and growth, and their response to environment factors make them applicable in a large range of situations (Colbach *et al.*, 2019). They simulate each plant individually, thus taking into account both the intra-specific and inter-specific variability existing in heterogeneous canopies.

Light is the main resource for which plants compete in intensive cropping systems (Wilson and Tilman, 1993; Perry *et al.*, 2003; Munier-Jolain *et al.*, 2013). Yet, competition for

58 belowground resources, particularly nitrogen, will become more and more frequent, as the use
59 of mineral fertilizers must be reduced due to environmental concerns (Sutton *et al.*, 2011).
60 Competition for nitrogen among plants in the field, whether crop or weed, might increase. It is
61 thus important to model this process but most individual-based models take into account
62 competition for light and rarely for nitrogen (Gaudio *et al.*, 2019) or need to be improved to
63 accurately predict plant nitrogen demand, particularly for isolated plants (Faverjon *et al.*,
64 2019).

65 Modelling nitrogen competition in individual-based models requires representing two
66 processes at the plant scale (1) the soil nitrogen supply and (2) the plant nitrogen demand. Soil
67 nitrogen supply depends on the quantity of nitrogen in the soil available to the root system
68 and on the root nitrogen uptake efficiency (Hodge *et al.*, 2009). These two aspects are already
69 well modelled, e.g., in soil nitrogen models (Brisson *et al.*, 1998; Brisson *et al.*, 2002; Brisson
70 *et al.*, 2003; Holzworth *et al.*, 2014) and in root architecture models (Dunbabin *et al.*, 2013;
71 Pages *et al.*, 2014). Plant nitrogen demand is defined as the quantity of nitrogen that a plant
72 needs to maximize its growth (Ulrich, 1952).

73 In homogeneous canopies, nitrogen demand is classically estimated from the critical nitrogen
74 dilution curve developed by Lemaire and Salette (1984). The critical nitrogen dilution curve
75 defines the minimum shoot nitrogen concentration (pN_{sh} in g nitrogen.g biomass^{-1}) required to
76 maximize shoot biomass (B_{sh} in g):

77 $$pN_{sh} = a \cdot B_{sh}{}^{b}$$

78 With a (in g^{-1}) the intrinsic ability of the crop to take up nitrogen during early growth
79 (Lemaire *et al.,* 2007) and b (dimensionless) the allometric coefficient between the nitrogen
80 concentration and the shoot biomass accumulation (Lemaire and Salette, 1984). The critical

nitrogen dilution curve is applicable at the individual scale. As shoot biomass increases, nitrogen is diluted in response to self-shading and to a higher proportion of stem than leaf biomass. Both processes decrease shoot nitrogen concentration (Lemaire and Gastal, 1997). Parameters *a* and *b* of the critical nitrogen dilution curves vary with the amount of light intercepted by plants and its impact on plant organ composition (Lemaire and Gastal, 1997; Seginer, 2004; Lemaire *et al.*, 2019). Thus, the nitrogen dilution curve, as represented by Lemaire and Salette (1984), cannot be used to estimate nitrogen demand at the plant scale in heterogeneous canopies.

To estimate nitrogen demand at the plant level, some models use biochemical processes linked to photosynthesis (Soussana *et al.*, 2012; Barillot *et al.*, 2016). These models are functional-structural models that explicitly represent plant architecture: they are adapted to investigate interactions among a small number of plant species (one for Barillot *et al.* (2016) and two plant functional types for Soussana *et al.* (2012)) on a relatively short time scale (several weeks or months). These models are not adapted to crop-weed modelling because (1) the numerous parameters required per species cannot be assessed for the several dozens (or more) contrasting weed species coexisting in arable fields (Fried *et al.*, 2008), and (2) plant representation is too detailed to run simulations over several years or decades, as required for analysing the effect of cropping systems on weed dynamics (Gardarin *et al.*, 2010).

Other models use empirical approaches to estimate plant nitrogen demand. The COMPETE model (Berger, 2009; Berger *et al.*, 2013) uses thresholds based on empirical data used to establish maximum and minimum nitrogen amount observed in leaves and stems at several development stage for two species only. Similar empirical approaches to estimate plant nitrogen demand using observed nitrogen concentrations are used in some root architecture

104 models (Lynch *et al.*, 1997; Postma *et al.*, 2017; Wu and McGechan, 1998; Wu *et al.*, 2007).
105 This approach is not generic which makes difficult its application to other species.

106 A third approach, used for instance in an individual-based model simulating grasslands with
107 forage legumes (Louarn and Faverjon, 2018), estimates shoot nitrogen concentration of the
108 plant species at the canopy level with the critical dilution curve using shoot biomass of all
109 plants in the mixture. Nitrogen demand of each plant then depends on its potential dry matter
110 production determined from the amount of intercepted light by the shoot. Using the shoot
111 biomass of the mixture (grass+legumes) allows estimating shoot nitrogen concentration with
112 the same dilution curve coefficients as for a homogeneous grass canopy (Soussana and
113 Arregui, 1995). But this relationship assumes that the species in the mixture have similar
114 height and neighbour shading capacity (Gastal *et al.*, 2015). This does not apply to weed-crop
115 canopy or many annual crop mixtures where plant height can greatly differ between species,
116 and individual plants receive different amounts of light, depending on their position in the
117 canopy (dominant or dominated plant).

118 Thus, it is necessary to find a new approach that allows the estimation of plant nitrogen
119 demand for many species and for plants that have different access to light depending on their
120 dominant or dominated position in the canopy.

121 At optimal plant nitrogen nutrition, Lemaire *et al.* (2005) found a linear relationship between
122 shoot nitrogen amount and leaf biomass of individual plants of *Medicago sativa* grown in a
123 dense stand in the field. The relationship was relatively stable regardless of the plant position
124 in the canopy, and thus the amount of light received by the plant (Lemaire *et al.*, 2005). In a
125 modelling perspective, this result could be used to simply simulate plant nitrogen demand
126 from leaf biomass, independently of light environment (by multiplying the simulated leaf
127 biomass by the slope of the relationship). Instead of leaf biomass, Lemaire *et al.* (2005) also

used leaf area. They identified a similar relationship between shoot nitrogen amount and leaf area at plant optimal nitrogen nutrition for *M. sativa*, stable across environments (greenhouse experiment at low density and field experiment at high density). At the homogeneous canopy level, this relationship varied between environments for several crop species (Lemaire *et al.*, 2007), this relationship might thus vary across environments at the plant scale.

The objective of this study was to identify a method to estimate nitrogen demand at the plant scale, independently of light environment. The linear relationship found by Lemaire *et al.* (2005) linking shoot nitrogen amount to leaf biomass at optimal nitrogen nutrition was analysed in order to determine whether (1) it was valid for other species, (2) its slope was stable irrespective of light environment, (3) its slope varied among a range of species, including both crops and weeds, (4) it was robust to growth conditions (particularly field versus greenhouse) and (5) leaf biomass was a more relevant variable than leaf area to estimate plant nitrogen demand independently of light environment.

2. Materials and methods

2.1. Controlled-environment experiments

2.1.1. Principle

Three greenhouse experiments were conducted in Dijon (France) in 2015, 2016 and 2018. Six annual species were chosen to include monocotyledonous and dicotyledonous as well as crop and weed species, and to cover the range of the N-number of Ellenberg (1974) (Table 1). This gradient ranks species from 1 (most oligotrophic species, mostly found in nitrogen-poor soils) to 9 (most nitrophilic species, mostly found in nitrogen-rich soils).

150 To obtain plants at optimal nitrogen nutrition, plants of all species were grown under different
151 concentrations of nitrogen solution. For two species used as references (*Triticum aestivum* and
152 *Polygonum aviculare*), plants were grown under two light treatments in order to test the
153 stability of the relationship between shoot nitrogen amount and leaf biomass across light
154 conditions. All the other species were grown in unshaded conditions only.

155

156 2.1.2. Experimental design

157 Plants from all six species were grown under four or five nutrient solutions, varying in their
158 nitrate concentration from 0.2 to 14 mM. For two species the different nutrient solutions were
159 applied under two contrasted light treatments: 100% (unshaded) and 40% (shaded) of incident
160 light (Table 1). Twenty-four plants were grown per species × nitrogen treatment × light
161 treatment combination, except for *T. aestivum* and *P. aviculare* in the shaded treatment at the
162 highest nitrogen concentration of the solution where twenty plants were grown. Plants were
163 grouped by nitrogen treatments in the greenhouse because each nitrogen treatment required its
164 own watering network. Within each nitrogen treatment, plant species were placed randomly
165 and spaced homogeneously. At the beginning of the experiment, the distance between pots
166 was 12cm in the unshaded treatment and 9cm in the shaded treatment (13pots/m^2 in the
167 unshaded treatment and 21pots/m^2 in the shaded treatment). Each sampling decreased the
168 stand density by 3pots/m^2 in the unshaded treatment and 5pots/m^2 in the shaded treatment.

