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RESEARCH PAPER

# 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

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## Abstract

**Shoot N concentration in plants decreases as they get bigger, due to the fact that N accumulates less rapidly than dry matter in plants during the plant growth process, leading to an allometric relationship between shoot N content ($N_{sh}$) and shoot mass ($W_{sh}$): $N_{sh} = a(W_{sh})^b$. The results obtained on lucerne plants growing either under controlled low density conditions or in dense stands under field conditions show that the value of the allometric coefficient *b* that represents the ratio between the relative N accumulation rate in shoots [$dN_{sh}/(N_{sh}dt)$] and the relative growth rate [$dW_{sh}/(W_{sh}dt)$], decreases from 0.88 for a low plant density to 0.72 for a dense stand. Therefore, the fractional increase of shoot N per unit of shoot dry matter is lower when plants are in competition for light in dense canopies. This decrease can be entirely explained by the parallel decline in the leaf area per unit of shoot mass. Thus, a remarkably constant linear relationship can be established between $N_{sh}$ and leaf area (*LA*): $N_{sh} = 1.7\ g\ m^{-2}\ LA$, regardless of the conditions (low versus high density, controlled versus field conditions). Moreover, in a field dense stand, the comparison of plants with contrasting positions between the top and the bottom of the canopy (dominant, intermediate or suppressed plants), also shows that the difference in $N_{sh}$ at similar shoot mass is explained by the proportion of leaf mass to shoot mass. These data support the idea that leaf growth drives the dynamics of shoot N accumulation. These results also indicate that competition for light among individual plants within a dense canopy induces developmental changes in plant morphology (leaf:stem ratio) that explain the differences observed in shoot N concentration. This last observation could be extrapolated to multispecific plant stands. Therefore, the sharing of N resources among plant species could partially be the result of the sharing of light within the canopy.**

Key words: Leaf area, leaf:stem ratio, *Medicago sativa* L., N dilution, plant density, shoot N accumulation.

## Introduction

Understanding of the N distribution within a canopy is relevant for the analysis of behaviour of an individual plant in a dense stand where competition for light, minerals, and water may be occurring between plants. For that it is necessary to know the developmental effect of growth on N acquisition and distribution within the plant in relation with the level of competition with neighbouring plants.

It is generally accepted that even when there is an ample supply of N, the shoot N concentration in plants within

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Abbreviations, *LA*, leaf area; *LAI*, crop leaf area index; *LAR*, leaf area ratio; *LMA*, leaf mass per unit of leaf area; *LWR*, leaf weight ratio; %N, plant N concentration; $N_{sh}$, N accumulation in the shoot.

a dense canopy decreases as the plants grow. Lemaire and Salette (1984) and Lemaire *et al.* (1985) showed that for perennial grasses and lucerne, the N accumulation ($N_{sh}$) in plant shoots at any given time was related to the shoot mass ($W_{sh}$) during growth process according to an allometric function:

$$N_{sh} = a(W_{sh})^{b} \quad (1)$$

or

$$[N_{sh}] = a(W_{sh})^{b-1} \quad (1')$$

where $[N_{sh}]$ is the N concentration in the shoot, $a$ represents the quantity of N required to produce the first unit of shoot mass ($W_{sh}$=1), and $b$ represents the ratio between the relative N accumulation rate ($dN_{sh}/N_{sh}dt$) and the relative growth rate ($dW_{sh}/W_{sh}dt$).

Two different processes may be involved in the decrease of shoot N concentration with shoot mass in a dense canopy. (i) According to Caloin and Yu (1984) and Greenwood *et al.* (1990), plants can be viewed as being composed of two compartments. The first, the metabolic compartment, is involved in growth processes with a high N concentration and, the second, the structural compartment, with a low N concentration. The proportion of these two compartments within the total plant mass would decrease as plants get bigger, leading to a decrease in shoot N concentration. Following Hardwick (1987) the metabolic compartment can be considered to scale with leaf area, while the shoot mass would scale with shoot volume, so the decrease in shoot N concentration should parallel the decrease in leaf area:shoot mass ratio (Lemaire and Gastal, 1997). (ii) According to Charles Edwards *et al.* (1987), Hirose *et al.* (1988), and Lemaire *et al.* (1991), the allocation of N in leaves within a dense canopy is not uniform and more or less parallels the light distribution profile. As a crop canopy develops, an increasing proportion of leaves are shaded and the average N concentration of plants within the canopy therefore declines.

The first objective of this paper is to analyse to what extent these two hypothesises can be combined in order to explain the plant N concentration decrease during the growth process. The second one is to analyse how the presence of neighbouring plants can affect the shoot N dynamics of any individual plant in a dense stand.

Previously published data on plant growth, shoot N accumulation dynamics, and *LA* expansion obtained on lucerne plants growing either under controlled conditions at low plant density or under field conditions in a dense canopy (Kim *et al.*, 1993; Avice *et al.*, 1997*a*) were used in order to analyse the effect of the intensity of competition. Lucerne is a non-clonal dicotelydonous herb showing the following features: (i) the separation between leaf and stem fraction is easy to achieve and each of these two fractions can be related to each of the two conceptual compartments, metabolic for the leaf and structural for the stem. This is not as evident for other species, such as grasses, where the morphological differences between photosynthetic and structural tissues are not as clear; (ii) individual plants can easily be identified within a canopy, which is not possible with plants with clonal propagation such as grasses; and (iii) it can be supposed that a steady-state non-limiting condition for plant N nutrition can be more easily achieved during the regrowth period under field conditions for a legume species with both $N_2$ fixation and soil N mineral sources. Thus, lucerne appears to be a good plant model for generating data as well as for explaining the relationships between individual plant development, canopy structure, and N accumulation dynamics in shoots.

## Materials and methods

Two types of experiments were used for producing data. The first type was based on a study carried out in a greenhouse where plants were grown using hydroponic solutions. Two plant densities, 20 and 40 plants $m^{-2}$, were considered. The second type of experiment was based on a field study with a plant density of about 300–500 plants $m^{-2}$.

### *Greenhouse experiment (low-density culture)*

These experiments were extensively described in Kim *et al.* (1993). Therefore only the most important aspects will be included in this document for data analysis purposes.

