



Dynamics of Vertical Leaf Nitrogen Distribution in a Vegetative Wheat Canopy. Impact on Canopy Photosynthesis

M. F. DRECCER*†, M. VAN OIJEN‡, A. H.C.M. SCHAPENDONK§, C. S. POT§
and R. RABBINGE†

†Laboratory of Theoretical Production Ecology, Wageningen University, P.O. Box 430, 6700 AK, Wageningen, The Netherlands, ‡Centre for Ecology and Hydrology, Edinburgh Research Station, Bush Estate, Penicuik, EH26 0QB, UK and §Plant Research International, P.O. Box 16, 6700 AA, Wageningen, The Netherlands

Received: 9 March 2000 Returned for revision: 16 May 2000 Accepted: 22 June 2000 Published electronically: 14 August 2000

The development of vertical canopy gradients of leaf N has been regarded as an adaptation to the light gradient that helps to maximize canopy photosynthesis. In this study we report the dynamics of vertical leaf N distribution during vegetative growth of wheat in response to changes in N availability and sowing density. The question of to what extent the observed vertical leaf N distribution maximized canopy photosynthesis was addressed with a leaf layer model of canopy photosynthesis that integrates N-dependent leaf photosynthesis according to the canopy light and leaf N distribution. Plants were grown hydroponically at two amounts of N, supplied in proportion to calculated growth rates. Photosynthesis at light saturation correlated with leaf N. The vertical leaf N distribution was associated with the gradient of absorbed light. The leaf N profile changed during crop development and was responsive to N availability. At high N supply, the leaf N profiles were constant during crop development. At low N supply, the leaf N profiles fluctuated between more uniform and steep distributions. These changes were associated with reduced leaf area expansion and increasing N remobilization from lower leaf layers. The distribution of leaf N with respect to the gradient of absorbed irradiance was close to the theoretical optimum maximizing canopy photosynthesis. Sensitivity analysis of the photosynthesis model suggested that plants maintain an optimal vertical leaf N distribution by balancing the capacity for photosynthesis at high and low light. © 2000 Annals of Botany Company

Key words: Canopy photosynthesis, leaf nitrogen distribution, nitrogen, *Triticum aestivum* L, wheat.

INTRODUCTION

Vertical gradients of leaf nitrogen (N) content are a common feature in canopies of crops (Shiraiwa and Sinclair, 1993; Connor *et al.*, 1995), herbaceous vegetation (Field, 1983; Hirose and Werger, 1987a) and trees (DeJong and Doyle, 1985; Hollinger, 1996). The leaf N gradients or profiles correspond well with the light distribution within the canopy, both in terms of amount (Hirose and Werger, 1987b) and spectral quality (Rousseaux *et al.*, 1999). Since leaf photosynthesis increases both with the amount of light absorbed and the leaf N content, the development of leaf N profiles can be viewed as a plastic response that optimizes N utilization with respect to carbon assimilation (Mooney and Gulmon, 1979).

In field crops such as wheat, maximizing biomass production with an efficient use of N is particularly relevant at early vegetative stages, when rapid canopy expansion is crucial for intercepting radiation and competing with weeds. During this period, the canopy undergoes large changes in leaf area and leaf orientation (Meinke, 1996). Accordingly, the vertical leaf N distribution in wheat is likely to change from emergence to full canopy cover. The

advantage for plant growth of different patterns of vertical leaf N distribution can be assessed by calculating daily canopy photosynthesis for a particular light distribution, based on N-dependent leaf photosynthetic rates (Hirose and Werger, 1987b). Calculated gains in canopy photosynthesis, for the optimal against an unchanged N profile, range from 0 to 12% in other species (Schieving *et al.*, 1992; Evans, 1993; Anten *et al.*, 1995; Connor *et al.*, 1995).

Besides changes in canopy structure, environmental factors such as N availability also influence the vertical leaf N distribution. Anten *et al.* (1995) derived analytically that the optimal leaf N profile is steeper in a canopy growing at high N supply, basically because there is more N available for redistribution to the better illuminated leaf layers (Hirose and Werger, 1987b). Experimental studies, however, seem to contradict this notion, since a steeper leaf N distribution has been observed in plants growing at low N due to higher N remobilization from basal to upper leaves (Hikosaka *et al.*, 1994). Another factor that may contribute to the formation of leaf N gradients is leaf age (Mooney *et al.*, 1981). Finally, most studies concerning the development of leaf N gradients assume that leaf photosynthesis is dependent on leaf N content in high light environments only (e.g. Anten *et al.*, 1995). However, canopy photosynthesis is strongly determined by the photosynthetic efficiency at low light (Ort and Baker,

* For correspondence at: Depto. de Producción Vegetal, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, 1417 Buenos Aires, Argentina. Fax 54 11 4 5148737, e-mail dreccer@agro.uba.ar

1988), which also depends on leaf N content (Hikosaka and Terashima, 1995; Dreccer *et al.*, 2000).

In this paper we address the question of whether the vertical leaf N distribution maximizes canopy photosynthesis in wheat at several stages during vegetative development. In addition, we try to assess whether, at low N availability, the development of light-associated leaf N profiles is limited by leaf N remobilization and leaf age. Canopy photosynthesis is calculated and compared between the observed and optimal leaf N distribution. Because of the level of detail and frequency of measurements required to investigate the dynamics of the association between light and leaf N gradients, the experiment was carried out under controlled conditions. In order to break the normally strong correlation between leaf age and light interception, and between leaf age and N content, we supplied different amounts of N and used different sowing densities. To prevent discontinuities in N supply and to overcome complex time-effects and dilution of N in plant biomass (Lemaire and Gastal, 1997), plants were grown hydroponically and N was provided in proportion to the calculated daily growth rate.

MATERIALS AND METHODS

Experimental set-up, design and statistics

Spring wheat (*Triticum aestivum* L., 'Minaret') was grown hydroponically in a greenhouse during the winter months in Wageningen, The Netherlands (51°58' N, 5°40' E). The light transmission of the roof was 60%. Natural irradiance was supplemented with high pressure sodium lamps (Phillips SON-T) supplying 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR, 400–700 nm). The photoperiod was 13 h, relative humidity 70% and average day/night temperatures 15/10°C.

