Annals of Botany **92**: 679–688, 2003
doi:10.1093/aob/mcg188, available online at www.aob.oupjournals.org

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

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Received: 2 July 2003 Returned for revision: 11 July 2003 Accepted: 28 July 2003 Published electronically: 10 September 2003

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_{SM} from N_{crit} 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.*, 1992*b*; 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.*, 1992*b*). In contrast, density effects on N_{LA} distribution were small in *Helianthus annuus* (Sadras *et al.*, 1993), *Glycine max* (Shiraiwa and Sinclair, 1993) and in *Triticum aestivum* stands (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 $(N_{LA}/N_0) = K_F(F/F_t)$] and (*b*) CN_{LA} distribution as a function of the relative irradiance [ln $CN_{LA}/N_0) = K_N$ ln (I/I_0)], where K_F and K_N 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_t, 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_t 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 inversed 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

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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_{LA} with leaf age in some species, even in leaves not exposed to shading. Hikosaka *et al.* (1994) found that N_{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 demand for N may be an important factor modifying the slope of the $N_{\mathrm{LA}} - I/I_0$ regression.

The objective of the present work was to investigate the N_{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 structure may affect the N_{LA} gradient of the individuals. It was hypothesized that the variability of the slope of the $N_{\mathrm{LA}} - I/I_0$ regression 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 negative deviation of actual N_{SM} from N_{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_2PO_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 m^2 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 week 7. The nutrient solution contained 7·5 mM nitrate-N (N+) or 1·5 mM nitrate-N (N–). At the beginning of the experiment 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, CO_2 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

$$I = I_0 \exp(-K_L F) \tag{1}$$

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

$$N_{LA} = a \exp(-K_{Fa} F) \tag{2a}$$

$$N_{LA} = b \exp(-K_{Fb} F) + n_b \tag{2b}$$

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

$$P_m = a_p (N_{LA} - n_b) \tag{3}$$

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

$$N_{LA} = a (I/I_0)^{Ka} \tag{4a}$$

$$N_{LA} = b (I/I_0)^{Kb} + n_b \tag{4b}$$

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: $\ln(N_{LA}) = \ln(a) + K_a \ln(I/I_0)$ and $\ln(N_{LA} - n_b) = \ln(b) + K_b \ln(I/I_0)$.

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):