#### *Plant material and culture*

Lucerne seeds (*Medicago sativa* L. var. Europe) were germinated on sand. After 15 d, when the primary trifoliate leaves appeared, seedlings were transplanted to plastic pots filled with sand and irrigated three times per week with 300 $cm^3$ of a full nutrient solution. The basic nutrient solution contained 0.4 $KH_2PO_4$, 1.0 $K_2SO_4$, 3.0 $CaCl_2$, 0.5 $MgSO_4$, 0.15 $K_2HPO_4$, and 0.2 Fe-Na EDTA in mol $m^{-3}$, and 14 $H_3BO_3$, 5 $MnSO_4$, 3 $ZnSO_4$, 0.7 $CuSO_4$, 0.7 $(NH_4)_6Mo_7O_{24}$, and 0.1 $CoCl_2$ in mmol $m^{-3}$ (Kim *et al.*, 1991). Nitrogen was supplied at 1 mol $m^{-3}$ of $NH_4NO_3$ to repress nodule formation. Plants were grown under greenhouse conditions with temperatures of 20 °C (day) and 18 °C (night) and a photoperiod of 16 h (day) and 8 h (night). After three months, plants were defoliated 6 cm above crown level and transferred to a continuously aerated nutrient solution in a plastic container of 8000 $cm^3$ (three plants per plastic container). After 30 d of regrowth, plants were again defoliated 6 cm above crown level and the regrowth after defoliation was studied by harvesting at days 3, 6, 9, 13, and 26 of growth. The density of the culture was either 20 or 40 plants $m^{-2}$. Throughout the entire experiment, the basic nutrient solution containing 1 mM $NH_4NO_3$ was renewed every 3 d and light was supplemented with high pressure sodium lamps (phytoclaude 400 W) supplying approximately 400 μmol photons $m^{-2}$ $s^{-1}$ 15 cm above crown level.

#### *Plant sampling and analysis*

Plant were harvested on the day of defoliation (day 0) and after 3, 6, 9, 13, and 26 d of regrowth, and were separated into leaves and stems. At the same time, and for the experiment with 40 plants $m^{-2}$ only, three independent samples per date of harvest were taken to determine the leaf area (*LA*, expressed as $cm^2$ of leaves per plant measured with Li-3100 area metre, Li-Cor, Inc., Lincoln, NE, USA).

Leaf and stem tissues were dried at 80 °C for 72 h. The dry weight of each tissue was determined. The leaves and stems were ground to a fine powder and stored in a vacuum with $CaCl_2$ until N analysis. The N concentration of stems and leaves was determined with an N analyser (Roboprep CN, PDZ Europa Scientific Ltd, Crewe, UK).

### *Field experiment (high-density culture)*

This experiment is extensively described in Avice *et al.* (1997*a*, *b*). In this document, only the most important aspects concerning the plant material are described and, the determination of three height categories of plants that present different hierarchical positions in the overall plant population.

### *Plant material and culture*

Lucerne stands (cv. Europe, or cv. Lodi, 5 blocks of 40 ×5 m each) were sown in field plots (16.5 cm between rows) in April 1993 in Lusignan, France (46.26° N, 0.07° E). Plant shoots were harvested in July, August, and November 1993. In 1994, plots received 80 kg P $ha^{-1}$ and 90 kg K $ha^{-1}$ at the end of February and they were first cut in May. On 6 July, the plants were cut a second time 6 cm above the soil level and the regrowth of the plants was observed at 7, 14, 21, 27, and 35 d after defoliation. The density was 325±13 plants $m^{-2}$. Between 24 June and 5 August 2004, plants were irrigated (177 mm $H_2O$) to prevent water deficit.

### *Plant sampling and analysis*

Throughout the experiment, sample plants were harvested along 2 m of a row. Plants were separated into leaves and stems (above the level of cutting). After the fresh weight was determined, tissue samples were dried at 70 °C for 72 h, ground to a fine powder, and stored in a vacuum with $CaCl_2$ until N analysis. Moreover, two independent samples were taken on an adjacent 0.5 m row to determine the leaf area index (*LAI*, expressed as $m^2$ of leaves $m^{-2}$ of soil), using a leaf area meter.

The changes of N accumulation in shoot dry matter were also studied in field experiments using the hierarchical position of plants within the canopy for light interception (Avice *et al.*, 1997*b*). The shorter plants, corresponding to the suppressed plant category, were shaded by the taller ones, the dominant plant category that intercepted most of the incident light. Therefore, at any given time during the regrowth period of the lucerne stand, it was possible to identify three categories of plants, according to the height of each plant: dominant (D), suppressed (S), and intermediary (I), representing the level of irradiance intercepted by their leaves. For the purpose of sampling plants from suppressed to dominant position categories during shoot regrowth after defoliation, all plants along 2 m of each row were harvested and then sorted according to their shoot length into one of the three categories: dominant (D), corresponding to the 20% tallest plants, suppressed (S) corresponding to the 20% shortest plants, and intermediate (I), corresponding to the 20% medium-height plants. Individual plant shoots from each category were separated into stem, leaves, and crown. The tissues were dried at 80 °C for 72 h, weighed for dry matter determination, ground to a fine powder, and stored in a vacuum with $CaCl_2$ dessicant until N analysis could be performed. The N concentration of stems and leaves was determined as described above.

### *Data analysis*

The greenhouse experiment was performed with three replicates (each replicate containing three plants) and the results were given as the mean of $n$=3. The field experiment was designed as randomized complete blocks with five replicates per plot and dominant, intermediate, and suppressed plants were harvested from two independent plots (Avice *et al.*, 1997*a*, *b*). The slope value of linear relationships for dominant, intermediate, and suppressed plants were statistically compared using Student's *t*-test (Statview Student software, Abacus Concepts, Berkeley, CA., USA).

## Results

### *Dynamics of shoot N, leaf area, and shoot mass for individual plants under controlled conditions*

The changes of N accumulation in shoots in relation to shoot mass for individual plants growing at low density (20 and 40 plants $m^{-2}$) under controlled conditions is shown in Fig. 1. It is possible to fit an allometric relationship between shoot N ($N_{sh}$) and shoot mass ($W_{sh}$) for both densities, according to equation 1:

$$20 \text{ plants m}^{-2}: \; N_{sh} = 0.114(W_{sh})^{0.881} \quad R^2 = 0.997 \quad (1.1)$$

$$40 \text{ plants m}^{-2}: \; N_{sh} = 0.112(W_{sh})^{0.840} \quad R^2 = 0.998 \quad (1.2)$$

The value of coefficient $a$ is similar for the two densities, indicating that the level of N supply for the two experiments was roughly the same. The value of coefficient $b$ is significantly higher at the low plant density (0.881) than at the high density (0.840), indicating that N accumulation in the shoot ($N_{sh}$) increases less rapidly with shoot mass when plant density increases from 20 to 40 plants $m^{-2}$.

