Vertical Leaf Nitrogen Distribution in Relation to Nitrogen Status in Grassland Plants

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Vertical gradients of leaf nitrogen (N) per unit leaf area \( N_{LA} \) are viewed as plastic responses that optimize N utilization with respect to carbon assimilation. However, it has been shown that plant species, sowing density and N availability affect the steepness of the \( N_{LA} \) gradient relative to the photon flux density (PFD) gradient. This paper tests the hypothesis that such variation is related to the N status of the plant. The N status was analysed using the concept of the critical N concentration \( N_{crit} \) in which shoot N per unit dry mass \( N_{SM} \) decreases with shoot mass, and a negative deviation of actual N supply \( N_{SM} \) indicates N shortage in the plant. The hypothesis was tested with contrasting grassland species Medicago sativa, Dactylis glomerata and Taraxacum officinale by varying PFD and N availability, plant density and hierarchical positions of individuals within stands. Combinations of all treatments showed a general negative correlation between the N allocation coefficient (i.e. the slope of the \( N_{LA} \)-PFD relationship) and \( N_{SM} \) for all three species. Thus, \( N_{LA} \) relative to PFD, gradients became steeper with increasing shoot mass and increasing N shortage in the plant. These data are consistent with the view that internal N availability is an important factor in modifying the \( N_{LA} \) gradient.

Key words: Dactylis glomerata, grassland species, light profile, leaf nitrogen distribution, Medicago sativa, plant nitrogen status, size hierarchy, Taraxacum officinale.

INTRODUCTION

Vertical gradients of leaf nitrogen (N) are a common feature of plant canopies. The partitioning of leaf N per unit leaf area \( N_{LA} \) parallels the vertical light distribution within the canopy. \( N_{LA} \) profiles are viewed as plastic responses that optimize N utilization with respect to carbon assimilation. Calculated gains in canopy photosynthesis resulting from an optimal, relative to a uniform, N profile range from 24 to 38 % (Hirose and Werger, 1987; Schieving et al., 1992b; Anten et al., 1995). The effects of N supply rate and plant density on leaf N profiles have been investigated for many species. Hirose and Werger (1987) concluded from their model calculations that the relative advantage of optimal \( N_{LA} \) distributions over uniform distributions increases with increasing density of the canopy and N availability. A less uniform \( N_{LA} \) distribution in dense, relative to open, stands was found in Lysimachia vulgaris (Hirose et al., 1988) and Carex acutiformis (Schieving et al., 1992b). In contrast, density effects on \( N_{LA} \) distribution were small in Helianthus annuus (Sadas et al., 1993), Glycine max (Shiraiwa and Sinclair, 1993) and in Triticum aestivum (Dreccer et al., 2000). An enhanced N supply led to a steeper \( N_{LA} \) gradient in high-, but not in low-productivity Carex species (Aerts and De Caluwe, 1994). More uniform \( N_{LA} \) distributions with increased N supply were reported by Dreccer et al. (2000) and Milroy et al. (2001) in Gossypium hirsutum stands, whereas variation in N supply rate did not alter the \( N_{LA} \) gradient in Glycine max (Shiraiwa and Sinclair, 1993).

These contradictory findings are partly explained by the different models used for analysis of \( N_{LA} \) gradients: (a) \( N_{LA} \) distribution as a function of the relative leaf area index \[ \ln \left( \frac{N_{LA}}{N_0} \right) = K_1 F \left( \frac{I}{I_0} \right) \] and (b) \( CN_{LA} \) distribution as a function of the relative irradiance \[ \ln \left( \frac{CN_{LA}}{N_0} \right) = K_2 \ln \left( \frac{I}{I_0} \right) \], where \( K_1 \) and \( K_2 \) are the N allocation coefficients and \( N_0 \) is the \( N_{LA} \) in the uppermost leaf layer (Hirose et al., 1988). When treatment effects on \( N_{LA} \) gradients are compared, the conclusions made from the two calculation methods can differ, particularly when plant densities are very different. Thus, \( N_{LA} \) distribution was less uniform in dense than in open canopies when \( N_{LA} \) was examined relative to \( F/F_0 \), but more uniform when \( N_{LA} \) was examined relative to \( I/I_0 \) (Hirose et al., 1988). This is explained by the different ranges of the independent variable in the regression equation. The variable \( F/F_0 \) always reaches unity, whereas \( I/I_0 \) depends on the fraction of the light that is absorbed by the canopy.