The hydroponics system consisted of four rows of 16 containers (25 l, 0.145 m²) connected by a pipeline. Each container was shielded with an aluminium screen. N addition was independently controlled per row. Ten days after germination (DAG), seedlings were arranged in three rows per container, with 0.09 m between rows, forming a canopy. PAR was measured every minute at a height equivalent to the top of the canopy by two linear sensors (1.1 m long, IMAG, Wageningen, The Netherlands) placed between containers. Steiner (1984) nutrient solution without N was added until 13 DAG, thereafter N was added as NO_3^- . The pH of the solution was adjusted to 5.5.

N was supplied in order to achieve the potential growth rate (high N, HN) or approx. 50% of its value (low N, LN). N treatments were combined with plant number per unit area, 165 (low density, LD) or 330 (high density, HD) plants m⁻². N was added hourly, according to the estimated actual growth rate. The growth rate was calculated based on intercepted radiation, assuming exponential light extinction and a radiation use efficiency equal to 5.0 g MJ⁻¹ (PAR) for the potential growth rate (HN) (Schapendonk *et al.*, 1990). Partitioning coefficients for biomass, initial leaf area, specific leaf area and target N concentrations in the different tissues were inputs for the calculations. Calculated

leaf area was frequently updated by non-destructive and destructive leaf area measurements.

Treatments were arranged in a nested design in randomized blocks. The two N applications were randomly allocated to two main blocks, consisting of two rows of containers each. Within each row of containers (or N application) two sub-blocks were distinguished where the two plant populations (densities) were randomly allocated. Each combination of N and density was thus replicated four times in the experiment. Treatment effects were tested by ANOVA; when *F*-tests were significant ($P < 0.05$), means were compared by the least significant differences test (LSD, $P = 0.05$). Differences between regression models were tested with a *t*-test, using Genstat 5 (1987).

Measurements and calculations

Total biomass (0.054 m²) was harvested 42 (17 Dec. 1996), 64, 78 and 92 (5 Feb. 1997) DAG. After cutting the roots, shoots were dissected at height intervals of 0.05 m, from which green and senescent leaves and stems (including leaf sheaths) were separated. Leaf area was measured (LI-3100, LI-COR, Inc., Lincoln, Nebraska, USA) after leaf discs were taken for determination of chlorophyll (Inskeep and Bloom, 1985). Plant parts were oven-dried at 70°C for 48 h and weighed. N content was analysed with a CHN-O analyser (Fisons Instruments, Interscience BV, Breda, The Netherlands).

PAR was measured three times a week (within 1 h of solar noon) above and inside the canopy (every 0.05 m height) with a linear ceptometer (SF-80, DELTA-T Devices Ltd, Cambridge, England). PAR attenuation through the canopy was fitted to an exponential model and the mean PAR absorbed per unit leaf area at a given height (I_{AL} , $\mu\text{mol quanta m}^{-2}$ leaf) was calculated (Thornley and Johnson, 1990):

$$I_{\text{AL}} = I_0 k \exp(-k\text{LAI}) / (1 - t) \quad (1)$$

where I_0 is the incident PAR on a horizontal plane at the top of the canopy ($\mu\text{mol quanta m}^{-2} \text{s}^{-1}$); k is the calculated coefficient of light extinction; LAI is the leaf area index (m² m⁻²) at the height of measurement; and t is the transmission coefficient. The fraction of PAR intercepted (f_{IPAR}) was calculated as 1 minus the ratio of PAR at the senescence line and PAR at the top of the canopy.

The net rate of photosynthesis at saturating irradiance ($A_{\text{max,n}}$, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was measured (ADC-LCA2, The Analytical Development Co. Ltd, UK) at 350 $\mu\text{l l}^{-1}$ CO₂. Photosynthesis, chlorophyll and leaf N were measured in leaves number 3 to 5 (order of appearance) on the main stem at 42 DAG, leaves 3 to 7 at 64 DAG, 5 to 7 at 78 DAG and 5 to 8 at 92 DAG.

Model for calculation of actual and optimal canopy photosynthesis

A static explanatory model was developed to calculate daytime total net CO₂ assimilation by the canopy of green

leaves (DTNA, mol CO₂ m⁻² d⁻¹). Incident PAR on sunlit and shaded leaves, light absorption and photosynthetic response to PAR and leaf N were calculated for ten layers of equal LAI, and the results were summed to give instantaneous canopy photosynthesis. Canopy photosynthesis was integrated five times over the photoperiod to yield DTNA (Goudriaan and van Laar, 1994). Leaf net photosynthesis (A_n , μmol CO₂ m⁻² s⁻¹) was assumed to follow an asymptotic negative exponential function of PAR, with two parameters: $A_{\max,n}$, the asymptote at high light, and EFF_A (mol CO₂ mol⁻¹ quanta), the initial slope of the curve, i.e. the photosynthetic efficiency based on absorbed radiation (Goudriaan and van Laar, 1994). According to observed data, leaf N (g N m⁻² leaf) declined linearly with cumulative LAI from the top of the canopy and $A_{\max,n}$ was linearly related to leaf N. The photosynthetic efficiency on an incident PAR basis (EFF_1) was calculated from the observed leaf N content, using the equation reported for the same cultivar by Dreccer et al. (2000); $EFF_1 = 0.045 [1 - \exp(-(\text{Leaf N (g N m}^{-2} \text{ leaf)} - 0.32)/0.76)]$, $P < 0.001$, $R^2 = 0.70$, $n = 17$. EFF_A was calculated as the ratio of EFF_1 and the absorbance of the leaf. Leaf absorbance was calculated from leaf chlorophyll contents: Absorbance = $1.0 - 0.7 [1 + 4.3 \text{ Chlorophyll (mmol m}^{-2})]$; $R^2 = 0.90$; $P < 0.001$, $n = 62$ (Dreccer et al., 2000).