On the set of plants with a density of 40 plants $m^{-2}$, it was possible to represent the changes of expansion of leaf area (*LA*) with the shoot mass. *LA* increases less than proportionally to shoot mass according to an allometric function:

$$LA = k_1(W_{sh})^{b'} \quad (2)$$

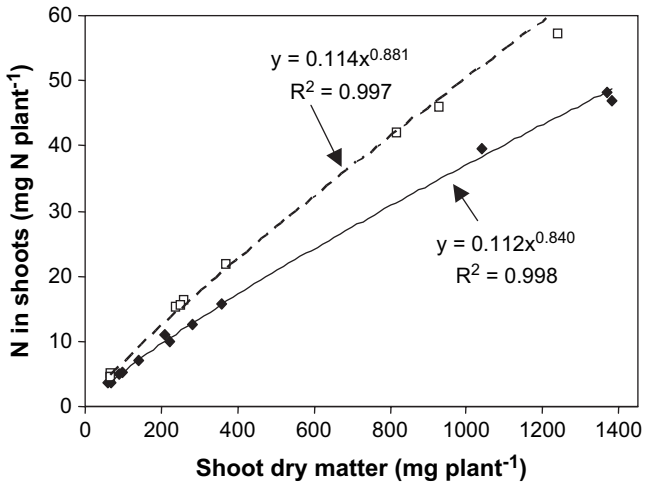


**Fig. 1.** Changes of the shoot N amount (mg $plant^{-1}$) as a function of the changes in the shoot dry matter (mg $plant^{-1}$) during the growth process in lucerne plants grown under hydroponic conditions at low density (20 or 40 plants $m^{-2}$). Density of 40 (——) or 20 (----) plants $m^{-2}$.

The corresponding fitted equation is:

$$LA = 0.671(W_{sh})^{0.849} \quad R^2 = 0.965 \qquad (2.1)$$

The allometric coefficient $b'$ is close to the value of $b$=0.840 in equation 1.2 in Fig. 1. As a result, if it is postulated that $b=b'$, it is possible to derive a linear relationship between N accumulation in the shoot and *LA* at the individual plant level:

$$N_{sh} = a/k_1(LA) \qquad (3)$$

where the coefficient $a/k_1$ represents the quantity of N that must accumulate in the shoot in order to elaborate a new *LA* unit. Figure 2 shows the fitted equation for the plants growing at a density of 40 plants $m^{-2}$. The intercept of this regression is not different from 0. The slope of the regression is equal to 1.67 g N $m^{-2}$, so the quantity of N accumulated in the shoot remains strictly proportional to *LA*. Unfortunately, the lack of data on *LA* for the experiment at the lower plant density does not make it possible to confirm such a relationship.

Coefficient $k_1$ of equation 2 represents the value of *LA* for a plant when $W$=1. Such a coefficient can be considered as the 'intrinsic leafiness' of the plant. Coefficient $a$ of equation 1 represents the shoot N content for a plant when $W$=1, corresponding to the 'intrinsic shoot N concentration'. According to equation 2, as the plant gets bigger, its *LA* increases at the same time as its shoot N content at the same fractional rate, leading to proportionality between shoot N accumulation and *LA* during plant development.

### *Dynamics of crop N, leaf area, and biomass at the stand level in the field*

The accumulation of N in a lucerne stand in relation to crop biomass was calculated according to equation 1:

$$N_{sh} = 0.161(W_{sh})^{0.723} \quad R^2 = 0.981 \qquad (1.3)$$

The value of coefficient $b$=0.723 is close to the range of values of 0.64–0.71 obtained by Lemaire *et al.* (1985) for different regrowth periods of lucerne in dense stands in the field. The relationships between leaf area index (*LAI*) and shoot mass corresponding to equation 2 reveal a similar pattern:

$$LAI = 0.052(W_{sh})^{0.809} \quad R^2 = 0.951 \qquad (2.2)$$

As a consequence, it is possible to derive a linear relationship between the accumulation of N in shoots and *LAI*, according to equation 3, as shown in Fig. 3. The slope of this regression (1.77 g N $m^{-2}$) is much closer to the slope obtained under controlled conditions at a much lower plant density (Fig. 2). Nevertheless, this value is obtained by a different combination of coefficients $a$ and $k_1$ where these ratios remain relatively unaffected by the differences in growth conditions: low versus high plant density and controlled versus field conditions. A lower value of $a$ (low intrinsic shoot N concentration) appears to have been entirely compensated for by a lower value of $k_1$ (low intrinsic 'leafiness'), leading to a remarkably constant shoot N content per unit of *LA*.

### *Partitioning of N between individual plants within the canopy*

For each group of plants, dominant (D), intermediate (I) or suppressed (S), a relationship was obtained between shoot N accumulation ($N_{sh}$) and shoot plant mass ($W_{sh}$) according to equation 1:

$$\text{D: } N_{sh} = 0.041(W_{sh})^{0.687} \quad R^2 = 0.972 \qquad (1.4)$$

$$\text{I: } N_{sh} = 0.035(W_{sh})^{0.674} \quad R^2 = 0.924 \qquad (1.5)$$

$$\text{S: } N_{sh} = 0.026(W_{sh})^{0.747} \quad R^2 = 0.862 \qquad (1.6)$$

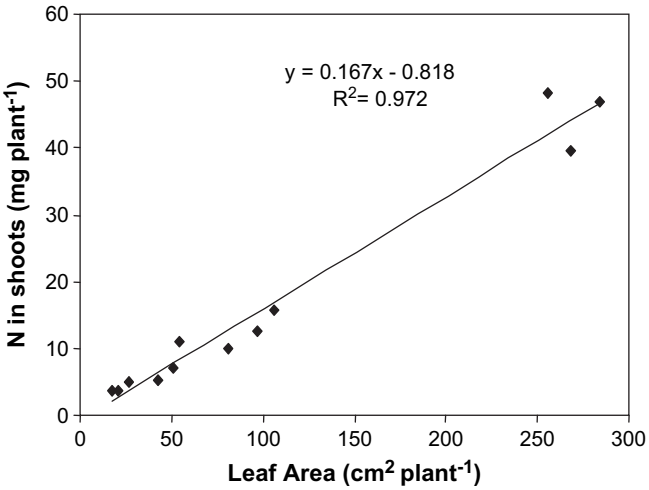


**Fig. 2.** Linear regression relationship between the accumulation of shoot N and the development of leaf area (*LA*) during the growth process in lucerne plants grown under hydroponic conditions at low density (40 plants $m^{-2}$).