Nevertheless, part of the variation of the \( N_{LA} \) gradients remains to be explained. Hikosaka et al. (1994) induced light gradients along leaves of horizontally growing vine, Ipomoea tricolor. When N supply rates were high, steeper light gradients resulted in steeper gradients of the \( N_{LA} \). Even when the light gradient was inverted so that younger leaves were more shaded than older ones, \( N_{LA} \) decreased according to the light gradient. This indicates that the \( N_{LA} \) gradient was a response to the light gradient, and the steepness of the light gradient should not affect the slope of the \( N_{LA} - I/I_0 \) regression. Thus, other factors such as species, leaf age and demand for N may modify the slope of the \( N_{LA} - I/I_0 \) regression. First, few studies have compared \( N_{LA} \) gradients...
of similarly treated species. Anten et al. (1995) found more uniform N distribution patterns in monocotyledonous compared with dicotyledonous herbs and concluded that the total amount of free N in the canopy determines the pattern of N distribution. Secondly, in erect growing dicotyledonous herbs, leaf age gradient is parallel to light gradient. Mooney et al. (1981) reported a marked decrease in $N_{\text{LA}}$ with leaf age in some species, even in leaves not exposed to shading. Hikosaka et al. (1994) found that $N_{\text{LA}}$ decreased during the ageing of the leaf when the N supply rate was low, but not with a high N supply. This indicates that during the ageing of the leaf when the N supply rate was low, but not with a high N supply. This indicates that demand for N may be an important factor modifying the variability of the slope of the $N_{\text{LA}} - I/I_0$ regression.

The objective of the present work was to investigate the $N_{\text{LA}}$ distribution relative to the light gradient in grassland species. Grassland systems are characterized by diversity of species and individuals in different hierarchical positions exposed to different availabilities of light and N. In such systems, species composition and stand architecture may affect the $N_{\text{LA}}$ gradient of the individuals. It was hypothesized that the variability of the slope of the $N_{\text{LA}} - I/I_0$ regression is related to the N status of the plant. The N status was analyzed using the concept of the critical N concentration ($N_{\text{crit}}$) in which shoot N per unit dry mass ($N_{\text{SM}}$) decreases with shoot mass, and negative deviation of actual $N_{\text{SM}}$ from $N_{\text{crit}}$ indicates N shortage of the plant (Lemaire and Gastal, 1997). The hypothesis was tested: (a) with contrasting growth forms common in grassland systems (monocotyledonous vs. dicotyledonous species, leaf rosette-forming vs. erect-growing plants), and (b) by varying light and N availability, plant density and hierarchical positions of the individuals within stands.

**MATERIALS AND METHODS**

There were three experiments in controlled conditions. Expts 1 and 2 were carried out in growth rooms (Heraeus Vötsch, Germany) adjusted to: 18/13 °C day/night temperature, 500–600 μmol m$^{-2}$ s$^{-1}$ photosynthetic photon flux density (PFD, 400–700 nm) at canopy height, 16-h photoperiod and 70 % relative humidity. Lighting was supplied by fluorescent tubes and tungsten bulbs, 90 % of the light intensity being provided by the fluorescent tubes. For expt 3, two growth cabinets (E15; Conviron, Winnipeg, Canada) were adjusted to 22/18 °C day/night temperature, 16-h photoperiod and 75 % relative humidity. Lighting was supplied by fluorescent tubes. Plant materials were non-nodulated Medicago sativa ‘Planet’, Dactylis glomerata ‘Lidacta’ and wild-type Taraxacum officinale.

**Experiment 1**

The aim of the experiment was to investigate the leaf N distribution of individuals growing in different hierarchical positions within plant stands. The grasses Lolium multiflorum, Lolium perenne and Poa pratensis were used to provide tall, intermediate and short background stands, respectively. The target individuals M. sativa, D. glomerata and T. officinale, introduced within these stands, were expected to grow in dominant, intermediate or subordinate positions.

Establishment of background stands: L. multiflorum ‘Ligrande’, L. perenne ‘Liprinta’ and P. pratensis ‘Limousine’ seeds were germinated in seed trays on sand. Three days after germination, seedlings were transplanted into pots (5 cm diameter, 35 cm long) filled with quartz sand (particle size 0.3–0.8 mm). Each pot contained three plants of a single species. Monospecific plant stands were formed by placing the pots into containers (76 cm long, 56 cm wide, 32 cm deep). In order to reduce border effects, curtains of opaque foil were stretched along the edges of the stands. The height of the curtains was adjusted weekly to the mean height of the stand. The stands were placed on trolleys and rearranged weekly within the growth rooms. Twice a day the containers were flooded for 1 h with nutrient solution (2.5 mM KNO$_3$, 2.5 mM Ca(NO$_3$)$_2$, 0.5 mM KH$_2$PO$_4$, 1 mM MgSO$_4$, 0.12 mM Fe as EDTA and micronutrients, pH 6.5). Once a week the pots were flushed with water and the nutrient solution was renewed. The stands grew for 3 weeks, then the plants were clipped to a stubble height of 3 cm.