$$N^*_{SM} = c (M_S)^{-d} \tag{5}$$

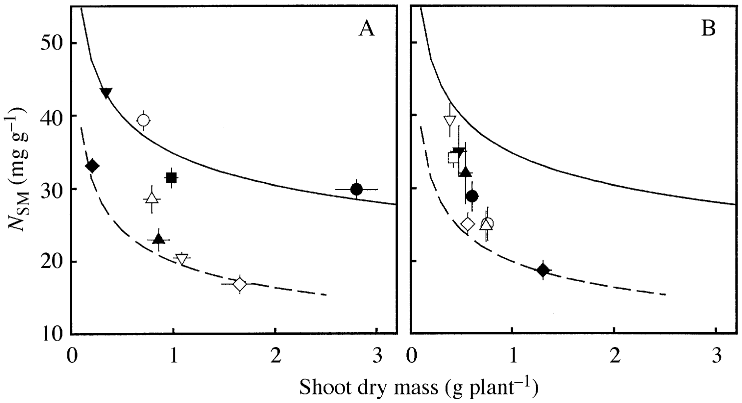

FIG. 1. Shoot N per unit dry mass (N_{SM}) in relation to shoot dry mass (M_S) of individuals. Lines denote N^*_{SM} during a 5-week regrowth period for *Medicago sativa*: plants grown with high PFD/high N supply (I+N+, solid line, $N^*_{SMN+} = 34{\cdot}8\ M_S^{-0{\cdot}196}$, $r^2 = 0{\cdot}73$, $n = 24$) or high PFD/low N supply (I+N–, dashed line, $N^*_{SMN-} = 19{\cdot}9\ M_S^{-0{\cdot}285}$, $r^2 = 0{\cdot}73$, $n = 24$). Symbols denote N_{SM} (mean ± s.e.) of individuals after 5 weeks regrowth. A, *M. sativa* grown with high PFD and high N supply (I+N+, filled circles), high PFD and low N supply (I+N–, filled upright triangles), low PFD and high N supply (I–N+, filled inverted triangles), low PFD and low N supply (I–N–, filled diamonds), in conditions changing from high to low PFD and N supply (cI–N–, filled squares) (expt 3, $n = 12$), grown in dense (open circles) and open (open upright triangles) monocultures and in *Lolium multiflorum* (open inverted triangles) and *Poa pratensis* (open diamonds) stands ($n = 6$). B, *Dactylis glomerata* (filled symbols) and *Taraxacum officinale* (open symbols) grown in dense (circles) and open (upright triangles) monocultures and in *L. multiflorum* (inverted triangles), *Lolium perenne* (open squares) and *P. pratensis* (diamonds) stands ($n = 6$).

where N^*_{SM} is the estimated N_{SM} at a given shoot mass; c and d are constants. A nitrogen nutrition index for individuals (NNI_i) was calculated as:

$$NNI_i = N_{SM}/N^*_{SMN+} \tag{6}$$

where N_{SM} is the actual shoot N concentration of an individual and N^*_{SMN+} the N_{SM} estimated for treatment I+N+ at the same shoot mass. It was assumed that N^*_{SMN+} was close to the critical N concentration (Lemaire and Gastal, 1997) at which plant growth rate is maximal. A value of NNI_i 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 NNI_i 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.

TABLE 1. *Coefficient of light extinction* (K_L)*, leaf area index* (F_t) *of the stand, height of the background stand* (h) *and mean height of the target individuals* Medicago sativa, Dactylis glomerata *and* Taraxacum officinale *relative to the background stand (rel* h_i*)* (n = *6, s.e. in parenthesis) in mixed and monospecific dense and open stands (expts 1 and 2)*

Background stand	K_L	F_t (m^2 m^{-2})	H (m)	rel h_i
M. sativa				
L. m.	0·372	16·3	0·80	0·76 (0·01)
P. p.	0·398	8·7	0·30	1·48 (0·11)
Dense	0·644	7·4	0·57	1
Open	0·675	5·4	0·51	1
D. glomerata				
L. m.	0·352	16·5	0·80	0·89 (0·08)
P. p.	0·371	9·5	0·23	1·64 (0·05)
Dense	0·408	8·9	0·60	1
Open	0·432	7·3	0·55	1
T. officinale				
L. m.	0·332	16·7	0·73	0·56 (0·01)
L. p.	0·309	16·0	0·44	0·83 (0·02)
P. p.	0·386	8·7	0·30	0·84 (0·03)
Dense	0·318	13·8	0·43	1
Open	0·361	9·6	0·29	1

L. m., Lolium multiflorum; *L. p., Lolium perenne*; *P. p., Poa pratensis.*

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 ($N_{stem} = 23{\cdot}0\ M_S^{-0{\cdot}284}$, $r^2 = 0{\cdot}86$ and $N_{lamina} = 48{\cdot}5\ M_S^{-0{\cdot}095}$, $r^2 = 0{\cdot}28$ 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,