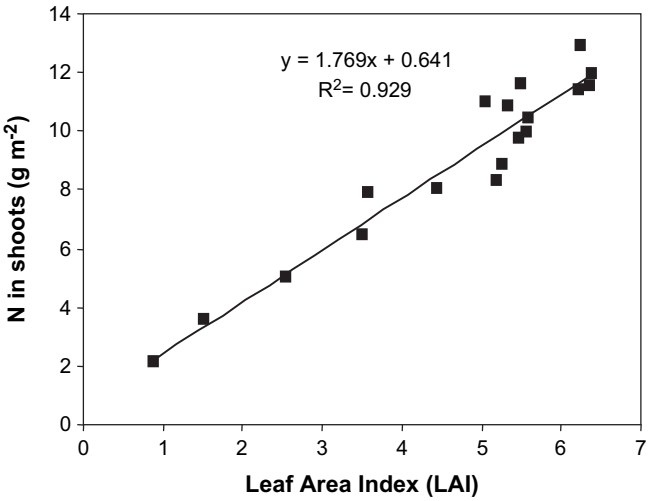


**Fig. 3.** Relationship between shoot N accumulation and crop *LAI* expansion during a regrowth process of a lucerne stand under field conditions at high density.

In Fig. 4A, these regressions were represented on a log–log scale to provide an easier comparison between plants with considerable size differences. For a given class of plants, each data point represents the average value of a set of plants of this class at a given time. The D and I classes of plants show a similar evolution: the values of coefficient *b* are not different ($P > 0.05$), but the values of the intercept are slightly different. This indicates that the intrinsic shoot N content (i.e. $N_{sh}$ for $W_{sh}$=1 g) of intermediate plants is slightly lower than that of D plants (35 mg N versus 41 mg N). Suppressed plants show a major reduction of shoot N when compared with other plant classes at similar plant mass, but the slope of the regression (coefficient *b*) is higher ($P < 0.05$). These data indicate that the relative difference in shoot N with other plant classes progressively diminishes as the crop develops. Nevertheless, care must be taken when analysing data for S plants. At the successive data points for each category, it was observed that some plants got bigger but maintained the same hierarchical position within the canopy. However, for the S class, the smaller plants progressively died as a result of self-thinning. This inevitably led to a drift in the population because more S plants, i.e. those with a lower intrinsic shoot N concentration, were progressively eliminated from the samples. Such a problem could explain the lower correlation coefficient for this category of plants and the fact that the slope of the regression appears to be different (the S plants remaining at the end of the period being progressively less suppressed than at the beginning). Nevertheless, the data in Fig. 4A confirm the hypothesis that the hierarchical position of plants within the canopy is a reflection of shoot N accumulation, i.e. plants in the dominant position have a higher shoot N content as opposed to plants in a more suppressed position, when compared at similar plant mass. Unfortunately, there was no possibility of measuring *LA* per plant for each height category. As a result, data on leaf mass were used instead of *LA* to analyse the developmental change in plant morphology. It was therefore possible to calculate an allometric relationship obtained between leaf mass ($W_L$) and shoot mass ($W_{sh}$), according to the general equation:

$$W_L = k_2 (W_{sh})^{b'} \quad (4)$$

For the different plant categories, the equations are as follows:

$$\text{D: } W_L = 0.505 (W_{sh})^{0.689} \quad R^2 = 0.960 \quad (4.1)$$

$$\text{I: } W_L = 0.448 (W_{sh})^{0.689} \quad R^2 = 0.873 \quad (4.2)$$

$$\text{S: } W_L = 0.351 (W_{sh})^{0.768} \quad R^2 = 0.831 \quad (4.3)$$

Figure 4B shows the regressions on a log–log scale to provide an easier comparison of plant categories with different sizes. As discussed above, the higher slope obtained for the S plant category could be due to the fact that more S plants progressively died as the canopy developed. The intercept of the regression (value of $k_2$) makes it possible to discriminate between the three categories of plants, revealing that S plants are less leafy than D plants for a similar plant mass. For a D plant with a shoot mass of W=1 mg, the ratio $W_L/W_{sh}$, that is, the leaf weight ratio (*LWR*), is 0.5, corresponding to a leaf:stem ratio (*L:S*) of 1, while at the same shoot mass of W=1 mg, an I plant should have a *LWR* of 0.45 (*L:S*=0.82), and a S plant should have a *LWR* of 0.35 (*L:S*=0.54). For the three categories, the allometric coefficients are very close to the corresponding values of coefficient *b* calculated with equations 1.4, 1.5, and 1.6. Therefore, by combining equations 1.4, 1.5, 1.6, and equations 4.1, 4.2, and 4.3, it is possible to derive a linear relationship between shoot N accumulation ($N_{sh}$) and leaf mass ($W_L$) for each of the three categories of plants:

$$\text{D: } N_{sh} = 0.083 W_L - 0.002 \quad R^2 = 0.976$$

$$\text{I: } N_{sh} = 0.073 W_L + 0.002 \quad R^2 = 0.938$$

$$\text{S: } N_{sh} = 0.073 W_L + 0.001 \quad R^2 = 0.961$$

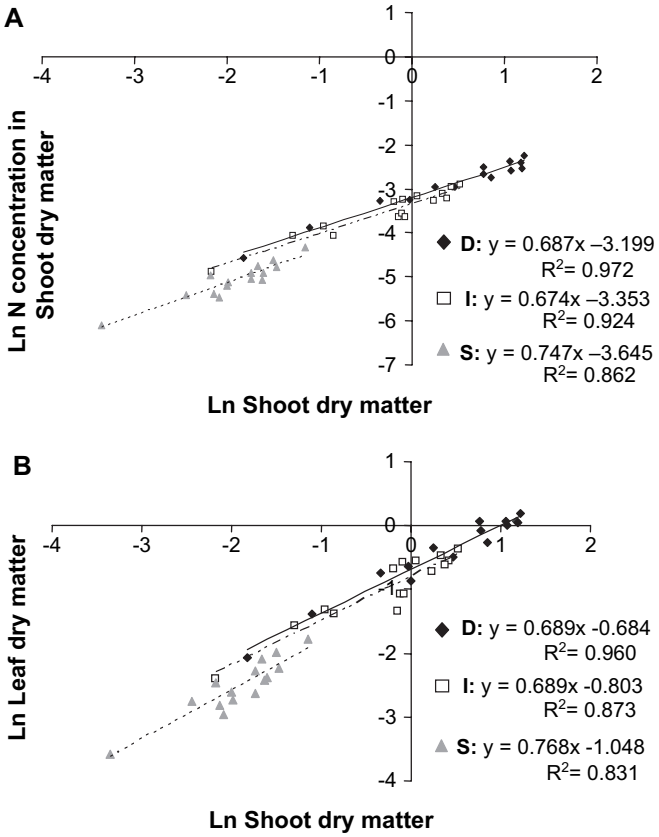


**Fig. 4.** Relationship between ln N concentration in shoot and ln shoot dry matter (A) and relationship between ln leaf dry matter and ln shoot dry matter (B) in plants with different hierarchical positions (D, dominant; I, intermediate; S, suppressed) in the canopy of a lucerne stand during a regrowth period under field conditions at high density.

The value of the intercept is not different from zero for the three plant categories. The slope of the regression appears significantly higher for D plants (0.083 g N $g^{-1}$, $P$=0.04) than for I and S plants (0.073 g N $g^{-1}$). Another way of estimating this ratio between shoot N accumulation and leaf mass is to calculate the ratio $a/k_2$ from equations 1.1, 1.2, 1.3, and 4.1, 4.2, and 4.3, respectively. This implicitly assumes that the two allometric coefficients $b$ and $b$' between $N_{sh}$ and $W_{sh}$ and $W_L$ and $W_{sh}$, respectively, are the same. The values of 0.082 g N $g^{-1}$, 0.078 g N $g^{-1}$, and 0.074 g N $g^{-1}$ for D, I and S plants, respectively, were obtained. Apparently, D plants accumulated slightly more N for a given leaf mass than the S plants, Furthermore, the difference in shoot N content between plants within a dense canopy appears to be largely determined (i) by their shoot mass, and (ii) given the same shoot mass, by the proportion of leaf mass to shoot mass, i.e. their *LWR* or their leaf:stem ratio.

## Discussion and conclusion

Data obtained under controlled conditions and in the field at different plant densities show that the allometric coefficient between shoot N accumulation and shoot mass decreases with plant density.

Moreover, the quantity of N accumulated in the shoot per unit of *LA* appears remarkably constant (1.7 g N $m^{-2}$ on average), regardless of the conditions: high versus low plant density, field versus controlled conditions, solution culture versus soil conditions. All of these factors could affect the developmental changes in the individual plant in terms of leaf:stem ratio. Consequently, these changes affect the dynamics of shoot N accumulation. It appears from these data that the developmental decrease in shoot N concentration as the plant gets bigger is the consequence of the developmental decline in leaf area ratio (*LAR*) or leaf weight ratio (*LWR*). This decline in *LAR* (or in *LWR*) is increased by plant density as a result of competition for light. These factors have a potential influence on biomass allocation, height growth, plant shape, and leaf morphology and physiology (Pons *et al.*, 1989). However, regardless of the cause of this change in plant development, it appears that the changes of shoot N accumulation by an individual plant seem to be determined by its *LA* or its leaf mass expansion. This type of empirical relationship between shoot N accumulation and *LAI* has been previously proposed for dense plant stands (Grindlay *et al.*, 1993, for wheat; Plénet and Lemaire, 1999, for maize), whereas, in this study, a generalization of such a relationship at the level of the individual plant is proposed, regardless of its density within a wide range of conditions and its hierarchical position within the canopy in relation to light interception (shaded versus unshaded plants).

From a mechanistic point of view, *LA* expansion should be considered as the consequence of shoot N accumulation and not the reverse if we consider that shoot N represents N availability for leaf expansion (Hirose *et al.*, 1996, 1997; Gastal and Nelson, 1994). This approach is correct when shoot N accumulation varies according to the level of N supply. Leaf expansion can then be analysed as a response to N supply. In this experiment, N supply is constant and what was observed through the relationships $N_{sh}$–$W_{sh}$ or/and $N_{sh}$–*LA* (or $N_{sh}$–$W_L$) is actually the feedback regulation of N uptake by plant growth or leaf expansion. Plant N uptake, regardless of the source of N supply (nitrate or ammonium uptake, or $N_2$ fixation), is regulated by shoot N and C signalling: a positive signal from photosynthesis C supply and a negative one from organic N recirculating from shoot to root through the phloem (Cooper and Clarkson, 1989; Ismande and Touraine, 1994; Lejay *et al.,* 1999; Touraine *et al.*, 2001; Forde 2002), which act as a N satiety signal. Therefore, the proportionality between *LA* expansion and shoot N accumulation can be explained by the fact that *LA* expansion (i) increases the photosynthetic activity of the plant that provides larger quantities of C compounds to roots for supporting their N uptake activity and (ii) increases the capacity of plants to store organic N in leaves as in Rubisco (Millard, 1988). This last action is crucial to avoid the depletion of root N uptake capacity by recirculating N compounds such as amino acids. Therefore, the relationship between $N_{sh}$ and *LA* could be interpreted as the consequence of the overall regulation of N uptake by plant growth itself. The slope of this relationship (1.7 g N $m^{-2}$) represents the quantity of N that the plant is able to accumulate in the shoots for each additional unit of *LA* expansion. According to equation 2, as the plant gets bigger, each additional *LA* unit is accompanied by a greater proportion of biomass not directly involved in area expansion (leaf thickness or stem fraction), which is mainly composed of supporting and structural tissue. Therefore, as a result, a greater proportion of the 1.7 g N is allocated to this structural component with a low N concentration. Thus, as the plant gets bigger, an increasing proportion of its N content is allocated to non-photosynthetic tissues and is 'diluted' within structural tissues, as reflected in equation 1, that supports clearly the first hypothesis presented in the Introduction. These data show that this 'dilution' effect is accelerated by plant density because the plants adapt to competition for light through an increasing allocation of dry matter to structural tissues, i.e. stems. The shoot N accumulation capacity of plants is determined by the coefficient $a/k_1$ of equation 2. The coefficient $a$ is very sensitive to the level of N supply (Lemaire and Gastal, 1997), while Plénet and Lemaire (1999) showed that $k_1$ was not affected by the level of N supply in maize. The coefficient $a/k_1$ therefore reflects the level of plant N nutrition.