Addition of target individuals to the background stands: M. sativa, D. glomerata and T. officinale were germinated and potted as described above. There was one plant per pot. Sowing dates were chosen so that target individuals had three leaves when background stands were clipped. After clipping, target individuals were placed into background stands by replacing individual pots of the background stand. Thus, the root medium of the target species was separate and any direct competitive effect on mineral resources between target and background species was avoided. Nine stands were formed with target species combined with each background species. Each stand consisted of 1000 plants m$^{-2}$ with 100 target individuals. Five weeks after the target individuals had been added to the stands, the light profile was measured and plants were harvested. As the mixed stands with M. sativa and D. glomerata grown in L. perenne tended to lodge shortly before the harvest, no data for these stands are presented.

**Experiment 2**

Leaf N distribution was investigated in monospecific open and dense stands of M. sativa, D. glomerata and T. officinale. After germination plants were potted and arranged in stands as described above. Nutrient supply was the same as in expt 1. In the open stands each pot contained a single plant, resulting in a density of 400 plants m$^{-2}$. In the dense stands half of the pots contained a single plant and the other half three plants per pot, resulting in a density of 800 plants m$^{-2}$. Only single-potted individuals were used as target individuals. They were harvested 7 weeks after sowing.

**Experiment 3**

The aim of expt 3 was to investigate the effects of light and N availability on leaf N distribution in M. sativa. Establishment procedures were similar to expt 1. There was one plant per pot. Three weeks after sowing, seedlings were
cut to a stubble height of 3 cm. For each treatment 36 plants were placed in a 0-4 m² stand formed by artificial plants. In order to achieve a vertical light gradient with neutral shading, the artificial plants were made of a green plastic stem and green, opaque plastic leaves arranged in layers at intervals of 10 cm. The stands contained 200 artificial plants m⁻² with a light extinction coefficient of 1-1. The leaf area index in each layer was 0-61. A new leaf layer was added to the artificial plants when 50 % of the natural plants overtopped the uppermost artificial leaves by 12 cm. There were five treatments: high PFD and high N supply (I+N+), low PFD and low N supply (I−N−), I+N− and I−N+, respectively. In the fifth treatment, cI−N±, conditions were changed gradually from I+N+ to I−N−. To this end, PFD and N supply were reduced weekly by 25 % from week 4 to 7. The nutrient solution contained 7·5 mM nitrate-N (N±). At the beginning of the pot height was between 0·02 (I+N+) and 0·14 (I−N±).

harvest 5 weeks after defoliation. By then, relative PFD at sampled weekly and 12 plants were sampled for the final plants (I−) and plants experiencing the transition to a growth conditions for dominant plants (I+), subordinate plants and green, opaque plastic leaves arranged in layers at shading, the artificial plants were made of a green plastic order to achieve a vertical light gradient with neutral light extinction coefﬁcient. Leaf area intervals of 10 cm. The stands contained 200 artiﬁcial plants index in each layer was 0·61. A new leaf layer was added to the artificial plants when 50 % of the natural plants overtopped the uppermost artificial leaves by 12 cm. There were five treatments: high PFD and high N supply (I+N+), low PFD and low N supply (I−N−), I+N− and I−N+, respectively. In the fifth treatment, cI−N±, conditions were changed gradually from I+N+ to I−N−. To this end, PFD and N supply were reduced weekly by 25 % from week 4 to 7. The nutrient solution contained 7·5 mM nitrate-N (N±). At the beginning of the experiment the lighting in the growth rooms was adjusted to 340 µmol m⁻² s⁻¹ PFD (I+) or 85 µmol m⁻² s⁻¹ PFD (I−) at pot height. The purpose of the treatments was to simulate growth conditions for dominant plants (I+), subordinate plants (I−) and plants experiencing the transition to a subordinate position (cI−). Four plants per treatment were sampled weekly and 12 plants were sampled for the final harvest 5 weeks after defoliation. By then, relative PFD at pot height was between 0·02 (I+N+) and 0·14 (I−N−).

Light measurement

The distribution of PFD was measured with a photon flux meter (sensor head 12 mm wide, 288 mm long; Solems, Palaiseau, France). PFD was measured at intervals of 2·5 cm (expts 1 and 2) and 10 cm (expt 3) from the ground level to the top of the leaf canopy 1 d before the final sampling and at similar heights in the empty growth rooms after sampling.

Sampling

In expts 1 and 2 target individuals were cut to obtain five horizontal layers. Cutting heights were calculated so that relative canopy light absorption was about 20 % in each layer. Hence, the thickness of the layers varied from 3 to 30 cm. Six target individuals were harvested from each stand. Plants were dissected into leaves, stems with petioles, and inflorescences. The stem of D. glomerata (pseudostem) consisted of leaf sheaths and enclosed young, developing leaf blades. Flowering was not induced and no true stems were found. At the first four harvests of expt 3 plants were cut at ground level and dissected into leaves and stems. At the final harvest, cutting heights were 15 cm in the I+N+ treatment and 10 cm in the other treatments. Plants were dissected into main stem, branches of the main stem and other stems. Each fraction was subdivided as described in expt 1. Leaf area was measured with a planimeter (LI3100; Li-Cor, Nebraska, USA). Dry mass was determined after oven drying at 70 °C for 72 h. Plant material was ground and total N content of sub-samples was determined with an NC-analyser (Carlo Erba 1110, Milan, Italy).