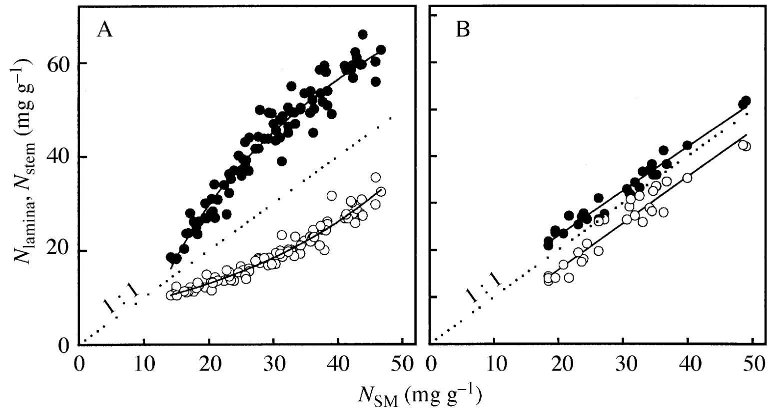

FIG. 2. N per unit dry mass of leaf lamina (filled circles) and stem (open circles) in relation to shoot N per unit dry mass (N_{SM}) of *Medicago sativa* (A) and *Dactylis glomerata* (B) individuals.

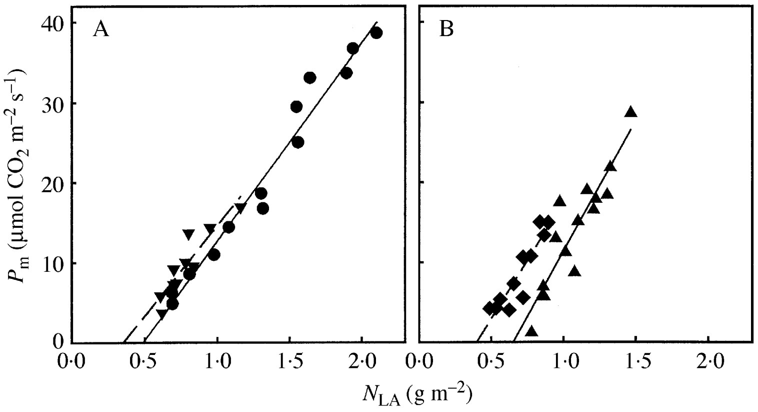

FIG. 3. Light saturated gross photosynthesis (P_{m}) as a function of leaf N per unit leaf area (N_{LA}) for *Medicago sativa* leaves. Plants grown with high/low incident PFD (I+/I–) and high/low N supply N+/N–): A, I+N+ (circles), I–N+ (inverted triangles); B, I+N– (upright triangles), I–N– (diamonds). Regression lines (eqn 3): I+N+, $P_{\text{m}} = 24{\cdot}8\ (N_{\text{LA}} - 0{\cdot}49)$, $r^2 = 0{\cdot}97$; I–N+, $P_{\text{m}} = 22{\cdot}5\ (N_{\text{LA}} - 0{\cdot}36)$, $r^2 = 0{\cdot}84$; I+N–, $P_{\text{m}} = 32{\cdot}7\ (N_{\text{LA}} - 0{\cdot}65)$, $r^2 = 0{\cdot}82$; I–N–, $P_{\text{m}} = 29{\cdot}1\ (N_{\text{LA}} - 0{\cdot}40)$, $r^2 = 0{\cdot}84$.

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 $N^*_{\text{SMN+}}$–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 $N^*_{\text{SMN+}}$ 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 *x*-axis (n_{b}, $P_{\text{m}} = 0$) 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