DTNA was calculated for the observed leaf N profiles and for a range of hypothetical vertical leaf N distributions, always using the observed LAI, total leaf N in the canopy, and the average incident radiation from the 4 days preceding the harvest. From these simulations, the optimal leaf N distribution was identified (corresponding to the maximum DTNA). In the hypothetical leaf N profiles, the lowest leaf N content per layer was such that $A_{\max,n} = 0$. The simulations were done for all treatments and harvest dates.

The sensitivity of the maximum DTNA to changes in the leaf N profile, represented by the slope of the linear relation between leaf N and cumulative LAI ($b_{N,LAI}$), was assessed as:

$$S \text{ DTNA}, b_{N,LAI} = (b/DTNA)dDTNA/db \quad (2)$$

$S \text{ DTNA}, b_{N,LAI}$ was estimated numerically by calculating the change in DTNA after a 1% change in $b_{N,LAI}$. The change of 1% was chosen because within that range, the changes in photosynthetic parameters as a function of leaf N can be assumed to be linear.

RESULTS

General

The harvests were made between the beginning of tillering and advanced stem elongation, DC21 and DC38 (Zadoks et al., 1974). During this period, the PAR incident over the canopy increased from 1.2 to 1.6 MJ m⁻² d⁻¹.

The N treatment had a greater effect on the variables under study than the plant populations (Fig. 1A–C). Total biomass was proportional to N uptake, so the N concentration in the whole canopy, and particularly in the foliage,

was nearly constant during the experiment. The regression for biomass as a function of N uptake at low N (LN) was: Biomass (g m⁻²) = $0.44 + 87.9 \text{ N uptake (g N m}^{-2})$ ($R^2 = 0.98$, $P < 0.001$, $n = 8$), while at high N (HN): Biomass (g m⁻²) = $-4.0 + 68.6 \text{ N uptake (g N m}^{-2})$ ($R^2 = 0.99$, $P < 0.001$, $n = 8$). On average, green leaf N contents (mass-based) were 1.7% at LN (s.e. = 0.10) and 2.5% at HN (s.e. = 0.06).

The LAI increased with abundant N supply and large plant populations, raising the fraction of radiation intercepted (Fig. 1C and E). The extinction coefficient for light (k) did not vary with height in the canopy, as indicated by strong linear relations between the log-transformed data of I/I_0 and LAI ($P < 0.001$). k tended to be higher at LN than at HN (Fig. 1D).

Differences in specific leaf mass were small among treatments (Fig. 1F), and did not vary systematically through the canopy (not shown). The percentage of dead leaves (in the bottom of the canopy) increased between harvests from 13 to 31% of total leaf mass at LN and from 5 to 14% at HN.

Vertical distribution of light and leaf N

The PAR absorbed per unit leaf area relative to the incident PAR at the top of the canopy (I_{AL}/I_0) decreased with depth in the canopy (Fig. 2A–D). The amount of PAR absorbed per leaf layer was higher at LN than HN, especially at the low sowing density (LD). This was a consequence of the lower LAI and more planophile leaf orientation in the low N treatment (Fig. 1C and D).

The leaf N distribution was linearly related to log-transformed I_{AL}/I_0 (Fig. 2E–H, Table 1). The dynamics of the light-associated leaf N profiles was different at HN than at LN. Density effects were marginal, so N treatments were compared, pooling the data across populations (Fig. 2E–H, Table 1). Judging from the slope values, the vertical distribution of leaf N with respect to absorbed light was more stable in time at HN (Fig. 2E–H). In LN canopies, the slope fluctuated in magnitude with time (Table 1). As a consequence, the vertical distribution of leaf N in LN compared to HN was less steep at 64 DAG, similar at 78 DAG and steeper at 92 DAG.

Light saturated assimilation rate in relation to leaf N and leaf position

The photosynthetic rate at saturating irradiance ($A_{\max,n}$) was linearly related to N content per unit area of leaves (Fig. 3). Deviations from the regression line suggested a possible effect of leaf position on $A_{\max,n}$, either related to leaf age or to acclimation to the lower PAR inside the canopy as it grows. An attempt to assess the effects of leaf N and age separately was made by plotting $A_{\max,n}$ as a function of time after leaf emergence (DALE, days) and as a function of leaf N (Fig. 4). Leaves number 5 and 6 were chosen for this analysis because photosynthesis was measured at the four harvest dates only on these leaves (by 42 DAG leaf number 6 had just expanded fully). The effect of leaf age on $A_{\max,n}$ was smaller than that of leaf N,