169

170 2.1.3. Growing conditions

171 Seeds were germinated in an incubator with photoperiod (from 16 hours of light per day to
172 total obscurity) and temperature (from 10 to 25°C) adapted to each species. When the radicle

protruded, germinated seeds were sown into 2-L pots, with one seedling per pot, and placed into the greenhouse (under natural light and cooled with cooling pads). Pots were filled with a solid, inert and draining substrate (60% attapulgite and 40% expanded clay, with clay balls added at the bottom of the pots except in 2018). For *T. aestivum* and *P. aviculare* in the shaded treatment, plants were grown under a shading net that transmitted 40% of incident light. Each nutrient solution was made up of nitrate, phosphorous, potassium, and oligo-elements (Table S1 section A [Supplementary Information]) and was provided by automatic watering several times a day at a frequency allowing the pots to drain, avoiding the accumulation of ions in the substrate and ensuring non-limiting irrigation. Air temperature (PT100 sensors in ventilated shelter in unshaded treatment; Pyro-Contrôle, Vaulx-en-Velin, France) and incident photosynthetically active radiation (PAR; silicon sensors; Solems, Palaiseau, France) were measured at 300-s (2015) or 600-s (2016, 2018) intervals and stored in a data logger (DL2e; Delta-T Devices, Cambridge, UK). Mean temperature and incident photosynthetically active radiation for each year of experiment are provided in Table S2 section A [Supplementary Information]. In 2018, the mean air temperature was 0.9°C (e.g. 4.2%) higher in the unshaded than in the shaded treatment while the mean incident photosynthetically active radiation in the shaded treatment was worth 41% of the value in the unshaded treatment. The main effect affecting plants growth between unshaded and shaded treatments was thus the incident light.

2.1.4. Plant measurements

For each species × nitrogen treatment × light treatment combination, four to six plants were sampled randomly at four dates, from 5 to 18-weeks after sowing for unshaded treatment and from 8 to 18-weeks after sowing for shaded treatment (Table S3 section A [Supplementary

197 Information]). Samplings were carried out during the vegetative stage for all species, except
198 for *M. sativa* (for two dates) and *P. aviculare* (for all dates) that were at reproductive stage.
199 Plants of *M. sativa* that were at vegetative stage (reproductive to shoot biomass ratio < 1%)
200 were considered separately from the plants at reproductive stage. For each plant, leaf area as
201 well as leaf, stem (i.e. lamina and sheath biomass for grass species) and reproductive (for *M.*
202 *sativa*) biomass were measured after drying during 48h at 80°C. The nitrogen concentration in
203 the aboveground plant part (Dumas method, ThermoScientific^{TM} FLASH 2000 CHNS/O
204 Analyzer) was measured. Shoot nitrogen amount was calculated for the vegetative parts of the
205 plants, except for *P. aviculare* where reproductive parts were included.

206

207 *2.2. Data analysis*

208 2.2.1. Principle

209 The objective was to analyse the relationship between shoot nitrogen amount and leaf biomass
210 at optimal nitrogen nutrition level, in order to determine whether this relationship was (1)
211 linear for a given species as showed for *M. sativa* by Lemaire *et al.* (2005), (2) stable
212 independently of light treatment (as individual plants differ in light access depending on their
213 position in the canopy), (3) different among species, (4) stable across a wide range of
214 environments (evaluate the stability of the relationship with independent data) and (5) more
215 independent on light conditions in comparison with using leaf area (instead of leaf biomass).
216 Indeed, in modelling perspective, if this relationship was proven stable across environments,
217 multiplying the simulated leaf biomass by the slope of the linear relationship could be used to
218 estimate plant nitrogen demand.

219 The approach consisted of four steps. The preliminary step (step 0) was to identify plants at
220 optimal nitrogen nutrition. The nitrogen nutrition index was calculated to quantify to which
221 extent plant nitrogen nutrition was suboptimal (nitrogen deficiency), optimal or supraoptimal
222 (nitrogen is not used by the plant to increase shoot biomass). It was determined, using the
223 method of the critical nitrogen dilution curve. In step 1, only plants at optimal nitrogen
224 nutrition were used in order to study the relationship between shoot nitrogen amount and leaf
225 biomass at optimal plant nitrogen nutrition level (objective (1) in section 2.2.1.). For *T.*
226 *aestivum* and *P. aviculare,* the significance of light treatment on the ratio between shoot
227 nitrogen amount and leaf biomass was analysed to assess if the relationship was independent
228 of light environment (objective (2)). Then, the species effect on the slope of the relationship
229 was studied (objective (3)). Step 2 consisted in evaluating the stability of the relationship
230 across environments using field data (objective (4)). In step 3, a similar relationship linking
231 shoot nitrogen amount to leaf area instead of leaf biomass at optimal plant nitrogen nutrition
232 level was studied (objective (5)).

233

234 2.2.2. Step 0. Identification of plants at optimal nitrogen nutrition

235 The critical nitrogen dilution curve was determined for each species × light treatment
236 combination as the critical nitrogen dilution curve depends on the species and on the light
237 environment (Seginer, 2004; Lemaire *et al.*, 2007). We used a method derived from the
238 reference method from Justes *et al.* (1994). This simplified method is illustrated in Figure 1
239 for *G. molle* in the unshaded treatment. For each sampling date, the nitrogen treatment
240 retained to compute the critical dilution curve (i.e. black points in Figure 1) corresponded to
241 the nitrogen treatment from which increasing solution nitrogen concentration did not result in
242 a significant increase in shoot biomass, while shoot nitrogen concentration may differ (the

nitrogen treatments used for the analysis are given in Table S4 section B [Supplementary Information]). To identify this nitrogen treatment at each sampling date, shoot biomass was compared between the four or five nitrogen treatments using analysis of variance and least significant difference test (aov and LSD.test functions of R (RCoreTeam, 2019)). After a $\log_n$ transformation for both variables, dilution curves were determined by fitting a linear regression (lm function of R) to shoot nitrogen concentration versus shoot biomass using the points corresponding to individual plants at the critical solution nitrogen concentration. The equations of the dilution curves obtained for each species × light treatment combination are given in Table S4 section B [Supplementary Information].

Then, these critical dilution curves were used to calculate the nitrogen nutrition index of each plant, which characterized to which extent plant nitrogen nutrition is suboptimal (nitrogen stress), optimal or supraoptimal (nitrogen excess) (Justes *et al.*, 1994). Nitrogen nutrition index (*NNI*) corresponded, for each plant, to the ratio between the measured shoot nitrogen concentration (pN_m) and the critical shoot nitrogen concentration (pN_c) read on the dilution curve at the corresponding shoot biomass:

$$NNI = pN_m / pN_c$$

If the nitrogen nutrition index was lower than 1, the plant was nitrogen deficient; if the ratio was equal to 1, the plant was at optimal nitrogen nutrition level; if the ratio exceeded 1, the plant nitrogen nutrition level was supraoptimal.

2.2.3. Step 1. Analysing the relationship between shoot nitrogen amount and leaf biomass

Objective 1. In order to analyse the relationship at optimal plant nitrogen nutrition level between the shoot nitrogen amount and leaf biomass, only plants with a nitrogen nutrition index close to optimum (between 0.9 and 1.1) were selected. Linear regressions between quantity of nitrogen in shoot (N_{sh} in g) and leaf biomass B_L (in g) were fitted through the origin, discriminating plants from shaded and unshaded conditions by species (GLM procedure of SAS (version 9.4, SAS Institute, Cary, NC) was used):

$$N_{sh} = B_L + B_L \times \text{Species} + B_L \times \text{Light} + B_L \times \text{Species} \times \text{Light} + \text{error} \quad (1)$$

Plot of residuals against fitted values was used to assess if a linear model was appropriate for the data.

Objective 2. To quantify the effect of species and light treatment independently of the correlation between shoot nitrogen amount and leaf biomass, an analysis of variance of the ratio between shoot nitrogen amount and leaf biomass was performed. Partial R^2 were calculated to quantify the effect of both factors on the variation of the ratio (Anova function of R):

$$N_{sh} / B_L = \text{constant} + \text{Species} + \text{Light} + \text{Species} \times \text{Light} + \text{error} \quad (2)$$

Mean of the ratios in unshaded and shaded treatment were compared with a Student test (t.test function of R).

Objective 3. As the effect of light was revealed to be minor (see 3.2.), model (1) was simplified to compare the slope value between species independently of light environment (lm function of R):

$$N_{sh} = B_L + B_L \times \text{Species} + \text{error} \quad (3)$$

2.2.4. Step 2. Evaluation with field data

Objective 4. To assess if the relationship between shoot nitrogen amount and leaf biomass at optimal nitrogen nutrition was stable not only across light treatments in our experiments, but also across a wider range of environments, our data were compared with data from literature or prior experiments. For *M. sativa*, data from greenhouse experiments where three light treatments were applied to *Medicago truncatula* (Moreau *et al.*, 2008) were used (Table 2 first line). Our data were also compared to values obtained in high-density lucerne (*M. sativa*) stands where three hierarchical positions (dominant, intermediate or suppressed by neighbouring plants) in the overall plant population were studied (and thus three different light conditions, Table 2 second line) (Lemaire *et al.*, 2005). For *T. aestivum*, data from a field experiment with a homogeneous wheat canopy were used (Moreau *et al.*, 2012) (Table 2 third line). In these data, nitrogen nutrition status was assessed at the canopy level for homogeneous canopies while, for heterogeneous canopies, it should be assessed at the plant level. We thus tested the indicator of the present study in the particular conditions of homogeneous canopies, in which we assumed that all plants were identical. If the relationship between shoot nitrogen amount and leaf biomass at optimal nitrogen nutrition was proven stable across light treatments at the plant level with our data, this relationship should remain stable for plants grown at optimal nitrogen nutrition in any light environment (including homogeneous canopy in the field, in which all plants were considered at optimal nitrogen nutrition). For *B. napus*, data from a field experiment in a homogeneous canopy and from a simulation study with APSIM-Canola model were used (Table 2 two last lines, see sections C.1 and C.2 [Supplementary Information] for more details). In all situations, individual plants (or the average plant over the canopy for homogeneous canopies) were at optimal nitrogen nutrition level (nitrogen nutrition index between 0.9 and 1.1). To compare data from our

experiments to data from literature or prior experiments, a linear regression of shoot nitrogen amount (N_{sh}) versus leaf biomass (B_L) was fitted, discriminating data by species and origin using lm function of R:

$$N_{sh} = B_L + B_L \times \text{Species} \times \text{Data Origin} + \text{error} \quad (4)$$