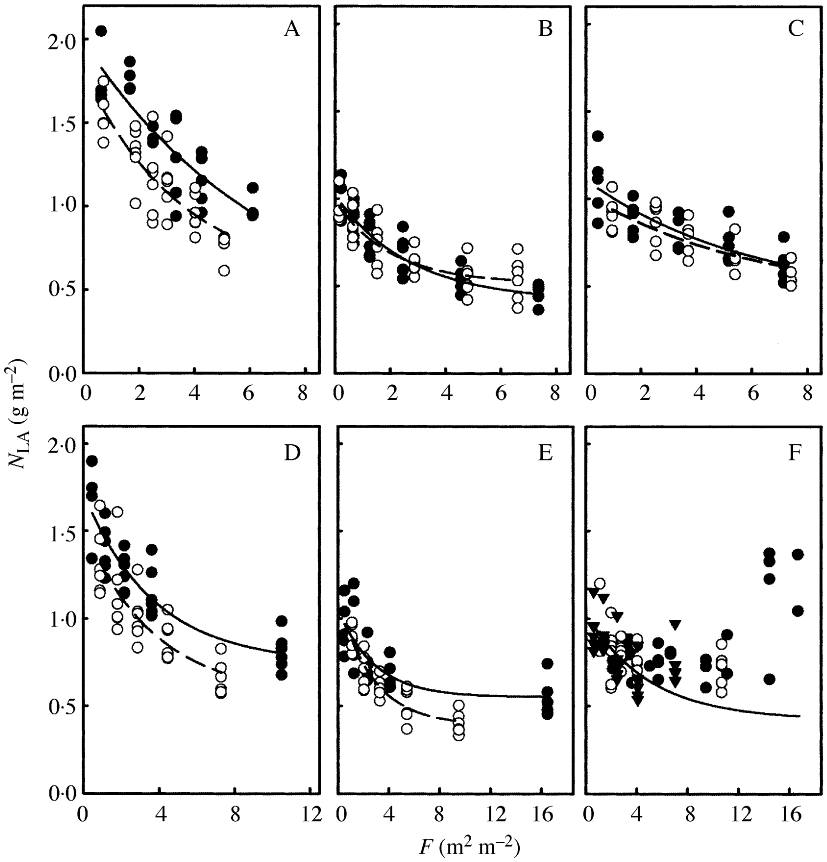

FIG. 4. Leaf N per unit leaf area (N_{LA}) in relation to the cumulative leaf area index (F) for *Medicago sativa* (A and D), *Dactylis glomerata* (B and E) and *Taraxacum officinale* (C and F). Plants grown in dense (filled circles) and open (open circles) monocultures (A–C), and in *Lolium multiflorum* (filled circles), *Poa pratensis* (open circles) and *Lolium perenne* (filled inverted triangles) stands (D–F).

TABLE 2. *Parameter* n_b *(g m*$^{-2}$*) of the relationship between leaf N per unit leaf area (*N_{LA}*) and relative incident PFD (*I/I_0*) for* Medicago sativa, Dactylis glomerata *and* Taraxacum officinale *individuals grown in monospecific dense and open stands and in* Lolium multiflorum, Lolium perenne *and* Poa pratensis *grass stands*

	n_b				
Species	Dense	Open	*L. m.*	*L. p.*	*P. p.*
M. s.	0·25 (n. s.)	0·55 (0·08)	0·75 (0·04)		0·56 (0·22)
D. g.	0·42 (0·06)	0·52 (0·05)	0·55 (0·06)		0·39 (0·05)
T. o.	0·42 (0·22)	0·42 (0·22)	0·42 (0·22)	0·42 (0·22)	0·42 (0·22)

Regression models (eqn 2b): $N_{LA} = b \exp(-K_{Fb} F) + n_b$.
Mean and s.e. in parenthesis.

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 (I+N–) to 0·99 (*M. sativa* in *L. multiflorum* stand). Variation of K_a and K_b within treatments was due to individuals which showed decreasing N_{LA} in the uppermost canopy layer (13 % for *D. glomerata* and 20 % for *T. officinale*) and increasing N_{LA} in the lowest layer (30 % *D. glomerata.*, 18 % *M. sativa*, 87 % *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–, I–N–), K_a and especially K_b, tended to be 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