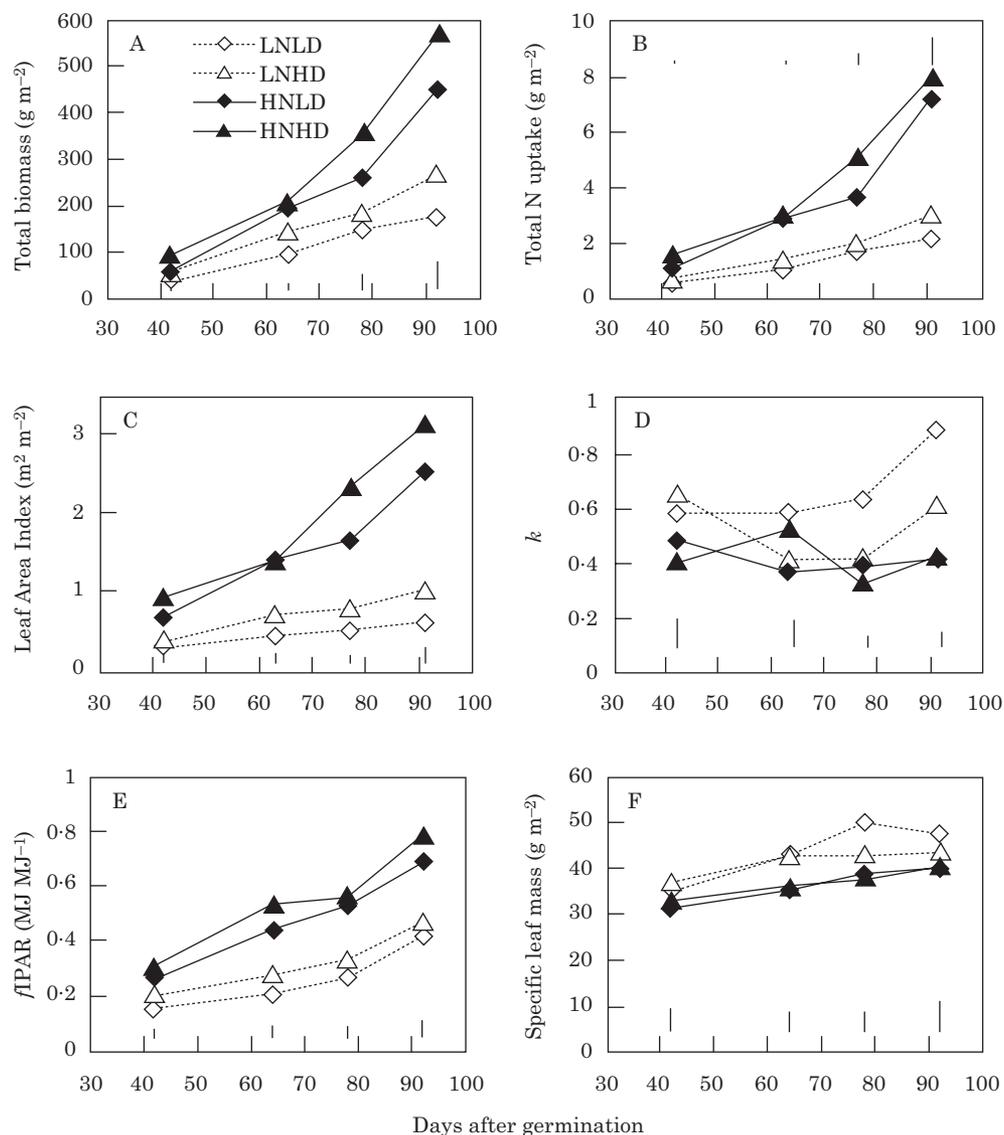


FIG. 1. Dynamics of total biomass (A), total N uptake (B), leaf area index (C), light extinction coefficient (D), fraction of intercepted PAR (E) and specific leaf mass (F). \diamond, \triangle , LN; $\blacklozenge, \blacktriangle$, HN; \diamond, \blacklozenge , LD; $\triangle, \blacktriangle$, HD. Each point is the average of three replicates. Bars indicate standard error of the differences between means.

and in fact age effects were only seen after 40–45 DALE. From 10 to 40 DALE, a leaf with a N content of 1.0 g N m⁻² had an $A_{\max,n}$ that was 1.6–1.7 times higher than a leaf with 0.5 g N m⁻². At 50 DALE, the same difference in leaf N gave a difference in $A_{\max,n}$ of a factor of 1.9, indicating that the nitrogen effect was amplified by age.

Photosynthetic acclimation to low PAR in lower leaf layers was not detected as an increase in the chlorophyll to leaf N ratio or a decrease in the ratio of chlorophyll a/b . The amount of chlorophyll per unit leaf N was similar for all treatments, canopy depths and harvests [Chlorophyll $a + b$ (mmol m⁻²) = $-0.16 + 0.62$ Leaf N (g N m⁻²), $R^2 = 0.70$, $P < 0.001$, $n = 113$]. The chlorophyll a/b ratio varied from 4.4 (s.e. = 0.12) at LN to 4.8 (s.e. = 0.04) at HN.

Canopy photosynthesis and sensitivity analysis

Given the small effects of leaf age on photosynthesis, we calculated daytime net canopy photosynthesis (DTNA) assuming that changes in $A_{\max,n}$ in space and time were mainly a response to leaf N (Fig. 3). Leaf N distribution was linearly related to cumulative LAI (Fig. 5). DTNA calculated with actual leaf N profiles was, from first to last harvest, 0.02–0.08 mol CO₂ m⁻² d⁻¹ at LNLD and 0.05 to 0.19 mol CO₂ m⁻² d⁻¹ at HNHD. The lines in Fig. 5 correspond to the simulated leaf N distribution that maximized DTNA. The measured leaf N distribution was generally within 1% of the calculated optimal distribution. For the range of situations explored, the advantage in DTNA of the optimal profile over a uniform distribution (i.e. when leaf N is the same at all leaf layers) never