2.2.5. Step 3. Analysing the relationship between shoot nitrogen amount and leaf area

Objective 5. Linear models (1) were fitted to our data (only for plants with a nitrogen nutrition index close to optimum) using leaf area (in cm^2) instead of leaf biomass B_L (GLM procedure of SAS). Plot of residuals against fitted values was used to assess if a linear model was appropriate for the data. To quantify the effect of species and light treatment, an analysis of variance was performed on the ratio between shoot nitrogen amount and leaf area. Partial R^2 were calculated to quantify the effect of both factors on the variation of the ratio (Anova function of R). Means of the ratios were compared between unshaded and shaded treatment for *T. aestivum* and *P. aviculare* (t.test function of R).

3. Results

3.1. Is shoot nitrogen amount proportional to leaf biomass at optimal nitrogen nutrition?

Linear regressions through the origin were fitted to the shoot nitrogen amount versus leaf biomass for plants at optimal nitrogen nutrition, for each species (for *M. sativa* at vegetative and reproductive stage, *B. napus*, *F. arundinacea* and *G. molle*) or each species × light treatment combination (for *T. aestivum* and *P. aviculare*) (Figure 2). For all species or species × light treatment except *M. sativa* at reproductive stage and *P. aviculare,* the residual plots

showed randomly distributed points, indicating the linear regressions were appropriate for the data. Thus, the quantity of nitrogen in the shoot was strictly proportional to leaf biomass at optimal plant nitrogen nutrition for the species at vegetative stage grown under unshaded or shaded treatment. For two species at reproductive stage (*P. aviculare* and *M. sativa*), a $\log_n$ transformation of shoot nitrogen amount and leaf biomass was necessary to satisfactorily fit the data (Figure 2B and D).

3.2. Does light affect the relationship between shoot nitrogen amount versus leaf biomass at optimal nitrogen nutrition?

For *T. aestivum* light treatment had a significant effect on the shoot nitrogen amount to leaf biomass ratio, but not for *P. aviculare* (Figure 3A and B). The value of the ratio increased by 9.2% from unshaded to shaded treatment for *T. aestivum* (Figure 3A). However, light treatment as a single factor explained less than 1% of the variability of the shoot nitrogen amount per unit of leaf biomass (Table 3). Species was the main factor explaining 63.2% of the variation of the ratio. Thus, shoot nitrogen amount per unit of leaf biomass allowed discriminating the species effect on the ratio, with a minor impact of light treatment.

3.3. How does the relationship between shoot nitrogen amount and leaf biomass at optimal nitrogen nutrition vary among species?

Four groups of species could be discriminated (Table 4A and B). The two first groups comprised *T. aestivum*, *M. sativa* at vegetative stage and *F. arundinacea* with high values of shoot nitrogen amount at 1g of leaf biomass (0.06-0.09 g nitrogen.g biomass^{-1}). The third group included *B. napus* and *G. molle*, with lower shoot nitrogen amount values at 1g of leaf

biomass (0.04-0.05 g nitrogen.g biomass^{-1}). Finally, the fourth group comprised *P. aviculare* and *M. sativa* plants that were at reproductive stage, and for which the values of shoot nitrogen amount at 1g of leaf biomass were the highest (0.10-0.15g). At optimal nitrogen nutrition, plants of the species from the two first groups accumulated more nitrogen for a given leaf biomass than plants of the species from the third group. For the first three groups, the slope between shoot nitrogen amount and leaf biomass was independent of leaf biomass, but not for the fourth group because of the allometric relationship between the two variables. We could not link these groups to clade (monocotyledonous/dicotyledonous species), crop versus weed species or preferential habitat nitrogen requirements (N Ellenberg) but the number of experimented species was small.

3.4. Do other environmental conditions affect the relationship between shoot nitrogen amount and leaf biomass at optimal nitrogen nutrition?

Our results could be compared to literature data or prior experiments for three crop species at vegetative stage, linking shoot nitrogen amount to leaf biomass at optimal nitrogen nutrition in different environments (Figure 4). For *B. napus*, the slope of the regression fitted to our data was not significantly different from the slope estimated in the field or estimated using the APSIM-Canola model at optimal nitrogen nutrition (Figure 4C). Similarly, the slope for *M. sativa* based on our data was not significantly different from the slopes estimated from field data from a dense stand or in greenhouse experiments with different light treatments at optimal nitrogen nutrition (Figure 4B). For *T. aestivum*, the slope in our study was significantly greater than the one obtained from field data at optimal nitrogen nutrition (Figure 4A) though, the difference between the two values was small (±7.2%) considering the various environments in which the data were obtained (greenhouse versus field, homogeneous versus

heterogeneous canopy, different light conditions). Thus, the slope of shoot nitrogen amount versus leaf biomass at optimal nitrogen nutrition was globally stable across various environmental conditions for the three studied crop species.

3.5. Is leaf biomass a better variable than leaf area to estimate optimal plant shoot nitrogen amount independently of light environment?

Similarly to leaf biomass, linear regressions through the origin were fitted to the shoot nitrogen amount versus leaf area for plants at optimal nitrogen nutrition, for each species or each species × light treatment combination (Figure S1 section D [Supplementary Information]). According to the residual plot, the quantity of nitrogen accumulated in the shoot was strictly proportional to leaf area at optimal plant nitrogen nutrition for each species × light treatment combination (except for *P. aviculare and M. sativa* at reproductive stage). For these two species at reproductive stage, a $\log_n$ transformation on shoot nitrogen amount and leaf area was necessary (Figure S1B and D section D [Supplementary Information]). For *T. aestivum* and *P. aviculare*, the ratio between shoot nitrogen amount and leaf area significantly decreased from unshaded to shaded treatment (8.6% and 32.3% respectively, Figure 3C and D). Light treatment, in interaction with species, was significant and explained 1.9% of the variation of the shoot quantity of nitrogen per unit of leaf area (Table 5) instead of 0.9% when using leaf biomass (Table 3).

The increase of specific leaf area with shading resulted from a higher decrease of leaf biomass than leaf area for *T. aestivum* and *P. aviculare*. From unshaded to shaded treatment, plants of *T. aestivum* primarily decreased leaf biomass (by 80%, Figure 3I) and, to a lesser extent, leaf area (by 76%, Figure 3G) in order to increase specific leaf area (Figure 3E). Interestingly, the

403 shoot nitrogen amount per unit of leaf biomass or area was more stable (respectively 9.2%
404 and 8.6% from unshaded to shaded treatment) (Figure 3A and C). The conclusions were
405 similar for *P. aviculare* concerning leaf biomass: shading decreased leaf biomass (by 74%,
406 Figure 3J) while the shoot nitrogen amount per unit of leaf biomass remained stable.
407 However, specific leaf area reacted much more to shading (Figure 3F). In this situation, the
408 shoot nitrogen amount per unit of leaf area was more sensitive to shading (Figure 3D). Leaf
409 biomass was thus the most relevant variable to estimate optimal shoot nitrogen amount
410 independently of light environment for the studied species, as plant response to shading was
411 mainly driven by the leaf compartment size.

412

413 **4. Discussion**

414 *4.1. Shoot nitrogen amount is proportional to leaf biomass at optimal nitrogen nutrition*

415 On the studied species, the present study showed an overall linear relationship linking shoot
416 nitrogen amount to leaf biomass at optimal nitrogen nutrition for plants at vegetative stage.
417 Lemaire *et al.* (2005) explained the proportionality of this relationship by the feedback
418 regulation of nitrogen uptake by plant leaf growth. As the plant leaf biomass increased, the
419 photosynthetic activity of the plant increased, requiring more nitrogen uptake. Larger
420 quantities of carbon were allocated to roots to satisfy the increased nitrogen demand. Also, the
421 expansion of plant leaf biomass increased the accumulation of nitrogen within leaves. This
422 relationship was valid for monocotyledonous and dicotyledonous crop and weed species,
423 covering a range of N-Ellenberg (from moderately oligotrophic to highly nitrophilic, see
424 Table 1). For each species, the relationship was established with plants sampled at different

dates, thus with different morphologies, showing the robustness of this relationship across vegetative development stage.

Two species (*P. aviculare* and *M. sativa*) started flowering during the experiment. Interestingly, for these two species at reproductive stage, the relationship linking shoot nitrogen amount to leaf biomass at optimal nitrogen nutrition was allometric. As the slope of the log-log regression was less than 1, this means that, with increasing leaf biomass, the ratio of shoot nitrogen amount to leaf biomass progressively decreased, pointing to a progressive reduction of nitrogen uptake by roots. This phenomenon may arise from modifications in the source-sinks relationships for carbon during the reproductive phase. Indeed, the reproductive compartment may have become a priority sink, at the expense of the root compartment, thereby reducing root (and nodule for *M. sativa*) activity (Rossato *et al.,* 2001).