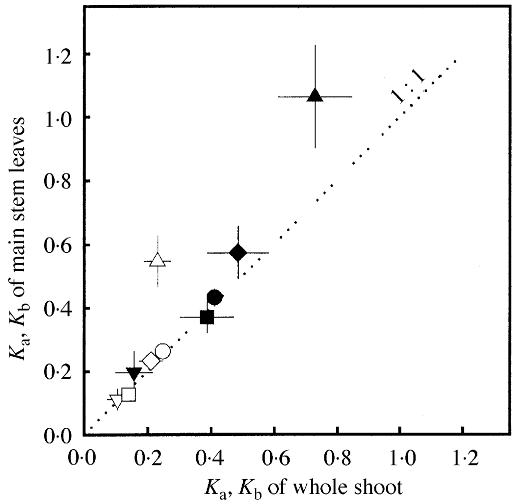

FIG. 5. Coefficient of leaf N allocation (K_a and K_b) for main stem leaves in relation to K_a and K_b of whole shoots (mean ± s.e.). Open symbols with K_a ($N_{\mathrm{LA}} = a\ (I/I_0)^{Ka}$) and closed symbols with K_b ($N_{\mathrm{LA}} = b\ (I/I_0)^{Kb} + n_b$). I+N+ (circles), I+N– (upright triangles), I–N+ (inverted triangles), I–N– (diamonds), and cI–N– (squares).

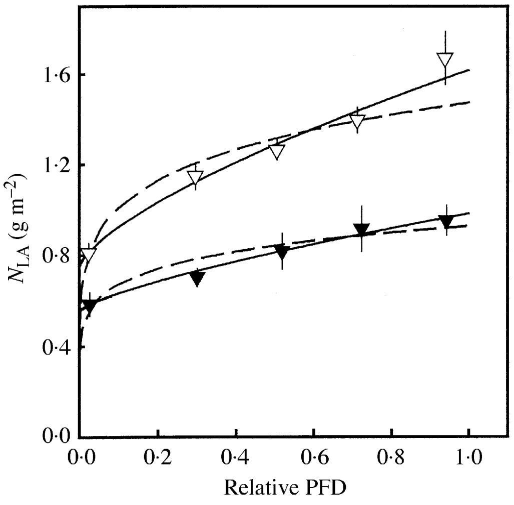

FIG. 6. Leaf N per unit leaf area (N_{LA}) as a function of relative PFD (I/I_0) of *Medicago sativa* (open triangles) and *Dactylis glomerata* (filled triangles) grown in *Lolium multiflorum* stands (mean ± s.e., n = 6). Regressions were analysed for each individual. Solid regression line for *M. s.*: N_{LA} = 0·87 (±0·10) $(I/I_0)^{0·69\,(\pm 0·06)}$ + 0·75 (±0·04), r^2 = 0·99 (±0·005) and *D. g.*: N_{LA} = 0·43 (±0·07) $(I/I_0)^{0·74\,(\pm 0·20)}$ + 0·55 (±0·06), r^2 = 0·80 (±0·09). Dashed regression line for *M. s.*: N_{LA} = 1·47 (±0·07) $(I/I_0)^{0·16\,(\pm 0·02)}$, r^2 = 0·84 (±0·03) and *D. g.*: N_{LA} = 0·93 (±0·06) $(I/I_0)^{0·14\,(\pm 0·008)}$, r^2 = 0·75 (±0·08).

shown, 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 K_b from 0·04 to 1·37. Although N_{LA} decreased in many treatments by about 50 % from the top to the bottom leaves (e.g. I+N+, *M. sativa* and *D. glomerata* in *P. pratensis*, Fig. 7), K_b varied among these treatments. A moderate K_b was manifested by a slow decrease of the N_{LA} in the upper leaf layers followed by a rapid decrease in the lower layers. High K_b values were found when N_{LA} decreased linearly or even exponentially with PFD. Thus, at a 50 % reduction of the relative PFD, plants with moderate K_b (e.g. I+N+) showed up to 20 % higher relative N_{LA} than plants with high K_b, although the overall decrease in N_{LA} was similar (Fig. 7B).