Gas exchange measurements

In expt 3 gas exchange was measured with a portable open gas exchange system (LI6400; Li-Cor) on attached leaves of the remaining plants 1 d after the final harvest. The middle leaflet of the youngest unfolded leaf on the main stem and every second older leaf were used for measurement. Photosynthetic light response curves were conducted by using a light source (LI6400-02 LED Source) mounted onto the sensor head. Leaf temperature was kept at 23–24 °C, CO₂ pressure in the cuvette was 35 Pa and leaf to air vapour pressure deficit was kept at about 0·9 kPa. Afterwards, the area of the detached leaflets was measured and readings recalculated with the correct leaf area. Dry mass and N content of the leaflets were determined as described above.

Calculations

The extinction coefficient of light was calculated after correction for the light gradient of the growth chambers as

\[ I = I_0 \exp (-K_L F) \]  

where \( F \) is the cumulative leaf area index from the top of the canopy and \( I_0 \) and \( I \) are the PFD on a horizontal level above the canopy and within the canopy at depth \( F \); \( K_L \) is the coefficient of light extinction. Leaf N per unit leaf area \( (N_{LA}) \) was related to \( F \) (Hirose et al., 1988, Anten et al., 1995) as

\[ N_{LA} = a \exp (-K_{FA} F) \]  

\[ N_{LA} = b \exp (-K_{FB} F) + n_b \]

where \( a \) and \( b + n_b \) are the \( N_{LA} \) values at the top of the plant, \( K_{FA} \) and \( K_{FB} \) are the coefficients of leaf N allocation in respect to cumulative \( F \). In expts 1 and 2, \( n_b \) was estimated by fitting the measured \( N_{LA} \) to eqn (2b). In expt 3, \( n_b \) was calculated as

\[ P_m = a_p (N_{LA} - n_b) \]

where \( P_m \) is the light saturated rate of gross photosynthesis and \( a_p \) and \( n_b \) are the slope and \( x \) intercept of the \( P_m - N_{LA} \) relationship. By eliminating \( F \) from eqns (1) and (2a, 2b) the relationship between \( N_{LA} \) and PFD is formulated as

\[ N_{LA} = a (H_0)^{K_a} \]

\[ N_{LA} = b (H_0)^{K_b} + n_b \]

where \( K_a \) and \( K_b \) are the coefficients of leaf N allocation with respect to the relative PFD, and \( K_a = K_{FA}/K_L \) and \( K_b = K_{FB}/K_L \). An optimal \( N_{LA} \) distribution is given when \( K_{FA} \) and \( K_{FB} \) equal \( K_L \), and \( K_a \) and \( K_b \) approach unity, respectively (Anten et al., 1995). \( K_a \) and \( K_b \) were calculated after logarithmic transformation: \( \ln (N_{LA}) = \ln (a) + K_a \ln (H_0) \) and \( \ln (N_{LA} - n_b) = \ln (b) + K_b \ln (H_0) \).

In expt 3, shoot N per unit shoot dry mass \( (N_{SM}) \) of the regrowing M. sativa plants was calculated as a function of shoot dry mass \( (M_S) \) (Lemaire and Gastal, 1997):

\[ N_{SM} = c (M_S)^{d} \]
where $N_{SM}$ is the actual shoot N concentration of an individual and $N_{SM+}$, the $N_{SM}$ estimated for treatment I+N+ at the same shoot mass. It was assumed that $N_{SM+}$ was close to the critical N concentration (Lemaire and Gastal, 1997) at which plant growth rate is maximal. A value of NNIi higher than or equal to 1 indicates that the individual was in a situation of non-limiting N supply at the time of sampling. A multiple regression analysis (REG Procedure; SAS Institute, NC, USA) was performed to estimate the contribution of NNIi and $N_{SM}$ to the variation in $N_{LA}$.

RESULTS

Plant size and shoot N concentration

In expt 1 the mixed stands were dense with leaf area indices of about 16 and 9 in the tall- and short-growing stands, respectively (Table 1). In consequence, at least 96% of the incident PFD was absorbed by the stands. The tall-growing grass L. multiflorum formed the highest and P. pratensis the shortest background stands with heights of about 0.8 and 0.3 m, respectively. The target individuals of M. sativa and D. glomerata were in dominant positions in P. pratensis stands, as they overtopped the background stand, but were in intermediate positions in L. multiflorum stands. T. officinale was subordinate in L. multiflorum and intermediate in L. perenne and P. pratensis stands.