4.2. The relationship between shoot nitrogen amount and leaf biomass is relatively stable across growth environments

In our experiments, the effect of light environment on the ratio between shoot nitrogen amount and leaf biomass was significant but minor compared to the species effect. Light treatment explained 0.9% of the variability of the ratio for a shading treatment of 60%. Beyond our experiments, comparison with literature data or data from prior experiments for three species showed that the relationship between shoot nitrogen amount and leaf biomass at vegetative stage was remarkably constant across very different environmental conditions at optimal nitrogen nutrition. Indeed, literature data and data from prior experiments differed greatly from our data on many levels related to environmental conditions: soil characteristics (texture, structure, composition), climate (temperature, humidity, incident light), nutrient form

448 (solution versus soil), cultural techniques, canopy versus individual plant, field versus pots in
449 greenhouse. Despite these major differences of environmental conditions, the relationship
450 between shoot nitrogen amount and leaf biomass at optimal nitrogen nutrition during
451 vegetative stage remained stable for a given species.

452 Our results show that, in response to shading, the size of the leaf compartment was strongly
453 affected, while the amount of shoot nitrogen per unit of leaf biomass was little affected even
454 though leaves have higher nitrogen concentration than stems (Greenwood *et al.*, 1990).
455 Consequently, at optimal nitrogen nutrition, when the light conditions or the growth stage
456 varied, the decrease (resp. increase) of the proportion of leaf biomass (rich in nitrogen) was
457 compensated by an increase (resp. decrease) of the proportion of stem biomass (poor in
458 nitrogen). The quantity of shoot nitrogen that would be accumulated per unit of leaf biomass
459 to reach optimal plant nitrogen nutrition for a given species could be an intrinsic property of
460 the plant.

461 Further studies will be necessary in the future as our greenhouse experiment did not evaluate
462 the effects of very strong shading as those reported from field studies (up to 90% including
463 self-shading) (Munier-Jolain *et al.*, 2014). Thus higher shading treatments should be
464 experimented. However highly shaded plants often die early during their growth cycle or
465 remain dwarfed, and thus little participate in competition with neighbouring plants. Finally,
466 we also need to compare greenhouse data to field data for more than the three species tested
467 here (and for *M. sativa*, few data points were available at vegetative stage in the present study,
468 affecting the precision of the relationship).

469

4.3. Shoot nitrogen amount per unit of leaf area at optimal nitrogen nutrition is more sensitive to light conditions

Light treatment had a greater effect on the shoot nitrogen amount that is accumulated at optimal nitrogen nutrition per unit of leaf area than per unit of leaf biomass. When shaded, plants of *P. aviculare* and *T. aestivum* decreased their metabolic compartments (leaf area and biomass). The specific leaf area (leaf area per unit of leaf biomass) varied with light environment at the plant scale: it increased when going from unshaded to shaded conditions in accordance with Mclachlan *et al.* (1993), Sims and Pearcy (1994), Harley and Bertness (1996), Gunn *et al.* (1999), Evans and Poorter (2001), Brainard *et al.* (2005). Specific leaf area reacted more to shading for *P. aviculare* than for *T. aestivum* in accordance with Colbach *et al.* (In revision). This reaction to shading induced that shoot nitrogen amount per unit of leaf area varied more with shading conditions than shoot nitrogen amount per unit of leaf biomass for *P. aviculare*. For the six studied species in the present study as well as other crop and weed species, specific leaf area is much more sensitive to shading than leaf biomass ratio (Colbach *et al.*, In revision). Thus, leaf area varies more than leaf biomass in response to shading. Leaf biomass seems a more relevant variable than leaf area to express nitrogen demand independently of light environment for a wide range of species. Note that only two species could be tested for light effect in the present study. So, more species with various morphological response to shading should be tested in the future to confirm this conclusion.

4.4. The relationship between shoot nitrogen amount and leaf biomass varies among species

Four species groups were discriminated in terms of shoot nitrogen amount needed per unit of leaf biomass to reach optimal plant nitrogen nutrition. We could not discriminate the groups by clade, weeds versus crops or preferential habitat nitrogen requirements, but we only had one or two species per group. Interestingly, species with a low shoot nitrogen accumulation per unit of leaf biomass at optimal nitrogen nutrition were those with a high leaf to shoot biomass ratio (*B. napus* and *G. molle* in Figure 5). At the opposite, species at reproductive stage with the highest shoot nitrogen amount for 1g of leaf biomass were those with the lowest leaf to shoot biomass ratio (*M. sativa* and *P. aviculare* in Figure 5). Finally, plants characterized by a high shoot nitrogen accumulation per unit of leaf biomass at optimal nitrogen nutrition had intermediate values of leaf to shoot biomass ratio (*T. aestivum, F. arundinacea* and *M. sativa* at vegetative stage). In the future, it will be necessary to study for more species the link between leaf to shoot biomass ratio and shoot nitrogen amount per unit of leaf biomass at optimal nitrogen nutrition.

4.5. Implications for crop modelling and management of heterogeneous canopies

Estimating nitrogen demand in heterogeneous canopies is challenging because plants have different access to light depending on their position in the canopy. The linear relationship linking shoot nitrogen amount to leaf biomass at optimal nitrogen nutrition could thus be widely used in individual-based model to estimate plant nitrogen demand in heterogeneous canopies. For each simulated plant, once leaf biomass is predicted, the slope of the relationship multiplied by this leaf biomass gives the optimal shoot nitrogen amount of the plant, i.e. the shoot nitrogen amount needed to maximize the plant growth. However, this approach requires that leaf biomass is well simulated by the model, otherwise it will induce a bias in the estimation of shoot nitrogen demand that will impact the shoot biomass, increasing

516 the error as the plant grows. Biomass partitioning between the different plant parts was
517 reported to be a weak point in several models aiming at very precise yield predictions (Rötter
518 *et al.,* 2012; Asseng *et al.,* 2013; Coucheney *et al.,* 2015). Yet, the much rougher partitioning
519 used in some crop-weed models was sufficient to adequately predict multiannual weed
520 dynamics and impacts on crop yield (Colbach *et al.,* 2016). Depending on the aim of the
521 model and the precision required to estimate plant nitrogen demand, our formalism might be
522 sufficient. A sensitivity analysis of nitrogen demand to light conditions would allow to
523 evaluate more precisely the effect of light for individual plants as well as for the canopy.

524 Beyond the modelling approaches, the relationship linking shoot nitrogen amount to leaf
525 biomass at optimal nitrogen nutrition could be used to estimate nitrogen nutrition level of any
526 plant or species in a canopy (virtual or not). For a given leaf biomass, the shoot nitrogen
527 amount observed can be compared to the optimal shoot nitrogen amount indicated by the
528 relationship. On the same principle as Justes *et al.* (1994), a nitrogen nutrition index at the
529 plant scale could be calculated as the ratio between the measured shoot nitrogen amount and
530 the optimal shoot nitrogen amount read on the relationship highlighted in this study. This
531 plant nitrogen nutrition index could help understand better heterogeneous canopies regarding
532 how the nitrogen resource is shared among individual plants or among species. With further
533 research, this index could improve nitrogen diagnostic and management in heterogeneous
534 canopies.

535

536 **5. Conclusions**

537 The linear relationship linking shoot nitrogen amount to leaf biomass at optimal nitrogen
538 nutrition allowed to estimate nitrogen demand of individual plants of a given species in

heterogeneous canopies at vegetative stage. This relationship became allometric when plants were at reproductive stage. Our study revealed that this relationship was remarkably constant across varying environmental conditions at optimal nitrogen nutrition. Using leaf area instead of leaf biomass in the relationship made it more dependent on light environment, confirming previous findings in homogeneous canopies.

Supplementary data

Supplementary data are available online and consist of the following. Section A. Table S1: Composition of the five nutrient solutions varying for their nitrate concentrations. Table S2: Air temperature and incident photosynthetically active radiation in the experiments. Table S3: Number of days since sowing date of all four sampling dates for each species × light treatment. Section B. Table S4: Equations of the critical nitrogen dilution curves for each species × light treatment. Section C.1 and C.2. Details about the oilseed rape experiments. Table S5: Phenological parameters used for the virtual oilseed rape experiment. Section D. Figure S1: Relationship between shoot nitrogen amount and leaf area.

Declaration of interest

None.

Acknowledgements

This work was supported by the French National Institute for Agricultural Research (INRA), the Environment & Agronomy Department of INRA, the French project CoSAC (ANR- 15-CE18-0007), the Casdar RAID project funded by the French Ministry in charge of Agriculture and Food (Ministère de l'Agriculture et de l'Alimentation, avec la contribution financière du compte d'affectation spéciale 'Développement agricole et rural') and the European Union's

562 Horizon 2020 Research and innovation programme under grant agreement N. 727217
563 (ReMIX project). Laurène Perthame benefited from a PhD studentship funded by the French
564 Ministry of Research. The authors are grateful to Gaëtan Louarn for helpful discussions about
565 experimental design and for providing some seed lots, as well as Danilo dos Reis Cardoso
566 Passos, Estelle Genottin, Laura Ponticelli and the greenhouse staff for their technical
567 assistance. The authors also thank Xavier Pinochet, Guillaume Jolly and Jérôme Pacquetet
568 from Terres Inovia for their involvement in the setting up of the field experiments in
569 Bretenières and for helping in sampling rapeseed plants.