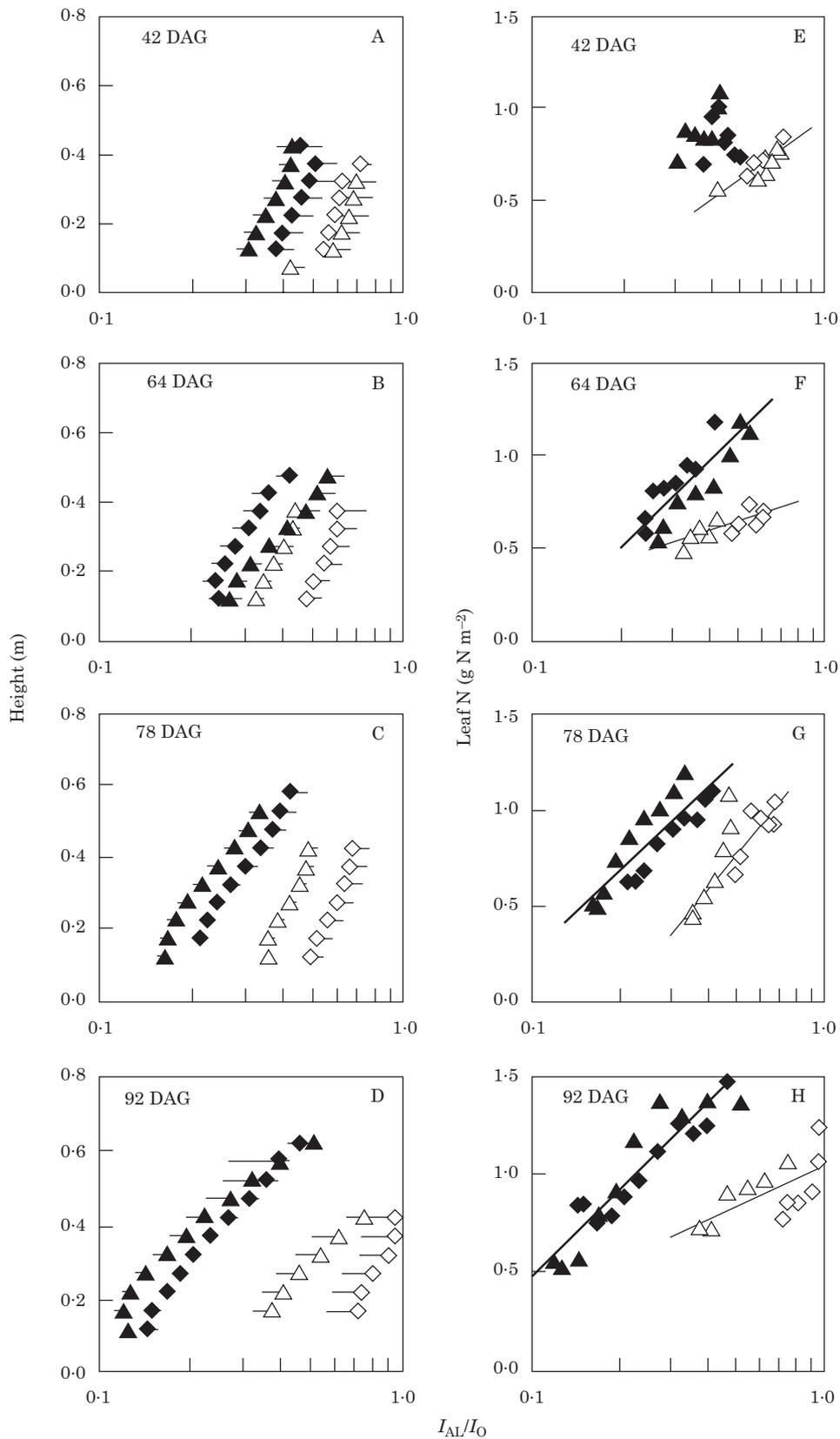


FIG. 2. Changes in absorbed PAR per unit leaf area relative to incident PAR above the canopy (I_{AL}/I_0), with canopy height (A–D), and leaf N content as a function of I_{AL}/I_0 (E–H). Treatment symbols as in Fig. 1. Each point is the average of three replicates. Bars indicate s.e. Regression lines for N applications are given in Table 1. Absence of lines indicates that residual variance exceeds that of y variate.

TABLE 1. Linear regressions between leaf N content (g N m^{-2} ; dependent variable) and relative incident PAR per unit leaf area (I_{AL}/I_0 after logarithmic transformation) on different days after germination (DAG)

		Intercept	Slope	R^2	P	Among slopes	Among lines
42 DAG	LNLD	1.04	0.65	0.719	0.021	NS	***
	LNHD	0.92	0.43	0.725	0.020		
	HNLD	0.60	-0.29	—	NS		
	HNHD	1.67	0.77	0.528	0.039		
	LN	0.95	0.48	0.717	<0.001	NS	***
	HN	0.92	0.05	—	NS		
64 DAG	LNLD	0.85	0.32	0.090	NS	NS	***
	LNHD	1.06	0.47	0.544	0.096		
	HNLD	1.93	0.90	0.868	<0.001		
	HNHD	1.65	0.82	0.932	<0.001		
	LN	0.80	0.22	0.483	0.011	**	
	HN	1.59	0.67	0.734	<0.001		
78 DAG	LNLD	1.39	0.91	0.653	0.017	***	
	LNHD	2.31	1.81	0.902	<0.001		
	HNLD	1.72	0.70	0.956	<0.001		
	HNHD	2.29	0.97	0.970	<0.001		
	LN	1.34	0.82	0.663	<0.001	NS	***
	HN	1.72	0.64	0.727	<0.001		
92 DAG	LNLD	1.17	1.19	0.651	0.032	NS	***
	LNHD	1.22	0.49	0.868	0.004		
	HNLD	1.89	0.61	0.918	<0.001		
	HNHD	2.04	0.69	0.852	<0.001		
	LN	1.05	0.30	0.875	<0.001	***	
	HN	1.97	0.65	0.874	<0.001		

LN, low nitrogen; HN, high nitrogen; LD, low density; HD, high density.

F -probability value: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS, not significant ($P > 0.05$).

— indicates that residual variance exceeds variance of y variate.

Equation: Leaf N = Intercept + Slope $\times \ln(I_{AL}/I_0)$.

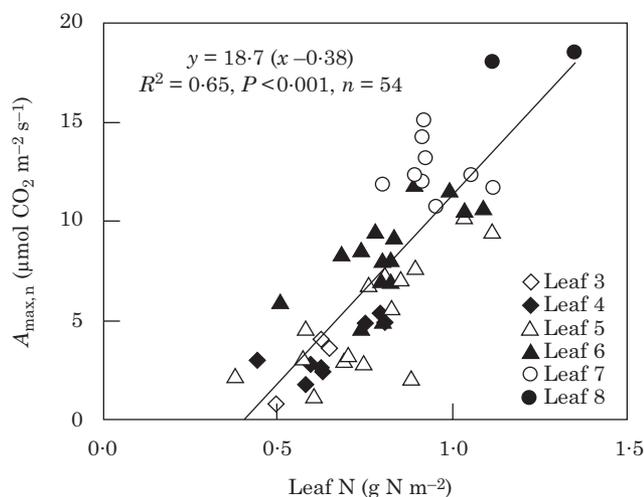


FIG. 3. Leaf photosynthetic rate at light saturation ($A_{\max,n}$) as a function of leaf N. All harvests, leaf positions and treatments included. Each point is the average of three replicates.