In general, K_b was negatively correlated with N content per unit shoot mass (N_{SM}), i.e. leaf N allocation was more uniform in treatments which induced high N_{SM} (Fig. 8). Similar results were found when K_b was set in relation to N_{lamina} and N_{stem}, respectively (data not shown). At comparable N_{SM}, K_b was significantly higher in *D. glomerata* than in *M. sativa*, whereas *T. officinale* showed rather lower K_b than *M. sativa* when N_{SM} was high.

N_{SM} declined with increasing shoot mass and decreasing NNI$_i$. Multi-regression analysis using the means of the treatments showed, for *M. sativa*, that 85 % of the variation in K_b was explained by the variation of NNI$_i$ and 12 % by variation of shoot mass (Table 3). Multi-regression analysis of individuals revealed a high variation within treatments, and contributions of NNI$_i$ 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 K_b was found (data not shown).

DISCUSSION

In the present study the gradient of leaf N per unit area (N_{LA}) relative to PFD gradient was analysed for grassland species grown in different combinations of PFD and N availability. As a measure for the N_{LA} gradient, coefficients of N allocation, as the slope of the linear relationship between ln (N_{LA}) and ln (I/I_0), were calculated. Introducing a minimal N_{LA} (n_b in eqn 4b) into the N_{LA} – (I/I_0) relationship improved the accuracy of the estimated coefficient of N allocation (K_b) (Fig. 6). That is, above a minimal N_{LA}, N_{LA} 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_m–N_{LA} 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.*, 1992*b*). The n_b values were comparable with N_{LA} values for dead leaves with about 0·45 for *Medicago sativa* (Lemaire *et al.*, 1991) and *Solidago altissima* (Hirose and Werger, 1987) and 0·28 for *Carex acutiformis* (Hirose *et al.*, 1989). Thus, n_b values reflect structural N which cannot be remobilized.

Regarding means of treatments, N_{LA} was reduced by 45 % (s.e. = 2·2, n = 14) from the top to the bottom leaves in treatments which induced steep N_{LA} gradients (K_b > 0·3) and by 21 % (10·4, n = 4) in treatments with more uniform N_{LA} distributions. However, despite a very similar overall decline in N_{LA} in many treatments, N_{LA} distribution relative

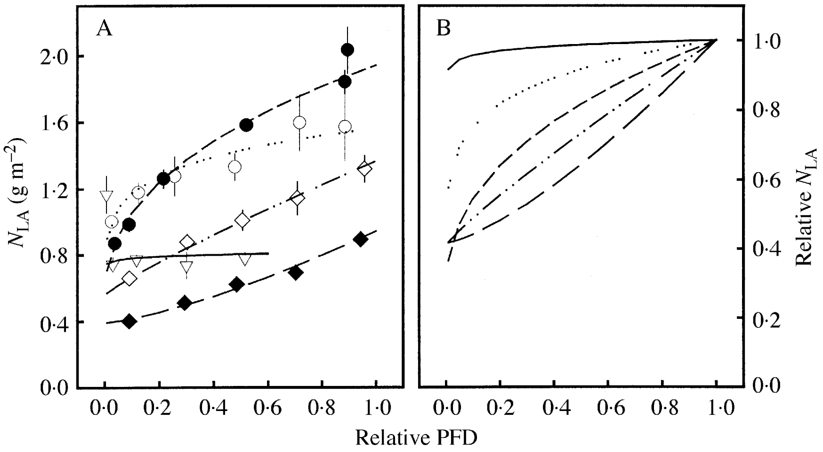

FIG. 7. A, Leaf N per unit leaf area (N_{LA}) (mean ± s.e.) and B, relative N_{LA} as a function of relative PFD (I/I_0) for selected treatments with increasing coefficient of leaf N allocation (K_b). *Taraxacum officinale* grown in a *Lolium multiflorum* stand (open triangles, solid line, K_b = 0·04), *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: $N_{LA} = b\ (I/I_0)^{Kb} + n_b$.