570 **References**

571 **Altieri MA, Nicholls CI, Henao A, Lana MA. 2015**. Agroecology and the design of climate
572 change-resilient farming systems. *Agronomy for Sustainable Development,* **35**: 869-
573 890.
574 **Asseng S, Ewert F, Rosenzweig C, *et al.* 2013.** Uncertainty in simulating wheat yields under
575 climate change. *Nature Climate Change,* **3**: 827-832.
576 **Barillot R, Chambon C, Andrieu B. 2016**. CN-Wheat, a functional-structural model of
577 carbon and nitrogen metabolism in wheat culms after anthesis. I. Model description.
578 *Annals of Botany,* **118**: 997-1013.
579 **Barot S, Allard V, Cantarel A, *et al.* 2017**. Designing mixtures of varieties for
580 multifunctional agriculture with the help of ecology. A review. *Agronomy for*
581 *Sustainable Development,* **37**.
582 **Berger AG. 2009**. *Competition for above and below ground resources among annual species*
583 *from the plant to the field: quantification analysis and modeling,* PhD Thesis, Cornell
584 University, Ithaca, New-York.
585 **Berger AG, McDonald AJ, Riha SJ. 2013.** Simulating Root Development and Soil Resource
586 Acquisition in Dynamic Models of Crop-Weed Competition. In: Timlin D, Ahuja LR,
587 eds. *Enhancing Understanding and Quantification of Soil-Root Growth Interactions,*
588 *Vol 4*.
589 **Brainard DC, Bellinder RR, DiTommaso A. 2005.** Effects of canopy shade on the
590 morphology, phenology, and seed characteristics of Powell amaranth (*Amaranthus*
591 *powellii*). *Weed Science Society of America*, **53**: 175-186.
592 **Brisson N, Gary C, Justes E, *et al.* 2003**. An overview of the crop model STICS. *European*
593 *Journal of Agronomy,* **18**: 309-332.
594 **Brisson N, Mary B, Ripoche D, *et al.* 1998**. STICS: a generic model for the simulation of
595 crops and their water and nitrogen balances. I. Theory and parameterization applied to
596 wheat and corn. *Agronomie,* **18**: 311-346.
597 **Brisson N, Ruget F, Gate P, *et al.* 2002**. STICS: a generic model for simulating crops and
598 their water and nitrogen balances. II. Model validation for wheat and maize.
599 *Agronomie,* **22**: 69-92.

Colbach N, Bertrand M, Busset H, Colas F, Dugué F, Farcy P, Fried G, Granger S, Meunier D, Munier-Jolain NM, Noilhan C, Strbik F, Gardarin A. 2016. Uncertainty analysis and evaluation of a complex, multi-specific weed dynamics model with diverse and incomplete data sets. *Environmental Modelling & Software,* **86**: 184-203.

Colbach N, Collard A, Guyot SHM, Meziere D, Munier-Jolain N. 2014. Assessing innovative sowing patterns for integrated weed management with a 3D crop:weed competition model. *European Journal of Agronomy,* **53**: 74-89.

Colbach N, Gardarin A, Moreau D. 2019. The response of weed and crop species to shading: Which parameters explain weed impacts on crop production? *Field Crops Research,* **238**: 45-55.

Colbach N, Moreau D, Dugué F, Gardarin A, Strbik F, Munier-Jolain N. In revision. The response of weed and crop species to shading. How to predict their morphology and plasticity from species traits and ecological indexes? *European Journal of Agronomy*.

Coucheney E, Buis S, Launay M, *et al.* 2015. Accuracy, robustness and behavior of the STICS soil-crop model for plant, water and nitrogen outpurs: Evaluation over a wide range of agro-environmental conditions in France. *Environmental Modelling and Software,* **64**: 177-190.

Duchene O, Vian JF, Celette F. 2017. Intercropping with legume for agroecological cropping systems: Complementarity and facilitation processes and the importance of soil microorganisms. A review. *Agriculture Ecosystems & Environment,* **240**: 148-161.

Dunbabin VM, Postma JA, Schnepf A, *et al.* 2013. Modelling root-soil interactions using three-dimensional models of root growth, architecture and function. *Plant and Soil,* **372**: 93-124.

Ellenberg H. 1974. Zeigerwerte der Gefässpflanzen Mitteleuropas. *Scripta geobotanica,* **IX**: 1-97.

Evans JR, Poorter H. 2001. Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell and Environment*, **24**: 755-767.

Faverjon L, Escobar-Gutiérrez A, Litrico I, Julier B, Louarn G. 2019. A generic individual-based model can predict yield, nitrogen content, and species abundance in experimental grassland communities. *Journal of Experimental Botany*, **70**: 2491-2504.

Fried G, Chauvel B, Reboud X. 2008. Evolution de la flore adventice des champs cultivés au cours des dernières décennies : vers la sélection de groupes d'espèces répondant aux systèmes de culture. *Innovations Agronomiques,* **3**: 15-26.

Gardarin A, Durr C, Mannino MR, Busset H, Colbach N. 2010. Seed mortality in the soil is related to seed coat thickness. *Seed Science Research,* **20**: 243-256.

Gastal F, Lemaire G, Durand JL, Louarn G. 2015. *Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency*.

Gaudio N, Escobar-Gutierrez AJ, Casadebaig P, *et al.* 2019. Current knowledge and future research opportunities for modeling annual crop mixtures. A review. *Agronomy for Sustainable Development,* **39**.

Gunn S, Farrar JF, Collis BE, Nason M. 1999. Specific leaf area in barley: individual leaves versus whole plants. *The New Phytologist*, **143**: 45-51.

Harley CDG, Bertness D. 1996. Structural interdependance: an ecological consequence of morphological responses to crowding in marsh plants. *Functional Ecology,* **10**: 654-661.

Hodge A, Berta G, Doussan C, Merchan F, Crespi M. 2009. Plant root growth, architecture and function. *Plant and Soil,* **321**: 153-187.

Holzworth DP, Huth NI, Devoil PG, *et al.* 2014. APSIM - Evolution towards a new generation of agricultural systems simulation. *Environmental Modelling & Software,* **62**: 327-350.

Justes E, Mary B, Meynard JM, Machet JM, Thelierhuche L. 1994. Determination of a critical nitrogen dilution curve for winter-wheat crops. *Annals of Botany,* **74**: 397-407.

Lemaire G, Avice JC, Kim TH, Ourry A. 2005. Developmental changes in shoot N dynamics of lucerne (Medicago sativa L.) in relation to leaf growth dynamics as a function of plant density and hierarchical position within the canopy. *Journal of Experimental Botany,* **56**: 935-943.

Lemaire G, Gastal F. 1997. N uptake and distribution of the nitrogen status in crops. *Springer, Berlin, Heidelberg,* 3-43.

Lemaire G, Oosterom E, Sheehy J, Jeuffroy MH, Massignam A, Rossato L. 2007. Is crop N demand more closely related to dry matter accumulation or leaf area expansion during vegetative growth? *Field Crops Research,* **100**: 91-106.

Lemaire G, Sinclair T, Sadras V, Bélanger G. **2019.** Allometric approach to crop nutrition and implications for crop diagnosis and phenotyping. A review. *Agronomy for Sustainable Development*, **39**: 27.

Lemaire G, Salette J. 1984. Relationship between growth and nitrogen uptake in a pure grass stand .1. Environmental-effects. *Agronomie,* **4**: 423-430.

Louarn G, Faverjon L. 2018. A generic individual-based model to simulate morphogenesis, C-N acquisition and population dynamics in contrasting forage legumes. *Annals of Botany,* **121**: 875-896.

Lynch JP, Nielsen KL, Davis RD, Jablokow AG. 1997. SimRoot: Modelling and visualization of root systems. *Plant and Soil,* **188**: 139-151.

Malezieux E, Crozat Y, Dupraz C*, et al.* 2009. Mixing plant species in cropping systems: concepts, tools and models. A review. *Agronomy for Sustainable Development,* **29**: 43-62.

Mclachlan SM, Tollenaar M, Swanton CJ, Weise SF. **1993.** Effect of corn-induced shading on dry matter accumulation, distribution, and architecture of Redroot pigweed (*Amaranthus retroflexus*). *Weed Science Society of America*, **41**: 568-573.

Moreau D, Allard V, Gaju O, Le Gouis J, Foulkes MJ, Martre P. 2012. Acclimation of Leaf Nitrogen to Vertical Light Gradient at Anthesis in Wheat Is a Whole-Plant Process That Scales with the Size of the Canopy. *Plant Physiology,* **160**: 1479-1490.

Moreau D, Milard G, Munier-Jolain N. 2013. A plant nitrophily index based on plant leaf area response to soil nitrogen availability. *Agronomy for Sustainable Development,* **33**: 809-815.**Moreau D, Voisin AS, Salon C, Munier-Jolain N. 2008**. The model symbiotic association between Medicago truncatula cv. Jemalong and Rhizobium meliloti strain 2011 leads to N-stressed plants when symbiotic N(2) fixation is the main N source for plant growth. *Journal of Experimental Botany,* **59**: 3509-3522.