The coefficient of light extinction ($K_L$) of the canopy was highest in the monospecific stands of M. sativa and lower in D. glomerata and T. officinale. In the mixed stands the effect of the target species on $K_L$ was rather small, and $K_L$ was comparable with values of pure grass stands (Table 1). In expt 3, $K_L$ of the artificial stands with introduced M. sativa was 0.91.

Shoot N per unit mass ($N_{SM}$) decreased during the regrowth with increasing plant size (Fig. 1). This decline was mainly due to a strong decrease in the N concentration of the stems ($N_{stem} = 23.0 M_S^{-0.284}$, $r^2 = 0.86$ and $N_{lamina} = 48.5 M_S^{-0.995}$, $r^2 = 0.28$ for the I+N+ treatment) and a decrease in the leaf : stem mass ratio (data not shown). Compared with the M. sativa plants of the I+N+ treatment,
low N supply (I+N−) reduced \( N_{SM} \) significantly at comparable shoot mass. In contrast, low PFD did not affect \( N_{SM} \), i.e. \( N_{SM} \) was the same for the high and low PFD treatments at comparable shoot mass and corresponding rates of N supply. Consequently, after 5 weeks of regrowth, individuals from the I+N+, cI−N± and I−N± treatments showed very similar \( N_{SM} \), although they differed considerably in shoot mass. In expts 1 and 2, rates of N supply were the same as in the I+N+ treatment of expt 3. Nevertheless, \( N_{SM} \) was in general lower than expected from the \( N_{SMN+} = M_S \) relationship of the I+N+ treatment, indicating that rates of N supply in expts 1 and 2 were not optimal. For \( D. \) glomerata and \( T. \) officinale, treatments induced a steep decline in \( N_{SM} \) with increasing shoot mass. Target species grown in \( P. \) pratensis stands showed the highest, and those grown in \( L. \) multiflorum stands the lowest, deviation from the \( N_{SMN+} \) of \( M. \) sativa.

N concentration was higher in the leaf lamina (\( N_{lamina} \)) than in the stem (\( N_{stem} \)) fraction (Fig. 2). In \( D. \) glomerata, N concentration of both organs decreased linearly with \( N_{SM} \). In \( M. \) sativa, the decline was steeper for \( N_{lamina} \) than for \( N_{stem} \). \( N_{lamina} \) and \( N_{stem} \) declined with decreasing \( N_{SM} \) towards 10 and 6 mg N g\(^{-1}\) DM, respectively.

**Evaluation of \( n_b \)**

Light saturated rate of gross photosynthesis (\( P_m \)) increased linearly with \( N_{LA} \) (Fig. 3). The intercept with the \( y \)-axis (\( n_b, P_m = 0 \)) was highest for the I+N− and lowest for the I−N− treatment. The high \( n_b \) in the I+N− treatment was reflected in the highest mass per unit leaf area (40 g m\(^{-2}\)) and the lowest N content per unit mass (16-3 mg N g\(^{-1}\)) in the lowest positioned leaves within the canopy. The opposite was found for the leaves of the I−N+ treatment (14-3 g m\(^{-2}\), 24-7 mg N g\(^{-1}\) ), whereas the values for the leaves of the other treatments were intermediate (data not shown). For expts 1 and 2, \( n_b \) was calculated using the \( N_{LA} - F \) relationship (Fig. 4; Table 2). No significant \( n_b \) was found...
for *M. sativa* grown in dense monoculture. In expt 2, *T. officinale* showed increasing \( N_{LA} \) in the lowest layers of the canopy. Thus, for the calculation of \( n_b \) the lowest layer was excluded and data from all treatments were pooled. 

**Coefficient of \( N \) allocation**

The mean coefficient of determination \( (r^2) \) of the \( N_{LA} \) (\( I/I_0 \)) regression varied among treatments from 0.59 for the *D. glomerata* and 20% for *T. officinale* and increasing \( N_{LA} \) in the lowest layer (30% for *D. glomerata*, 18% for *M. sativa*, 7% for *T. officinale*). Furthermore, \( K_a \) and \( K_b \) were influenced by the branching status of the *M. sativa* plants. In expt 3, \( K_a \) and \( K_b \) of the main stem leaves and of the whole shoot of *M. sativa* were analysed. In plants with low \( N \) supply (I+\( N^- \)), \( K_a \) was higher for main stem leaves than for the whole shoot (Fig. 5).