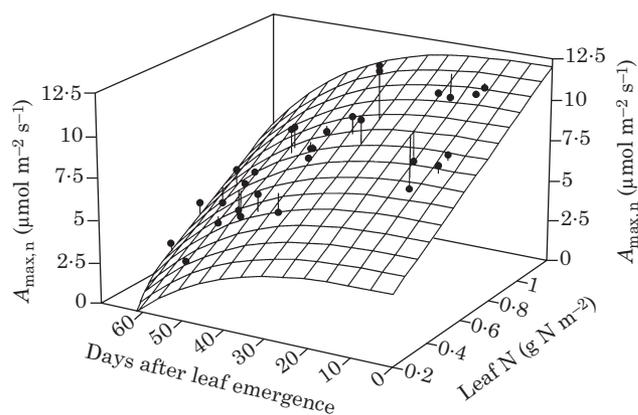


FIG. 4. Changes in $A_{\max,n}$ with leaf N content and days after leaf emergence (DALE), for leaves number 5 and 6. Bars are distance to the plane: $A_{\max,n} = 2.7 + 7.7 \text{ Leaf N} - 1.9E - 05 \text{ DALE}^2$, $R^2 = 0.82$, $n = 30$.

exceeded 2%, except at 64 DAG at HNHD when the advantage was 7%.

To analyse the extent to which treatment differences in maximum DTNA and the associated leaf N profiles

would change at higher light intensities, we compared simulated DTNA between the LNLD and the HNHD treatments on 5 February (92 DAG, Global radiation: $2.9 \text{ MJ m}^{-2} \text{ d}^{-1}$) and a clear summer day (15 July, Global

radiation: 17 MJ m⁻² d⁻¹). In February, DTNA was 2.3-times higher at HNHD than LNLN (0.18 vs. 0.08 mol CO₂ m⁻² d⁻¹), while in July the difference rose to 2.6 (0.44 vs. 0.17 mol CO₂ m⁻² d⁻¹). The slope of the optimal leaf N distribution as a function of cumulative LAI ($b_{N,LAI,opt}$) was steeper at high than at low light, and steeper at LNLN than HNHD (0.55 vs. 0.27 in February, 0.81 vs. 0.35 in July).

Finally, we considered how leaf N affects both photosynthesis at high light and the photosynthetic efficiency at low light. The importance for canopy photosynthesis of maintaining a leaf N profile that optimizes the distribution of the photosynthetic capacity at low (EFF_A) or high light ($A_{max,n}$) may depend on the intensity of incident light. To test this we ran the model with either (1) an EFF_A profile corresponding to the optimal leaf N profile, while $A_{max,n}$ changed as a function of leaf N; or (2) with an $A_{max,n}$ profile corresponding to the optimal leaf N profile, while the EFF_A changed with leaf N (Fig. 6A and B). The simulations were done for 5 February and 15 July for a canopy with the characteristics of HNHD and a range of hypothetical leaf N profiles (given by variations in $b_{N,LAI}$). DTNA was more sensitive to a change in the slope of the leaf N distribution at high than low light. For the whole range of leaf N profiles considered (x -axis), variations in EFF_A with leaf N closely followed the pattern of DTNA at low irradiance, while changes in $A_{max,n}$ did so at high irradiance (Fig. 6A and B). However, near the maximum DTNA, leaf N distribution was between those at which $A_{max,n}$ and EFF_A were optimized. Around the optimum, a change of 1% in the slope of leaf N distribution resulted in a similar drop in DTNA due to changes in either EFF_A or $A_{max,n}$ as a function of leaf N (Fig. 6C and D).

DISCUSSION

The dynamics of light-associated leaf N distribution depends on N supply

During vegetative growth of wheat, the vertical distribution of leaf N changed dynamically with the gradient of absorbed irradiance and was responsive to N supply (Fig. 2E–H, Table 1). At high N supply, leaf N distribution was quite stable in time, as observed in other vegetative canopies (Evans, 1993). This means that, in a canopy during active leaf area expansion, N redistribution was controlled by changes in light intensity as leaves became shaded. In contrast, at low N supply, the relation between leaf N distribution and absorbed irradiance fluctuated in time from steeper to more uniform, even when changes in radiation profiles were small. In a crop growing under N deficiency, older leaves reach low N contents sooner than if N availability is high, and the leaf N profile can be uniform when older leaves senesce completely. In addition, at low N supply, leaf area expansion (Gastal et al., 1992) and branching (Fischer, 1993) are reduced, favouring the preservation of higher leaf N content in the last-developed leaves (Vos and van der Putten, 1998). The co-ordination of canopy expansion and N mobilization seems to play a major role in the definition of the vertical leaf N

distribution under N shortage. Leuning et al. (1995) calculated that at low N supply, canopy photosynthesis was significantly increased if leaf N followed light attenuation compared to a uniform pattern, while gains were negligible at high N. Our analysis also indicates that at low N supply a steeper leaf N distribution narrowed the gap in maximum canopy photosynthesis compared to the high N treatment, particularly at high irradiance.

Plant density had a negligible effect on the vertical distribution of leaf N (see also Shiraiwa and Sinclair, 1993; Del Pozo and Dennett, 1999). This is probably related to compensation in canopy expansion through the production of tillers that led to a quite close radiation distribution between population densities.