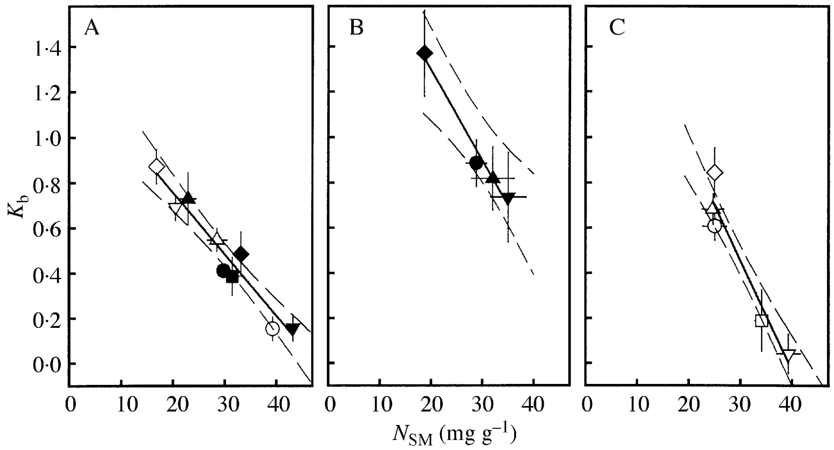

FIG. 8. Coefficient of leaf N allocation (K_b) in relation to shoot N per unit mass (N_{SM}) for A, *Medicago sativa*, B, *Dactylis glomerata* and C, *Taraxacum officinale* individuals (mean ± s.e.). For explanation of symbols see Fig. 1. Regression lines for means of treatments in A, K_b = −0·028 N_{SM} + 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.

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 sunflecks might be high.

A linear decline in N_{LA} with PFD ($K_b \approx 1$) 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.*, 1992*a*, *b*; Anten *et al.*, 1995; Rousseaux *et al.*, 1999). The PFD gradient reflects the *average* PFD with increasing depth in the canopy. However, a leaf may experience higher than average PFD due to sunflecks and heliotropic movement (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 NLA 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

TABLE 3. *Summary of regression analyses among N nutrition index (NNI$_i$), shoot dry mass (M$_S$) and coefficient of N allocation (K$_b$) for* Medicago sativa

Parameter	Value	s.e.	SS	*F*
c_1	1·311	0·070	0·775	351·4***
c_2	–1·152	0·083	0·426	193·2***
c_3	0·109	0·021	0·057	25·8**

Stepwise selection

Variable entered	No. of variables	Partial r^2	Model r^2	*F*
NNI$_i$	1	0·851	0·851	39·8***
M_S	2	0·121	0·972	25·8**

Model: $K_b = c_1 + c_2$ NNI$_i$ + 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^*_{SMN+}) seemed to be close to the critical N concentration where additional N supply would not result in extra growth. A negative deviation from N^*_{SMN+}, reflected by a low N nutrition index (NNI$_i$ < 1), would indicate a shortage of N. In expt 3, low NNI$_i$ 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 NNI$_i$. 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 NNI$_i$. 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 stands. Small effects of plant density on the N_{LA} gradient were also observed by Dreccer *et al*. (2000), Del Pozo and Dennett (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_i (≈ 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 regrowth 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_i, 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_{lamina} 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– and cI–N– *vs*. I+N+) reduced NNI_i 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_{lamina} which could be interpreted as increasing N shortage (decreasing NNI_i) 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.

ACKNOWLEDGEMENTS

We thank Dr G. Lemaire and an anonymous referee for helpful comments on an earlier version of the manuscript. The work was supported by the Federal Ministry of Research and Technology (BMBF 0339370) and by the Deutsche Forschungsgemeinschaft (SFB 607).

LITERATURE CITED

Aerts R, De Caluwe H. 1994. Effects of nitrogen supply on canopy structure and leaf nitrogen distribution in *Carex* species. *Ecology* **75**: 1482–1490.