In general, introducing the parameter \( n_b \) into the \( N_{LA} \) (\( I/I_0 \)) relationship (eqn 4b) improved the goodness-of-fit as
Similar results were found when variation within treatments (Table 3). Multi-regression analysis showed, for example, in Fig. 6. Thus, in the following, results from eqn 4b only are presented. Figure 7 shows a range of treatments that induced increasing values of leaf N allocation (Kb) with PFD. Thus, at a 50% reduction of relative PFD, leaf N allocation was more than in M. sativa. In general, Kb was negatively correlated with N content per unit shoot mass (N Ms), i.e. leaf N allocation was more uniform in treatments which induced high N Ms (Fig. 8). Similar results were found when Kb was set in relation to N lamina and N stem, respectively (data not shown). At comparable N Ms, Kb was significantly higher in D. glomerata than in M. sativa, whereas T. officinale showed rather lower Kb than M. sativa was high.

N Ms declined with increasing shoot mass and decreasing NNI. Multi-regression analysis using the means of the treatments showed, for M. sativa, that 85% of the variation in Kb was explained by the variation of NNI, and 12% by variation of shoot mass (Table 3). Multi-regression analysis of individuals revealed a high variation within treatments, and contributions of NNI, and shoot mass to the variation of N LA were 37 and 10%, respectively. For D. glomerata and T. officinale no significant contribution of shoot mass to the variation of Kb was found (data not shown).

**DISCUSSION**

In the present study the gradient of leaf N per unit area (NL A) relative to PFD gradient was analysed for grassland species grown in different combinations of PFD and N availability. As a measure for the NL A gradient, coefficients of N allocation, as the slope of the linear relationship between ln (NL A) and ln (IL A), were calculated. Introducing a minimal NL A (n b in eqn 4b) into the NL A – (IL A) relationship improved the accuracy of the estimated coefficient of N allocation (K b) (Fig. 6). That is, above a minimal NL A, NL A distribution relative to the PFD gradient was often better explained by a more linear or even positive curvilinear pattern (Fig. 7). Estimates of n b using eqn 2b in expts 1 and 2 and the x intercept of the P = = = NL A regression in expt 1 resulted in similar values for M. sativa. As n b was positively correlated with leaf mass per unit leaf area, high n b values in the lowest leaves indicated low N per unit leaf mass. The n b values (g m⁻²) used in the present study were comparable with those of other plants: 0.36, Oryza sativa; 0.41, Glycine max; 0.18, Sorghum bicolor; 0.35, Amaranthus cruentus (Anten et al., 1995); 0.50 and 0.48 for dominant and subordinate Amaranthus dubius (Anten and Werger, 1996); 0.28, Xanthium canadense (Hikosaka et al., 1999) and 0.77, Carex acutiformis (Schieving et al., 1992b).

Regarding means of treatments, NL A was reduced by 45% (s.e. = 2.2, n = 14) from the top to the bottom leaves in treatments which induced steep NL A gradients (K b > 0.3) and by 21% (10.4, n = 4) in treatments with more uniform NL A distributions. However, despite a very similar overall decline in NL A in many treatments, NL A distribution relative
to the PFD gradient altered with increasing $N_{SM}$ from negatively to positively curvilinear. This indicates that, within a given relative decline of $N_{LA}$ from the top to the bottom leaves, adaptation of $N_{LA}$ to the PFD gradient depended on the $N$ status of the plant, i.e. the internal $N$ availability. Interestingly, due to the negatively curvilinear decline $N_{LA}$ was mainly higher in the middle layers of the canopy where the probability of sunscald might be high.

A linear decline in $N_{LA}$ with PFD ($K_b = 1$) appeared only in treatments which induced low $N_{SM}$; most treatments showed $K_b < 1$. A more uniform, relative to the optimal, distribution has been found in many studies (Hirose and Werger, 1987; Schieving et al., 1992a, b; Anten et al., 1995; Rousseaux et al., 1999). With higher $N_{LA}$ the above-average PFD can be used to increase photosynthesis (Leuning et al., 1995). Thus, high $N_{LA}$ in the middle of the canopy would increase the photochemical efficiency of plants. When internal availability of $N$ becomes scarce (lower $N_{SM}$), this extra $N$ in middle leaves may be utilized to support the growth of upper leaves, thus decreasing $N_{LA}$ linearly or even more steeply with the PFD gradient.

**Variation of $N_{LA}$ distribution within treatments**

Variation of $N_{LA}$ distribution within treatments was partly attributable to the branching status of the individuals and leaf senescence. In erect-growing dicots, leaves are placed in different positions of the canopy and leaf senescence on the main axis occurs along the light gradient, promoting a steep vertical $N_{LA}$ gradient. However, in

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**Fig. 7.** A, Leaf N per unit leaf area ($N_{LA}$) (mean ± s.e.) and B, relative $N_{LA}$ as a function of relative PFD ($I/I_0$) for selected treatments with increasing coefficient of leaf N allocation ($K_b$). *Taraxacum officinale* grown in a *Lolium multiflorum* stand (open triangles, solid line, $K_b = 0.04$), *Medicago sativa* grown in dense monoculture (open circles, dotted line, $K_b = 0.15$), grown with high PFD and high $N$ supply (filled circles, short dashed line; $K_b = 0.41$), grown in a *Poa pratensis* stand (open diamonds, dashed-dotted line, $K_b = 0.87$), and *Dactylis glomerata* grown in a *P. pratensis* stand (filled diamonds, long dashed line, $K_b = 1.37$). Regression model: $N_{LA} = b (I/I_0)^{K_b} + n_b$.