Munier-Jolain N, Deytieux V, Guillemin JP, Granger S, Gaba S. 2008. Multi-criteria evaluation of cropping systems prototypes based on integrated weed management. *Innovations Agronomiques,* **3**: 75-88.

Munier-Jolain NM, Guyot SHM, Colbach N. 2013. A 3D model for light interception in heterogeneous crop:weed canopies: Model structure and evaluation. *Ecological Modelling,* **250**: 101-110.

Munier-Jolain NM, Collard A, Busset H, Guyot SHM, Colbach N. 2014. Investigating and modelling the morphological plasticity of weeds. *Field Crops Research,* **155**: 90-98.

Pages L, Becel C, Boukcim H, Moreau D, Nguyen C, Voisin AS. 2014. Calibration and evaluation of ArchiSimple, a simple model of root system architecture. *Ecological Modelling,* **290**: 76-84.

Perry LG, Neuhauser C, Galatowitsch SM. 2003. Founder control and coexistence in a simple model of asymmetric competition for light. *Journal of Theoretical Biology,* **222**: 425-436.

Postma JA, Kuppe C, Owen MR, *et al.* 2017. OPENSIMROOT: widening the scope and application of root architectural models. *New Phytologist,* **215**: 1274-1286.

RCoreTeam. 2019. R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. Vienna, Austria.

Rossato L, Laine P, Ourry A. 2001. Nitrogen storage and remobilization in Brassica napus L. during the growth cycle: nitrogen fluxes within the plant and changes in soluble protein patterns. *Journal of Experimental Botany*, **52**: 1655-1663.

Rötter RP, Palosuo T, Kersebaum KC, *et al.* 2012. Simulation of pring barley yield in different climatic zones of Northern and Central Europe: A comparison of nine crop models. *Field Crops Research,* **133**: 23-36.

Seginer I. 2004. Plant spacing effect on the nitrogen concentration of a crop. *European Journal of Agronomy,* **21**: 369-377.

Sims DA, Pearcy RW. **1994.** Scaling sun and shade photosynthetic acclimation of *Alocasia macrorrhiza* to whole-plant performance – I. Carbon balance and allocation at different daily photon flux densities. *Plant, Cell and Environment,* **17**: 881-887.

Soussana JF, Arregui MC. 1995. Impact de l'association sur le niveau de nutrition azotée et la croissance du ray-grass anglais et du trèfle blanc. *Agronomie,* **15**: 81-96.

Soussana JF, Maire V, Gross N, *et al.* 2012. Gemini: A grassland model simulating the role of plant traits for community dynamics and ecosystem functioning. Parameterization and evaluation. *Ecological Modelling,* **231**: 134-145.

Sutton MA, Oenema O, Erisman JW, Leip A, van Grinsven H, Winiwarter W. 2011. Too much of a good thing. *Nature,* **472**: 159-161.

Ulrich A. 1952. Physiological bases for assessing the nutritional requirements of plants. *Annual Review of Plant Physiology*: 207-228.

Wilson SD, Tilman D. 1993. Plant competition and resource availability in response to disturbance and fertilization. *Ecology,* **74**: 599-611.

Wu L, McGechan MB. 1998. Simulation of biomass, carbon and nitrogen accumulation in grass to link with a soil nitrogen dynamics model. *Grass and Forage Science,* **53**: 233-249.

Wu L, McGechan MB, McRoberts N, Baddeley JA, Watson CA. 2007. SPACSYS: Integration of a 3D root architecture component to carbon, nitrogen and water cycling-model description. *Ecological Modelling,* **200**: 343-359.

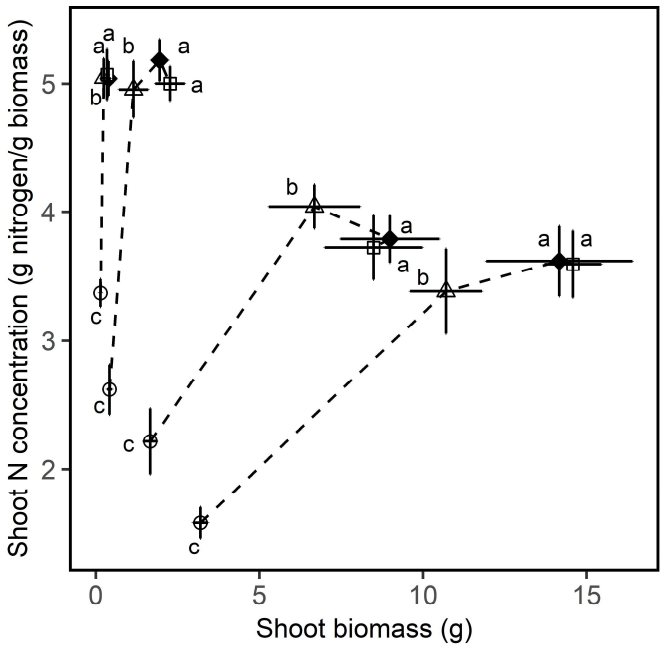

Figure 1: Method to identify the critical solution nitrogen concentration at each sampling date for *Geranium molle* in unshaded treatment. Symbol shapes indicate nitrogen treatment (○ 0.4mM, Δ 1mM, ◇ 5mM and □ 10mM). Each point is the mean value of four to six plants corresponding to a nitrogen treatment × sampling date combination, error bars are standard-deviation. Nitrogen treatments of a given sampling date are linked by a dashed line. At each sampling date, the critical solution nitrogen concentration (allowing to reach the minimum plant nitrogen concentration required to maximize shoot biomass) is filled in black. The shoot biomass of points sharing the same letter are not significantly different.

Single column fitting image.

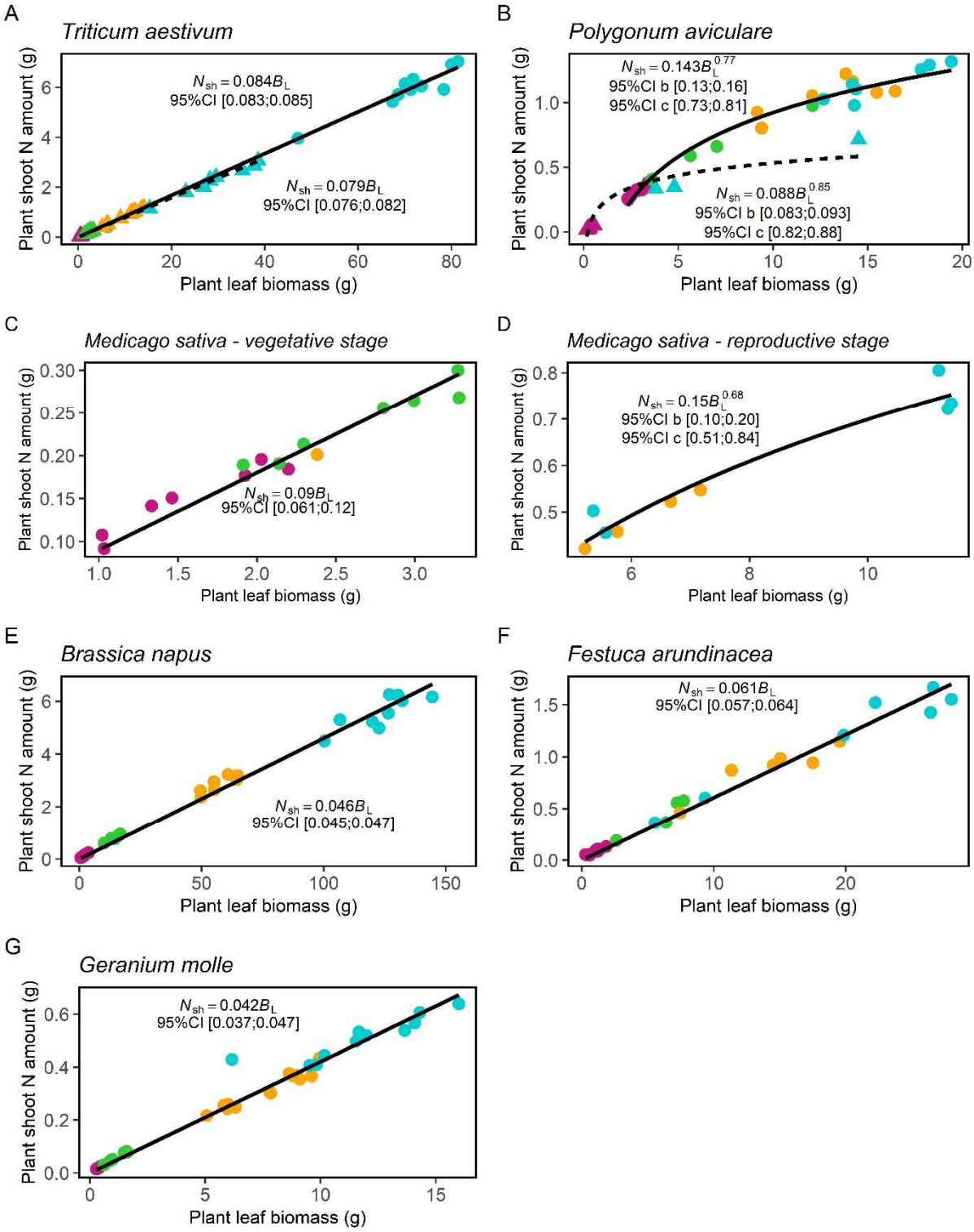
A
Triticum aestivum
$N_{sh} = 0.084B_L$
95%CI [0.083;0.085]
$N_{sh} = 0.079B_L$
95%CI [0.076;0.082]
Plant shoot N amount (g)
Plant leaf biomass (g)
B
Polygonum aviculare
$N_{sh} = 0.143B_L^{0.77}$
95%CI b [0.13;0.16]
95%CI c [0.73;0.81]
$N_{sh} = 0.088B_L^{0.85}$
95%CI b [0.083;0.093]
95%CI c [0.82;0.88]
Plant shoot N amount (g)
Plant leaf biomass (g)
C
Medicago sativa - vegetative stage
$N_{sh} = 0.09B_L$
95%CI [0.061;0.12]
Plant shoot N amount (g)
Plant leaf biomass (g)
D
Medicago sativa - reproductive stage
$N_{sh} = 0.15B_L^{0.68}$
95%CI b [0.10;0.20]
95%CI c [0.51;0.84]
Plant shoot N amount (g)
Plant leaf biomass (g)
E
Brassica napus
$N_{sh} = 0.046B_L$
95%CI [0.045;0.047]
Plant shoot N amount (g)
Plant leaf biomass (g)
F
Festuca arundinacea
$N_{sh} = 0.061B_L$
95%CI [0.057;0.064]
Plant shoot N amount (g)
Plant leaf biomass (g)
G
Geranium molle
$N_{sh} = 0.042B_L$
95%CI [0.037;0.047]
Plant shoot N amount (g)
Plant leaf biomass (g)