The dynamics of light-associated leaf N distribution is close to optimum

The vertical distribution of leaf N was always such that the actual canopy daytime assimilation was within 1% of the optimum, both at high and low N availability (Fig. 5). Independent of the gain in canopy photosynthesis, leaf N distribution has been found to be more uniform in measured than optimal patterns (e.g. Anten et al., 1995). There are different explanations for this discrepancy. The characterization of the light environment is, in many cases, inaccurate and the occurrence of sunflecks (brief episodes of direct light penetrating to basal leaves) may affect the light gradient (Rousseaux et al., 1999). In addition, many factors that affect the photosynthetic capacity per unit leaf N and could influence the optimal leaf N distribution are generally not taken into account, such as photoinhibition, leaf ageing (Field, 1983) or acclimation to low light (Evans, 1993). Accounting for sunflecks or photoinhibition was not a priority for our experimental conditions, with low light intensity and low solar elevation. We did analyse the effects of leaf ageing on maximum photosynthetic capacity, but these were small and only noticeable when leaves were relatively old and deficient in N. According to our results (Fig. 4) and those of others (Hikosaka et al., 1994), effects of leaf age on photosynthesis which are not related to N will not change the distribution of photosynthetic capacity in the canopy. Therefore, these effects may be neglected. Furthermore, no N-independent effects of low light on photosynthesis could be distinguished. When acclimation to low irradiance occurs, leaves at the bottom of the canopy are likely to have a higher proportion of chlorophyll per unit leaf N, a lower chlorophyll a/b ratio and a lower specific leaf mass than the more-illuminated top leaves (Evans, 1996). In our experiment the canopy was adapted to low irradiance, as judged by the absence of any vertical gradient in the above-mentioned characteristics. According to Evans (1993), accounting for acclimation of canopy photosynthesis to low light changed the calculated potential canopy photosynthesis of lucerne by only a few per cent. In fact, the prevailing low light level might have limited nitrogen assimilation directly (Foyer et al., 1995), leading to the observed low plant N concentrations in our experiment compared to field conditions. Still, the N-treatments did cause a difference in N concentration in fully expanded

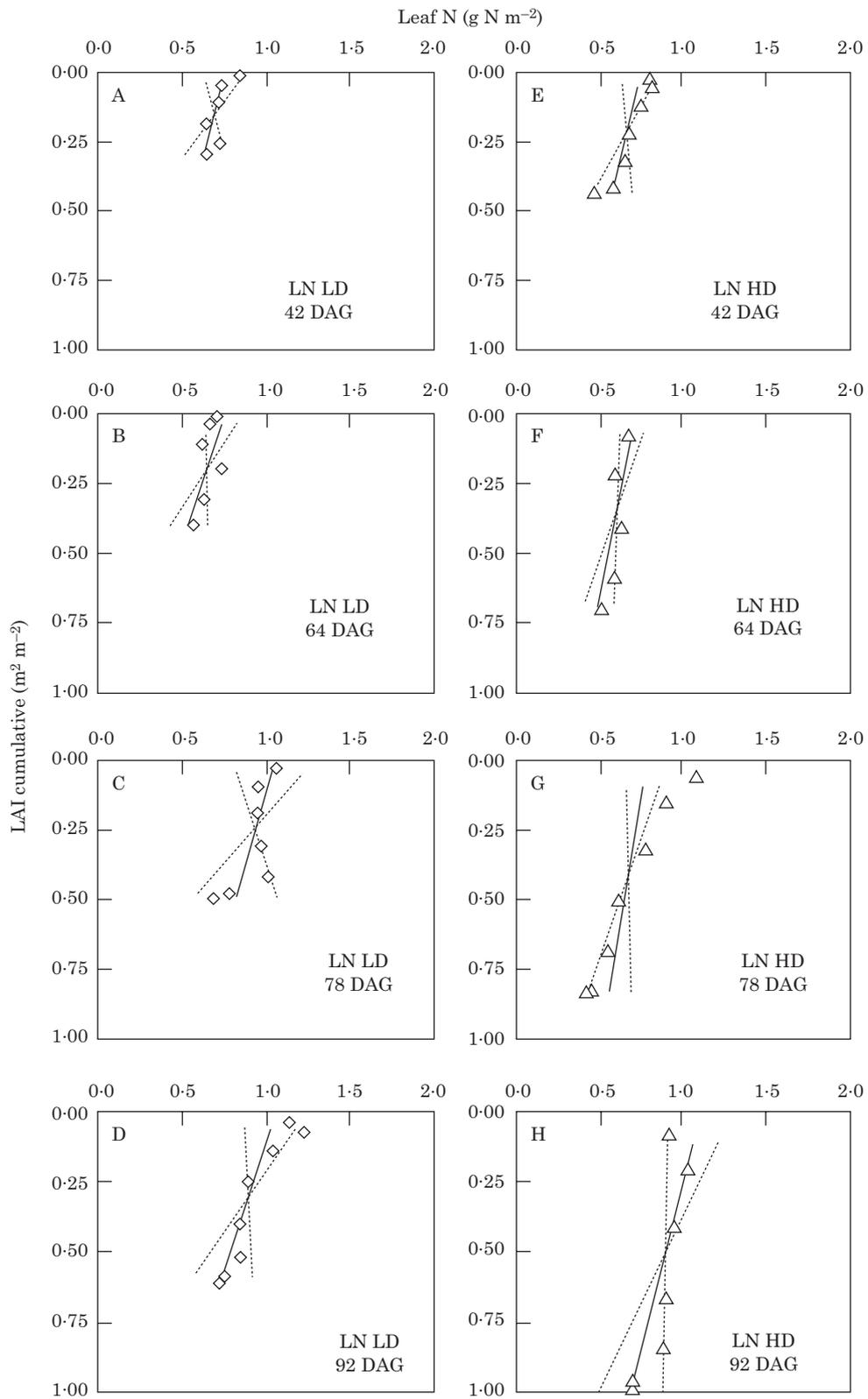


FIG. 5. Legend on facing page.