The results obtained on individual plants within a dense canopy also support the hypothesis that leaf growth determines the dynamics of N shoot accumulation (Fig. 5).

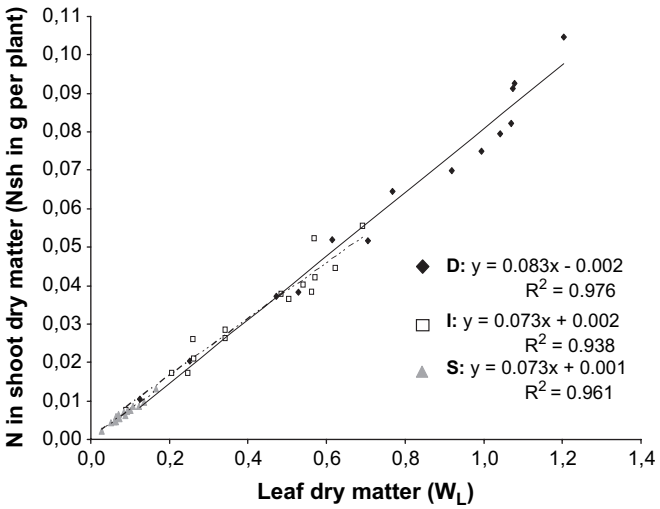


**Fig. 5.** Relationship between shoot N accumulation ($N_{sh}$) and leaf mass

Regardless of their hierarchical position within the canopy and, subsequently, independently of their access to light, plants with the same leaf mass accumulate similar quantities of N in shoots (leaf+stem). Suppressed plants have less leaf per unit shoot mass than dominant plants and, consequently, a lower N content for a similar shoot mass. This result supports the hypothesis that N is partitioned among individual plants within a dense stand in proportion to their contribution to the leaf area of the whole canopy. This result appears to be in contradiction with most of the recorded data that show that leaf N is not distributed uniformly within the canopy and that shaded leaves at the bottom of the canopy have a much lower N per unit area than unshaded leaves at the top (Hirose *et al*., 1988). However, this contradiction is only apparent. In Fig. 6, it can be observed that at the same leaf mass, suppressed plants have both a lower leaf and stem N concentration than dominant plants because they are shaded (Fig. 6A, B), in keeping with the second hypothesis presented in Introduction section. However, the suppressed plants develop a lower leaf:stem ratio because of a stronger competition for light. Therefore, for a similar leaf area (or leaf mass) they accumulate a greater quantity of N in their stems, leading to a shoot N per unit of leaf mass similar to that of dominant plants. Thus, within a dense stand, the competition for light between plants produces two different effects: (i) a decrease in leaf and stem N concentration of the more shaded plants that should lead to a decrease in shoot N per unit of leaf area or leaf mass, and (ii) a decrease in the leaf:stem ratio that leads to an increase in shoot N per unit leaf area or leaf mass. These two opposite effects result in a more or less constant shoot N per unit of leaf mass or leaf area. Therefore, the two hypotheses presented in the Introduction to explain N dilution in a dense canopy are not mutually exclusive and their combination leads to a remarkably constant plant N accumulation per unit of leaf area or leaf mass.

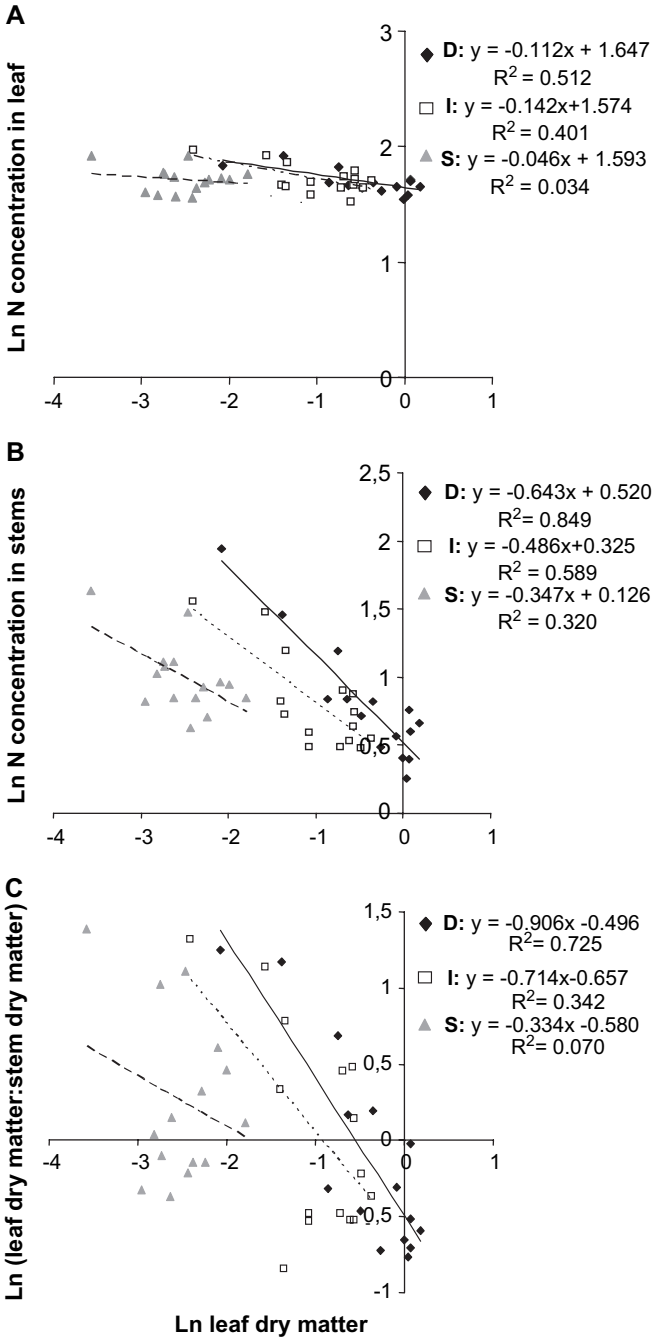


**Fig. 6.** Relationship between ln leaf dry matter and ln leaf N concentration (A), ln stem N concentration (B) and ln leaf dry matter: stem dry matter (C) for the different plant categories (D, dominant; I, intermediate; S, suppressed) within a dense stand.