**Fig. 8.** Coefficient of leaf N allocation ($K_b$) in relation to shoot N per unit mass ($N_{SM}$) for A, *Medicago sativa*, B, *Dactylis glomerata* and C, *Taraxacum officinale* individuals (mean ± s.e.). For explanation of symbols see Fig. 1. Regression lines for means of treatments in A, $K_b = -0.028 N_{SM} + 1.32$, $r^2 = 0.94$, in B, $K_b = -0.040 N_{SM} + 2.09$, $r^2 = 0.98$, and in C, $K_b = -0.049 N_{SM} + 1.92$, $r^2 = 0.92$. Dashed lines, 95 % confidence interval for individual plants.
**TABLE 3. Summary of regression analyses among N nutrition index (NNIi), shoot dry mass (M_S) and coefficient of N allocation (K_b) for Medicago sativa**

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<th>Parameter</th>
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<tr>
<td>c_1</td>
<td>1.311</td>
<td>0.070</td>
<td>0.775</td>
<td>351.4***</td>
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<tr>
<td>c_2</td>
<td>1.152</td>
<td>0.083</td>
<td>0.426</td>
<td>193.2***</td>
</tr>
<tr>
<td>c_3</td>
<td>0.109</td>
<td>0.021</td>
<td>0.057</td>
<td>25.8**</td>
</tr>
</tbody>
</table>

Stepwise selection

<table>
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<tr>
<th>Variable entered</th>
<th>No. of variables</th>
<th>Partial r^2</th>
<th>Model r^2</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>NNIi</td>
<td>1</td>
<td>0.851</td>
<td>0.851</td>
<td>39.8***</td>
</tr>
<tr>
<td>M_S</td>
<td>2</td>
<td>0.121</td>
<td>0.972</td>
<td>25.8**</td>
</tr>
</tbody>
</table>

Model: K_b = c_1 + c_2 NNIi + c_3 M_S.

**P < 0.01; ***P < 0.001.

*M. sativa*, senescent leaves of the main axis were replaced by younger leaves on branches. This was obvious in treatments with low N supply (I+N−, I−N−), where N_LA gradients were steeper in leaves of the main stem compared with the gradient of the whole shoot (Fig. 5). The increase in N_LA in the lowest layer of the canopy found in many D. glomerata and *T. officinale* plants might be related to developing tillers or leaves on the main axis, respectively. A further factor contributing to the variation of the N_LA gradient within treatments was the decline of N_LA in the top layer in some individuals of *D. glomerata* and *T. officinale*. This might indicate the beginning of senescence in the leaf tips of these species resulting in a more uniform N_LA distribution. Besides these factors inducing variation of the N_LA gradient within treatments, shoot size and N nutrition status of the plant strongly affected the steepness of the N_LA gradient.

**Effect of plant size and N nutrition status on N_LA distribution**

The coefficient of leaf N allocation K_b was negatively correlated with shoot N per unit mass (N_SM). There are two factors affecting N_SM: plant size and N nutrition status. In general, N_SM declines with increasing shoot mass (M_S) (Lemaire and Gastal, 1997). This decline is mainly due to a decrease in N_{stem} and a decrease in the leaf : stem ratio; i.e. N_SM declines with increasing proportion of structural tissues. In the present study, the slope of the relationship between ln (N_SM) and ln (M_S) was –0.196 for plants grown with high PFD and high N supply (Fig. 1). This value is very similar to that of dominant plants of a sweet sorghum stand in which rates of N supply resulted in maximal growth rate of the stand, and where N_SM of the stand was equal to the critical N concentration (Lemaire and Gastal, 1997). Thus, N concentration in plants of the I+N+ treatment (N_{SM}+) seemed to be close to the critical N concentration where additional N supply would not result in extra growth. A negative deviation from N_{SM}+, reflected by a low N nutrition index (NNI < 1), would indicate a shortage of N. In expt 3, low NNIi was induced by low rates of N supply (I+N−, I−N−, cI−N−). In expt 1 and 2 increasing shoot mass was accompanied by decreasing NNIi. In these experiments plant density was much higher than in expt 3. Thus, the rate of N supply may have been suboptimal. Whatever the cause, in all three species, N_LA gradient was affected by N_SM in that it increased with increasing shoot mass and with increasing deviation of the N_SM from the critical N concentration of shoots. K_b was higher in *D. glomerata* than in *M. sativa* and *T. officinale* at the whole shoot level (Fig. 8). However, K_b values for main stem leaves of *M. sativa* were higher and closer to the values of *D. glomerata* in plants with low NNIi. In contrast, Anten *et al.* (1995) reported less uniform N_LA distribution in dicots than in monocots.