Figure 2: Relationship between shoot nitrogen amount (N_{sh}) and leaf biomass (B_L) at optimal nitrogen nutrition for different species grown in unshaded (●) and shaded treatment (▲). Each point represents a plant. Colours indicate plants sampled at different dates: first sampling (red), second sampling (green), third sampling (orange) and fourth sampling (blue). (A) (C) (E) (F) (G) Species at vegetative stage. Equations and black lines result from fitting linear model (1) to our data (with the 95% confidence interval of the slopes) (R^2=0.994). (B) (C) Species at reproductive stage. Equations and black lines result from fitting $N_{sh}=b\times B_L^{c}$ to our data after a log-n transformation (with the 95% confidence intervals) (R^2=0.996).

2-column fitting image.

Can be printed in grey-shades and produced in colour in the pdf-version of the paper.

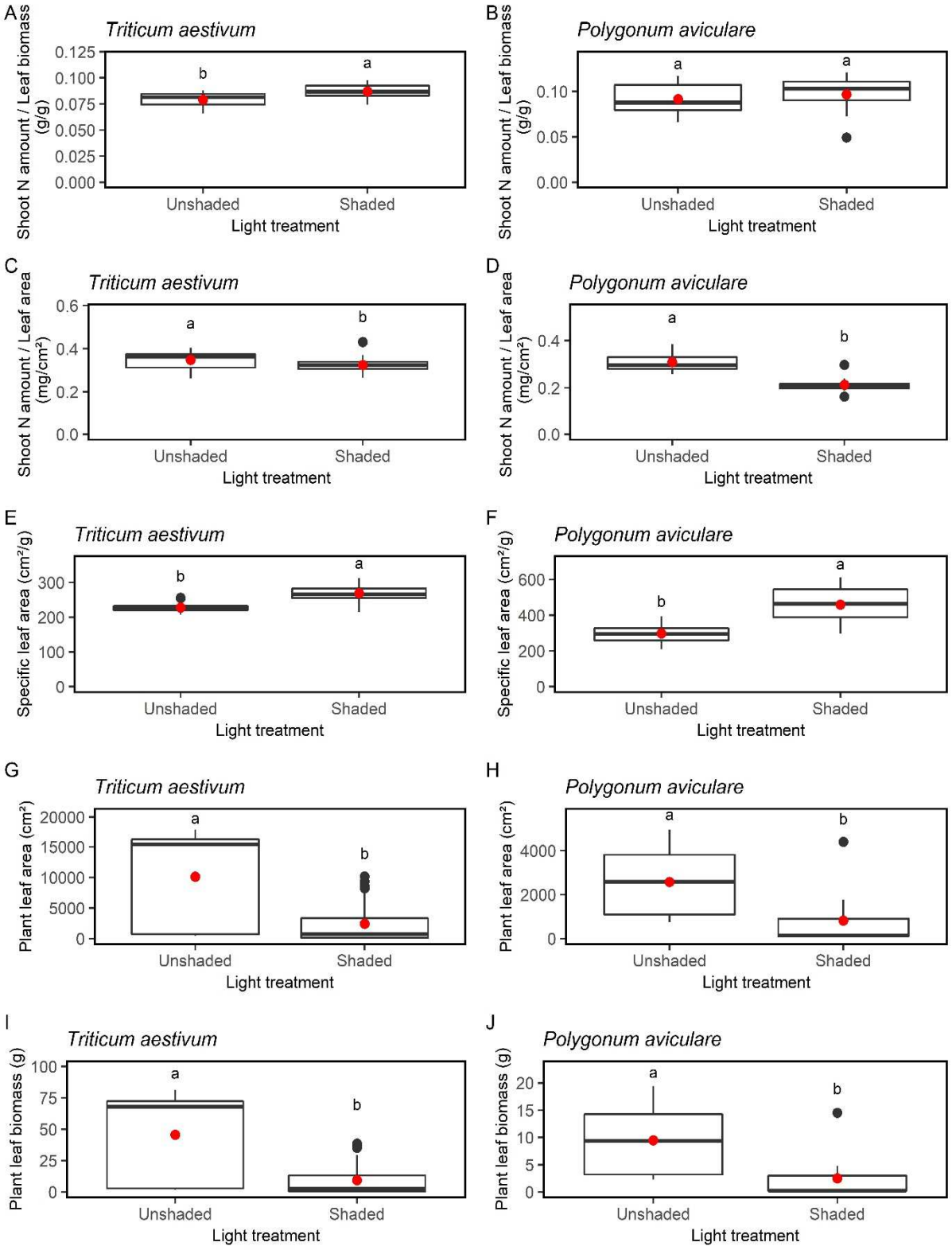

Figure 3: (A) (B) Shoot nitrogen amount to leaf biomass ratio, (C) (D) shoot nitrogen amount to leaf area ratio, (E) (F) specific leaf area, (G) (H) leaf area and (I) (J) leaf biomass for plants at optimal nitrogen nutrition for two species grown in unshaded and shaded treatment. Boxplots showing minimum, third quartile, median, first quartile and maximum ratio values (with outliers outside four times the interquartile range). Red points show means. Same letters indicate that means are not significantly different.

Two columns fitting image.

Can be printed in grey-shades and produced in colour in the pdf-version of the paper.

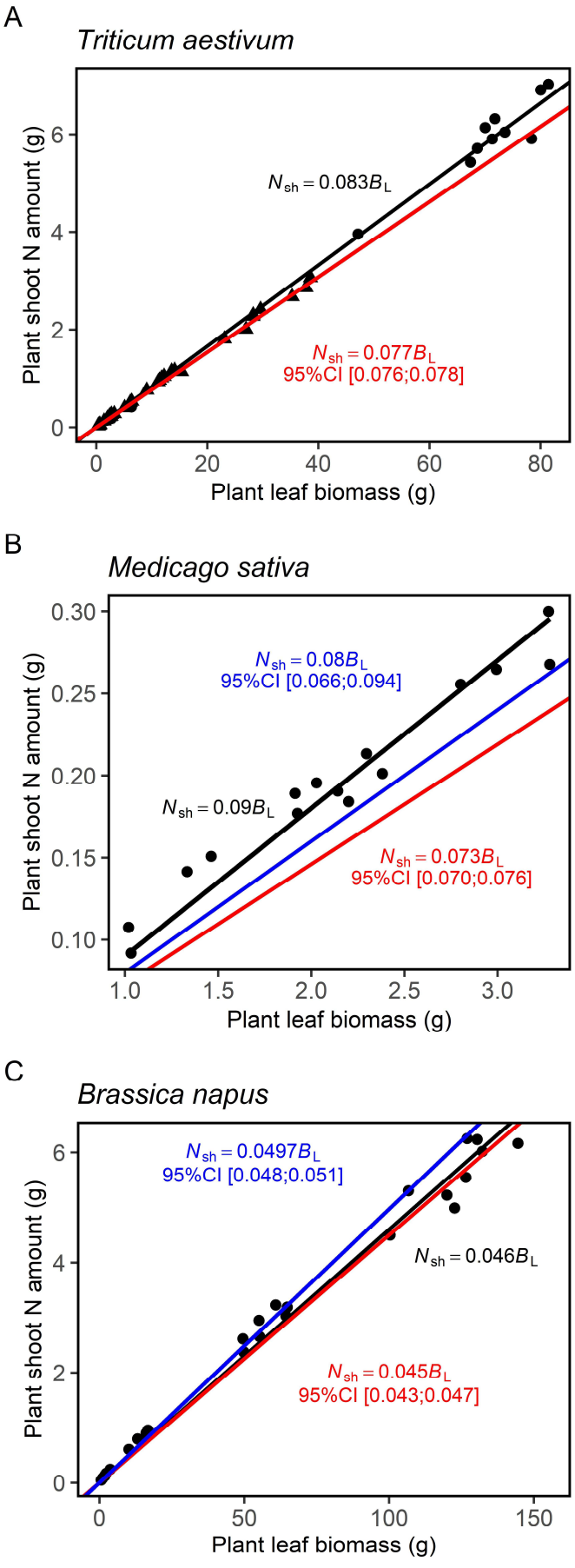
A
Triticum aestivum
Plant shoot N amount (g)
Plant leaf biomass (g)
$N_{sh} = 0.083B_L$
$N_{sh} = 0.077B_L$
95%CI [0.076;0.078]
B
Medicago sativa
Plant shoot N amount (g)
Plant leaf biomass (g)
$N_{sh} = 0.08B_L$
95%CI [0.066;0.094]
$N_{sh} = 0.09B_L$
$N_{sh} = 0.073B_L$
95%CI [0.070;0.076]
C
Brassica napus
Plant shoot N amount (g)
Plant leaf biomass (g)
$N_{sh} = 0.0497B_L$
95%CI [0.048;0.051]
$N_{sh} = 0.046B_L$
$N_{sh} = 0.045B_L$
95%CI [0.043;0.047]