Unfortunately, these data do not allow it to be determined if it is leaf area or leaf mass that is the most relevant variable. A previous study by Lemaire *et al.* (1991) showed that in a dense lucerne crop, leaf mass per unit leaf area (*LMA*) decreases from 4 to 2 mg $cm^{-2}$ from the top to the bottom of the canopy, respectively. A similar result was also reported by Anten *et al.* (1998) for another dicotyledonous herb (*Xanthium canadense*). It can therefore be

assumed that the leaves of the suppressed plants that grow within the lower layers of the canopy have lower *LMA* than those of the dominant plants. Therefore, equation 4 can be rewritten as:

$$N_{sh} = a/k_2\ LMA\ LA \quad (5)$$

that is the equivalent of equation 2. If it is assumed that the *LMA* of dominant plants is significantly higher than that of suppressed plants, then the quantity of N accumulated in shoot per unit of *LA* (coefficient $a/k_1$ of equation 2) in dominant plants should be higher than in suppressed plants, explaining the small differences observed in terms of quantity of shoot N per unit leaf mass in Fig. 5.

These data support the idea that competition for light among individual plants within a dense canopy induces developmental changes in plant morphology (leaf versus stem) and explains the differences observed in shoot N concentration. Different allometries among individual plants within different size categories were discussed earlier by Anten and Hirose (1998) in relation to light partitioning. Plants detect neighbour density and respond by an increase in stem or petiole elongation through a phytochrome-mediated shade avoidance response (Weiner, 1990; Varlet-Grancher and Gautier, 1995). This type of response leads to a lower leaf:stem ratio. Nevertheless, Anten and Hirose (1998) found that suppressed plants allocated a higher fraction of mass to leaves than dominant ones, which is apparently contradictory with this study's results. However, in Fig. 4B, comparisons between plant categories are made at similar shoot mass. When compared at similar dates, it is possible that the suppressed plants have higher leaf:stem ratios than the dominant plants only because they are smaller in size. This point demonstrates that comparisons of morphological plant traits such as leaf:stem ratio, *LAR* or *LWR* are only relevant if plants of a similar size are compared, and that the coefficients $k_1$ and $k_2$ of equations 2 and 4 provide intrinsic morphological plant traits for interspecific comparisons. In the same way, when the comparison between plant classes is made at the same date, the suppressed plants should have higher shoot N concentrations because they are smaller than the dominant plants, according to the 'dilution effect' described by equation 1. In fact, they have a slightly lower N% because they have a much lower leaf:stem ratio. Therefore, the partition of N among the population of plants within a dense canopy follows the partition of light. *LA* per plant should be a more relevant variable than leaf mass because it more closely reflects the contribution of each plant category in terms of light interception. If it is taken into account that suppressed plants intercept less light per unit of *LA* than dominant plants because they are more shaded, it can then be explained why they accumulate less N in their shoots than dominant plants at similar *LA*.

These results could be widely used in plant and crop modelling. It has been demonstrated that shoot N accumulation increases linearly with leaf area, both at the individual plant level as well as at the canopy level. In most crop models such as STICS (Brisson *et al*., 2003), APSIM (McCown *et al*., 1996), and CERES (Jones and Kiniry, 1986), *LAI* is calculated through a morphological sub-model related to temperature and water availability. *LAI* is then used to calculate the intercepted radiation. The relationship between shoot N and *LAI* that is proposed here could then be used in crop models for estimating the dynamics of crop N demand, i.e. the crop N uptake necessary to produce optimum *LAI* expansion and to provide the maximum interception of light.

It can be postulated that these observations within a population of plants of the same species could be extrapolated to multispecific plant populations, when plants of a given species are dominated by plants of other species (Hirose and Werger, 1994; Anten and Hirose, 1999). Some models are now able to simulate the proportion of incident light intercepted by the species components of a crop mixture (Sinoquet *et al*., 2000). In these types of situations, it could, therefore, be hypothesized that the sharing of N among the species within a crop mixture should be proportional to their respective contribution to the light interception, as suggested by the data on lucerne. It would be interesting to test this hypothesis with non $N_2$-fixing species with different levels of N supply in the soil in order to analyse to what extent competition for light within plant communities can interfere with competition for soil N resources when soil N supply is limited.

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## References

**Anten NPR, Hirose T.** 1998. Biomass allocation and light partitioning among dominant and subordinate individuals in *Xanthium canadense* stands. *Annals of Botany* **82,** 665–673.

**Anten NPR, Hirose T.** 1999. Interspecific differences in aboveground growth patterns results in spatial and temporal partitioning of light among species in a tall-grass meadow. *Journal of Ecology* **87,** 583–597.

**Anten NPR, Miyazawa K, Hikosaka K, Nagashima H, Hirose T.** 1998. Leaf nitrogen distribution in relation to leaf age and photon flux density in dominant and subordinate plants in dense stands of a dicotyledonous herb. *Oecologia* **113,** 314–324.

**Avice JC, Lemaire G, Ourry A, Boucaud J.** 1997*a*. Effects of the previous shoot removal frequency on subsequent shoot regrowth in two *Medicago sativa* L. cultivars. *Plant and Soil* **188,** 189–198.

**Avice JC, Ourry A, Lemaire G, Volenec JJ, Boucaud J.** 1997*b*. Root protein and vegetative storage proteins are key organic nutrients for alfalfa shoot regrowth. *Crop Science* **37,** 1187–1193.

**Brisson N, Gary C, Justes E, *et al*.** 2003. An overview of the crop model STICS. *European Journal of Agronomy* **18,** 309–332.