The correlation between N_LA gradient and N_SM suggests a general explanation for the variation of N_LA due to variation in N fertilization rate, plant density, plant size and the hierarchical position of individuals within the canopy. First, when compared with high N supply, low N supply resulted in reduced N_SM and steeper N_LA gradients in both high and low PFD treatments. In agreement with the present results, Dreccer *et al.* (2000) found that a steeper N_LA gradient at low N supply in wheat narrowed the gap in maximum canopy photosynthesis compared with the high N treatment, particularly at high PFD. Compared with high N supply, a steeper N_LA gradient was found at low N supply in cotton (Milroy *et al.*, 2001). Leuning *et al.* (1995) showed that, at low N supply, a less uniform, compared with a uniform, N_LA distribution increased simulated canopy photosynthesis significantly, whereas gains were negligible at high N. When site fertility was manipulated by mowing, the mown and nutrient-depleted site produced *Solidago altissima* stands with a less uniform N_LA distribution compared with the non-mown stands (Egli and Schmid, 2000).

Secondly, an effect of plant density on N_LA distribution was found for *M. sativa*. N_SM was higher and N_LA more uniformly distributed in dense stands (Fig. 8). This relationship was also found in *Lysimachia vulgaris* stands where plant density increased N_SM and decreased the coefficient of N allocation (Hirose *et al.*, 1988). However, when plant density did not affect N_SM, as for *D. glomerata* and *T. officinale*, N_LA gradients were similar in open and...
dense plants. Small effects of plant density on the $N_{LA}$ gradient were also observed by Dreccer et al. (2000), Del Pozo and Denny (1999), Sadras et al. (1993) and Shiraiwa and Sinclair (1993).

Thirdly, as increasing shoot mass results in decreasing $N_{SM}$, the $N_{LA}$ gradient should also increase with plant growth. In treatments resulting in similar NNI ($= 1.0$ for $I^{+}N^{+}, I^{–}N^{–}$ and $M$. sativa grown in dense monoculture, Fig. 1), $N_{SM}$ decreased and $K_b$ increased with shoot mass. In a Carex acutiformis stand $N_{SM}$ declined and the $N_{LA}$ gradient became steeper during a growth period (Hirose et al., 1989).

Fourthly, delayed germination, slow growth and low N availability are factors which result in plants being located at subordinate positions within the canopy. Once shaded, growth rate is further slowed and plant size reduced relative to dominant plants. Realised $N_{SM}$ depends on shoot size and N availability, which affect $N_{SM}$ in opposite directions. Thus, analysis of the $N_{LA}$ gradient in individuals from differential hierarchical positions should take into account shoot size and nutrient status. For plants with similar NNI, $N_{SM}$ decreased and $K_b$ increased from subordinate to dominant plants due to the increasing shoot mass ($I^{–}N^{–}$ vs. $I^{+}N^{+}, I^{–}N^{–}$ vs. $I^{+}N^{+}$, M. sativa grown in L. multiflorum vs. P. pratensis). In an Amaranthus dubius stand, in which dominant plants showed lower $N_{SM}$ and $N_{lamin}$ than subordinate plants, the $N_{LA}$ gradient was steeper for dominant than for subordinate plants (Anten et al., 1996). In comparison, reduced availability of both PFD and N ($I^{–}N^{–}$ vs. cl–$N^{–}$ vs. $I^{+}N^{+}$) reduced NNI and shoot mass, resulting in similar $N_{SM}$ and $K_b$ values for dominant and subordinate individuals. Anten et al. (1998) found the steepest $N_{LA}$ gradients among the most subordinate plants of a Xanthium canadense stand. However, all size classes showed similar $N_{lamin}$ which could be interpreted as increasing N shortage (decreasing NNI) towards the smallest, most subordinate plants inducing steep $N_{LA}$ gradients.

CONCLUSIONS

The general result of the correlation between $K_b$ and $N_{SM}$ explains the variation in the $N_{LA}$ gradients for a broad selection of plant growth conditions, including temporary and spatial heterogeneity of resource availability below and above ground. $N_{SM}$ reflects the relationship between the cumulative net assimilation of N and C. $N_{SM}$ decreases with increasing shoot mass and is related to the time-integrated competition for N and PFD among plants. Thus, the present data indicate that the $N_{LA}$ gradient is not only a result of the light climate and leaf age but is an integrated result of plant size and availability of above- and below-ground resources.

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LITERATURE CITED