Figure 4: Relationship between shoot nitrogen amount (N_{sh}) and leaf biomass (B_L) at optimal nitrogen nutrition for three crop species at vegetative stage grown in unshaded (●) and shaded treatment (▲) in our experiments. Equations and lines result from fitting linear model (4) for our experimental data (in black) and for field or simulated data obtained from the literature or prior experiments (in red or blue): (A) field data obtained in a homogeneous wheat canopy at vegetative stage (Moreau *et al*., 2012), (B) field data obtained in a dense *Medicago sativa* stand at vegetative stage (in blue) (Lemaire *et al*., 2005) and data of *Medicago truncatula* at vegetative stage obtained in greenhouse experiments under three light treatments (in red) (Moreau *et al.*, 2008), (C) field data obtained in a homogeneous oilseed rape field at vegetative stage (in red) and simulated data obtained with APSIM-Canola at vegetative stage (in blue) (Sections C.1 and C.2 [Supplementary Information]).

Single column fitting image.

Can be printed in grey-shades and produced in colour in the pdf-version of the paper.

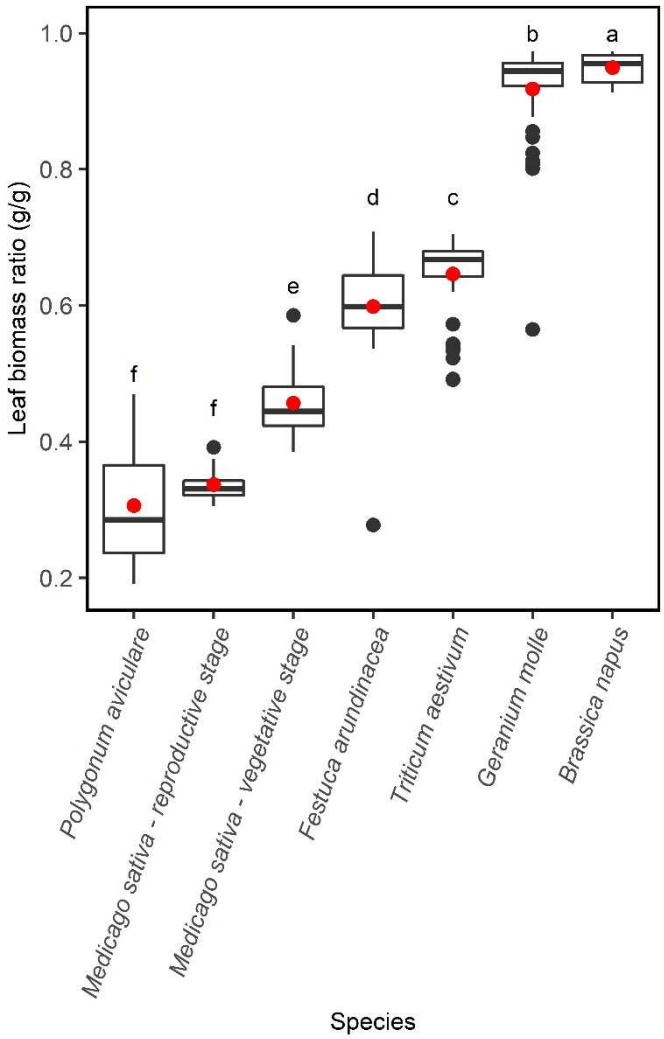

Figure 5: Leaf to shoot biomass ratio per species. Boxplots showing minimum, third quartile, median, first quartile and maximum leaf biomass ratio values (with outliers outside four times the interquartile range). Red points show mean leaf biomass ratio. Same letters indicate no significantly different means.

Single column fitting image.

Can be printed in grey-shades and produced in colour in the pdf-version of the paper.

Table 1: Details of the experiments performed in greenhouse in Dijon (France). Plants were grown under four or five nitrogen (N) treatments. *Triticum aestivum* and *Polygonum aviculare* were grown under two light treatments (unshaded or shaded).

Year	Species (cultivar for crops)	Monocotyledonous or dicotyledonous species	Ellenberg N-number[a]	Nitrogen treatments (mM)		Percentage of incident light available to plants	
				Unshaded treatment	Shaded treatment	Unshaded treatment	Shaded treatment
2015	*Festuca arundinacea* (Soni)	Monocotyledon	5	0.4, 1, 5 and 10		100%	
	Medicago sativa (Agathe NF[b])	Dicotyledon	NA				
2016	*Brassica napus* (Kadore)	Dicotyledon	12.4	1, 5, 10 and 14		100%	
	Geranium molle	Dicotyledon	4				
2018	*Triticum aestivum* (Caphorn)	Monocotyledon	4.4	1, 5, 10 and 14	0.4, 1, 5, 10 and 14	100%	40%
	Polygonum aviculare	Dicotyledon	6				

[a] Ellenberg N-number estimated from Ellenberg (1974) for all species except *B. napus* and *T. aestivum* (Moreau *et al.*, 2013).
[b] Non-N_2-fixing

Table 2: Description of the experiments from literature or prior experiments used to study the stability to environmental conditions of the relationship between shoot nitrogen amount and leaf biomass at optimal nitrogen nutrition.

Source	Crop species	Type of experiment	Measurement scale	Light environment
Moreau *et al.* (2008)	*Medicago truncatula*	Greenhouse	Individual plant	Three light treatments (no-shade, low-shade and high-shade)
Lemaire *et al.* (2005)	*Medicago sativa*	Field	Individual plant	Dense canopy with three hierarchical positions (dominant, intermediate and suppressed)
Moreau *et al.* (2012)	*Triticum aestivum*	Field	Average plant over the canopy	Homogeneous canopy
Section C.1 [Supplementary Information]	*Brassica napus*	Field	Average plant over the canopy	Homogeneous canopy
Section C.2 [Supplementary Information]	*Brassica napus*	Simulations (APSIM-Canola model)	Average plant over the canopy	Homogeneous canopy

Table 3: Analysis of variance of shoot nitrogen amount to leaf biomass ratio as a function of species, light treatment and their interaction (model (2)). Partial R^2 (calculated from the type III sum of square of Anova function in R) indicate the proportion of variance of ratio explained by each factor. NS indicate non-significant factor.

	Shoot N amount to leaf biomass ratio	
Factors	Partial R^2	p-value
Species	0.632	<0.0001
Light	0.009	0.0451
Species × Light	0.000	NS
Total	0.641	<0.0001

Table 4: (A) Slope of the linear relationship linking shoot nitrogen amount to leaf biomass at optimal nitrogen nutrition for six species fitted with model (3) (i.e. the effect of light treatment is neglected) (R^2=0.996). Two slope values followed by the same letter (a, b or c) are not significantly different.

Species	Slope value (g nitrogen/g biomass) and 95%CI		
Triticum aestivum	0.083	[0.082;0.084]	a
Medicago sativa – vegetative stage	0.090	[0.061;0.12]	ab
Festuca arundinacea	0.061	[0.057;0.064]	b
Brassica napus	0.046	[0.045;0.047]	c
Geranium molle	0.042	[0.037;0.047]	c

(B) Parameters of the allometric relationship linking shoot nitrogen amount (N_{sh}) to leaf biomass (B_L) at optimal nitrogen nutrition for two species at reproductive stage fitted with the model $N_{sh}=b\times B_L^c$ neglecting light effect (R^2=0.988). Two parameter values followed by the same letter are not significantly different.

Species	Shape parameter (c) and 95%CI			Shoot nitrogen amount at 1g of leaf biomass (b) and 95%CI		
Polygonum aviculare	0.90	[0.87;0.93]	a	0.10	[0.097;0.11]	a
Medicago sativa – reproductive stage	0.68	[0.51;0.84]	b	0.15	[0.10;0.20]	a

Table 5: Analysis of variance of shoot nitrogen amount to leaf area ratio as a function of species, light treatment and their interaction (model (2) using leaf area instead of leaf biomass). Partial R^2 (calculated from the type III sum of Anova function in R) indicate the proportion of variance of the ratio explained by each factor. NS indicate non-significant factor.

	Shoot N amount to leaf area ratio	
Factors	Partial R^2	p-value
Species	0.647	<0.0001
Light	0.004	NS
Species × Light	0.015	0.0036
Total	0.666	<0.0001