Anten NPR, Werger MJA. 1996. Canopy structure and nitrogen distribution in dominant and subordinate plants in a dense stand of *Amaranthus dubius* L. with a size hierarchy of individuals. *Oecologia* **105**: 30–37.

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.

Anten NPR, Schieving F, Werger MJA. 1995. Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C_3 and C_4 mono- and dicotyledonous species. *Oecologia* **101**: 504–513.

Del Pozo A, Dennett MD. 1999. Analysis of the distribution of light, leaf nitrogen, and photosynthesis within the canopy of *Vicia faba* L. at two contrasting plant densities. *Australian Journal of Agricultural Research* **50**: 183–189.

Dreccer MF, Van Oijen M, Schapendonk AHCM, Pot CS, Rabbinge R. 2000. Dynamics of vertical leaf nitrogen distribution in a vegetative wheat canopy. Impact on canopy photosynthesis. *Annals of Botany* **86**: 821–831.

Egli P, Schmid B. 2000. Seasonal dynamics of biomass and nitrogen in canopies of *Solidago altissima* and effects of a yearly mowing treatment. *Acta Oecologica* **21**: 63–77.

Hikosaka K, Sudoh S, Hirose T. 1999. Light acquisition and use by individuals competing in a dense stand of an annual herb, *Xanthium canadense*. *Oecologia* **118**: 388–396.

Hikosaka K, Terashima I, Katoh S. 1994. Effects of leaf age, nitrogen nutrition and photon flux density on the distribution of nitrogen among leaves of a vine (*Ipomoea tricolor* Cav.) grown horizontally to avoid mutual shading of leaves. *Oecologia* **97**: 451–457.

Hirose T, Werger MJA. 1987. Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oecologia* **72**: 520–526.

Hirose T, Werger MJA, Pons TL, van 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.

Hirose T, Werger MJA, van Rheenen JWA. 1989. Canopy development and leaf nitrogen distribution in a stand of *Carex acutiformis*. *Ecology* **70**: 1610–1618.

Lemaire G, Gastal F. 1997. N uptake and distribution in plant canopies. In: Lemaire G, ed. *Diagnosis of the nitrogen status in crops*. Berlin, Heidelberg: 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.

Leuning R, Kelliher FM, De Pury DGG, Schulze E-D. 1995. Leaf nitrogen, photosynthesis, conductance and transpiration: scaling from leaves to canopies. *Plant, Cell and Environment* **18**: 1183–1200.

Milroy SP, Bange MP, Sadras VO. 2001. Profiles of leaf nitrogen and light in reproductive canopies of cotton (*Gossypium hirsutum*). *Annals of Botany* **87**: 325–333.

Mooney HA, Field C, Gulmon SL, Bazzaz FA. 1981. Photosynthetic capacity in relation to leaf position in desert versus old-field annuals. *Oecologia* **50**: 109–112.

Rousseaux MC, Hall AJ, Sánchez RA. 1999. Light environment, nitrogen content, and carbon balance of basal leaves of sunflower canopies. *Crop Science* **39**: 1093–1100.

Sadras VO, Hall AJ, Connor DJ. 1993. Light-associated nitrogen distribution profile in flowering canopies of sunflower (*Helianthus annuus* L.) altered during grain growth. *Oecologia* **95**: 488–494.

Schieving F, Werger MJA, Hirose T. 1992*a*. Canopy structure, nitrogen distribution and whole canopy photosynthetic carbon gain in growing and flowering stands of tall herbs. *Vegetation* **102**: 173–181.

Schieving F, Pons TL, Werger MJA, Hirose T. 1992*b*. The vertical distribution of nitrogen and photosynthetic activity at different plant densities in *Carex acutiformis*. *Plant and Soil* **14**: 9–17.

Shiraiwa T, Sinclair TR. 1993. Distribution of nitrogen among leaves in soybean canopies. *Crop Science* **33**: 804–808.