**Caloin M, Yu O.** 1984. Analysis of the time-course of change in nitrogen content in *Dactylis glomerata* L. using a model of plant growth. *Annals of Botany* **54,** 69–76.

**Charles-Edwards DA, Stutzel H, Fenaris R, Beech DF.** 1987. An analysis of spatial variation in the nitrogen content of leaves from different horizons within a canopy. *Annals of Botany* **60,** 421–426.

**Cooper HD, Clarkson DT.** 1989. Carbon and nitrogen sensing and signalling in plants: emerging 'matrix effects'. *Current Opinion in Plant Biology* **4,** 247–253.

**Forde BG.** 2002. The role of long-distance signalling in plant response to nitrate and other nutrients. *Journal of Experimental Botany* **53,** 39–43.

**Gastal F, Nelson CJ.** 1994. Nitrogen use within the growing blade of tall fescue. *Plant Physiology* **105,** 191–197.

**Greenwood JS, Lemaire G, Gosse G, Cruz P, Draycott A, Neeteson JJ.** 1990. Decline in percentage N of $C_3$ and $C_4$ crops with increasing plant mass. *Annals of Botany* **66,** 425–436.

**Grindlay DJC, Sylvester-Bradley R, Scott RK.** 1993. Nitrogen uptake of young vegetative plants in relation to green area. *Journal of the Science and Food Agriculture* **63,** 116.

**Hardwick RC.** 1987. The nitrogen content of plants and the self-thinning rule of plant ecology: a test of the core-skin hypothesis. *Annals of Botany* **60,** 439–446.

**Hirose T, Ackerly DD, Traw MB, Bazzaz FA.** 1996. Effect of $CO_2$ elevation on canopy development in stands of two co-occurring annuals. *Oecologia* **108,** 215–223.

**Hirose T, Ackerly DD, Traw MB, Ramseier D, Bazzaz FA.** 1997. $CO_2$ elevation, canopy photosynthesis and optimal leaf area index. *Ecology* **78,** 2339–2350.

**Hirose T, Werger MJA.** 1994. Photosynthetic capacity and nitrogen partitioning among species in the canopy of a herbaceous plant community. *Oecologia* **100,** 203–212.

**Hirose T, Werger MJA, Pons TL, Rheenen JWA.** 1988. Canopy structure and leaf nitrogen distribution in a stand of *Lysimachia vulgaris* L. as influenced by stand density. *Oecologia* **77,** 145–150.

**Ismande J, Touraine B.** 1994. N demand and the regulation of nitrate uptake. *Plant Physiology* **105,** 3–7.

**Jones CA, Kiniry JR.** 1986. C*ERES-maize: a simulation model of maize growth and development*. Texas A&M University Press, College Station.

**Kim TH, Ourry A, Boucaud J, Lemaire G.** 1991. Changes in source–sink relationship for nitrogen during regrowth of lucerne (*Medicago sativa* L.) following removal of shoots. *Australian Journal of Plant Physiology* **18,** 593–602.

**Kim TH, Ourry A, Boucaud J, Lemaire G.** 1993. Partitioning of nitrogen derived from $N_2$ fixation and reserves in nodulated *Medicago sativa* L. during regrowth. *Journal of Experimental Botany* **44,** 555–562.

**Lejay L, Tillard P, Lepetit M, Olive FD, Filleur S, Daniel-Vedele F, Gojon A.** 1999. Molecular and functional regulation of two $NO_3^-$ uptake systems by N and C status of *Arabidopsis* plants. *The Plant Journal* **18,** 509–519.

**Lemaire G, Cruz P, Gosse G, Chartier M.** 1985. Etudes des relations entre la dynamique de prélèvement d'azote et la dynamique de croissance en matière sèche d'un peuplement de luzerne (*Medicago sativa* L.). *Agronomie* **5,** 685–692.

**Lemaire G, Gastal A.** 1997. N uptake and distribution in plant canopies. In: Lemaire G, ed. *Diagnosis of the nitrogen status in crops.* Berlin: Springer-Verlag, 3–43.

**Lemaire G, Onillon B, Gosse G, Chartier M, Allirand JM.** 1991. Nitrogen distribution within a lucerne canopy during regrowth: relation with light distribution. *Annals of Botany* **68,** 483–488.

**Lemaire G, Salette J.** 1984. Relation entre dynamique de croissance et dynamique de prélèvement d'azote pour un peuplement de graminées fourragères. I. Etude de l'effet du milieu. *Agronomie* **4,** 423–430.

**McCown RL, Hammer GL, Hargreaves JNG, Holzworth DP, Freebairn DM.** 1996. APSIM: a novel software system for model development, model testing and simulation in agricultural systems research. *Agricultural Systems* **50,** 255–271.

**Millard P.** 1988 The accumulation and storage of nitrogen by herbaceous plants. *Plant, Cell and Environment* **11,** 1–8.

**Plénet D, Lemaire G.** 1999. Relationships between dynamics of nitrogen uptake and dry matter accumulation in maize crops. Determination of critical nitrogen concentration. *Plant and Soil* **216,** 65–82.

**Pons TL, Schieving F, Hirose T, Werger MJA.** 1989. Optimization of leaf nitrogen allocation for canopy photosynthesis in *Lysimachia vulgaris.* In: Lambers H, *et al*., eds. *Causes and consequences of variation in growth rate and productivity of higher plants*. The Hague: SPB Academic, 175–186.

**Sinoquet H, Rakocevic M, Varlet-Grancher.** 2000. Comparison of models for daily light partitioning in multispecies canopies. *Agricultural and Forest Meteorology* **101,** 251–263.

**Touraine B, Daniel-Vedèle F, Forde BG.** 2001. Nitrate uptake and its regulation. In: Lea PJ, Morot-Gaudry JF, eds. *Plant nitrogen*. Berlin: Springer-Verlag, 1–36.

**Varlet-Grancher C, Gautier H.** 1995. Plant morphogenetic responses to light quality and consequences for intercropping. In: Sinoquet H, Cruz P, eds. *Ecophysiology of tropical intercropping*. Paris: INRA, 232–256.

**Weiner J.** 1990. Asymmetric competition in plant populations. *Trends in Ecology and Evolution* **5,** 360–364.