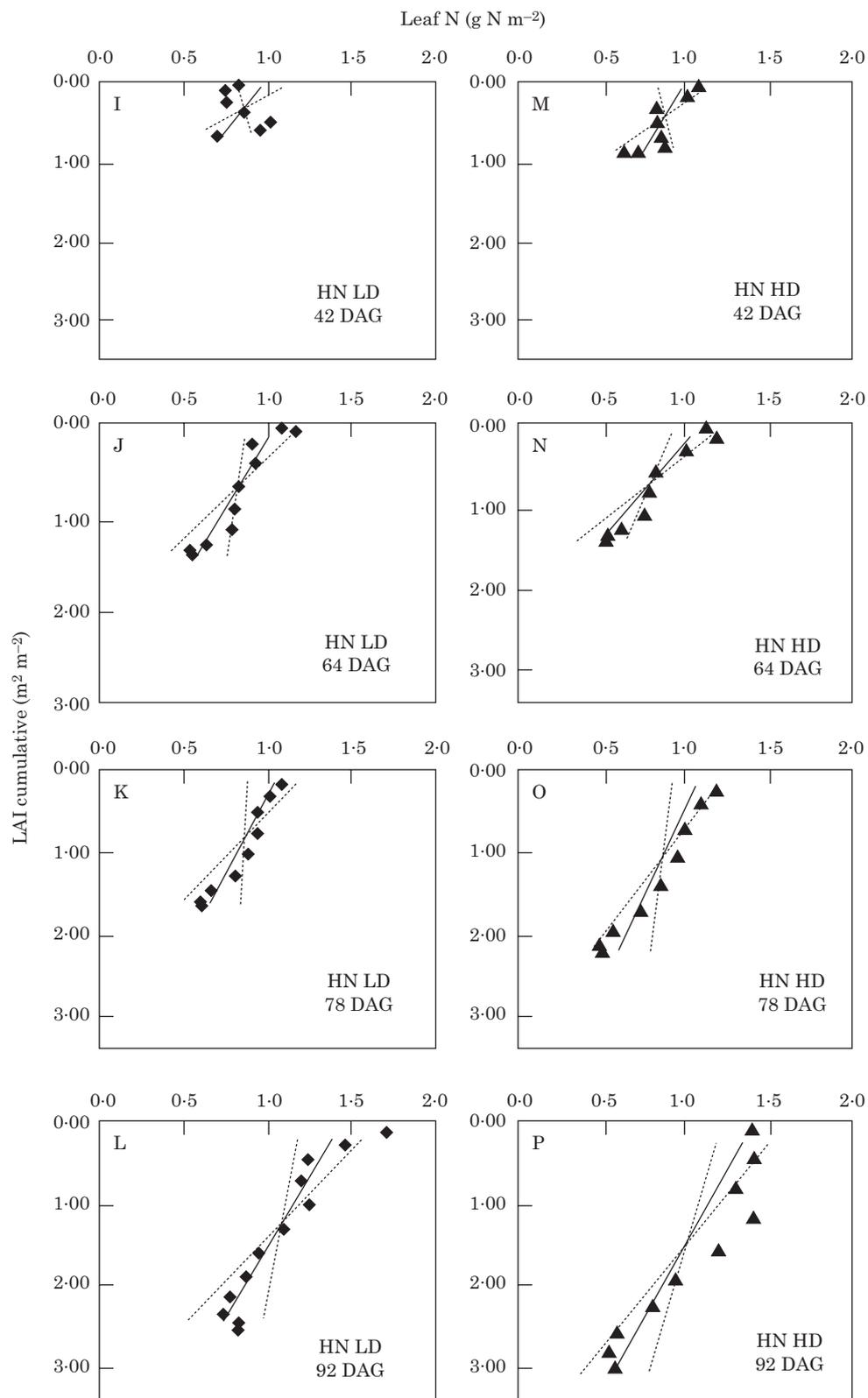


FIG. 5. Variation in leaf N with cumulative LAI from the top of the canopy. Cumulative LAI is pictured on the y-axis to facilitate the analogy with a canopy. Treatment symbols as in Fig. 1. Solid line indicates model-calculated distribution for maximum canopy net assimilation during photoperiod. Dotted lines indicate deviation of 1% from the maximum.

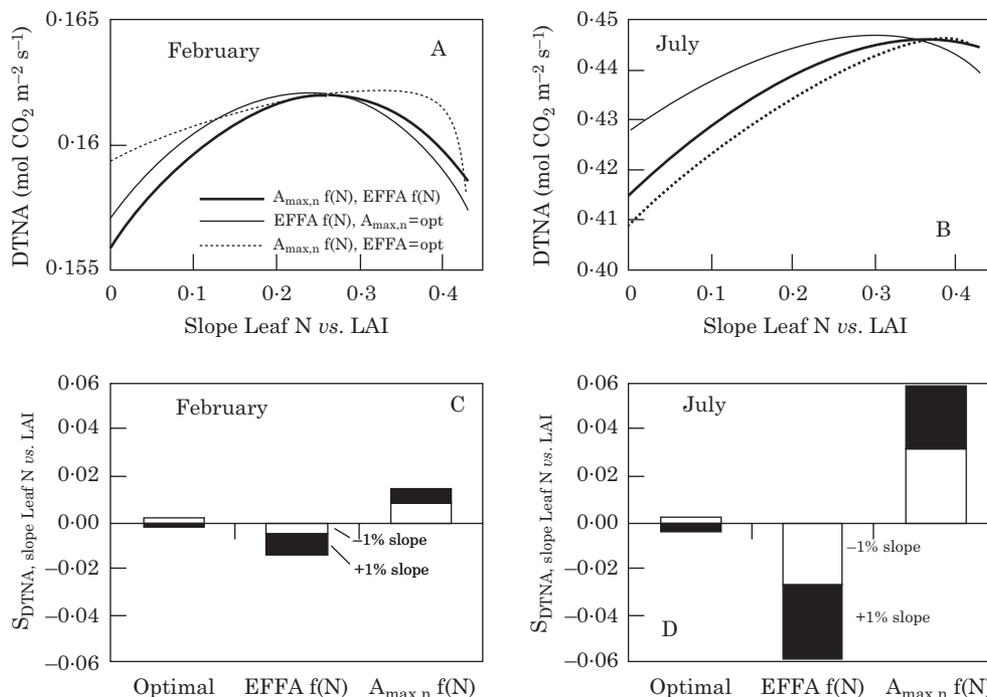


FIG. 6. Changes in canopy photosynthesis (DTNA) with the slope of the leaf N distribution within the canopy ($b_{N,LAi}$) for a day in winter (February) (A, C) and summer (July) (B, D) in the northern hemisphere. In A and B thick lines are DTNA when both $A_{max,n}$ and EFF_A vary with leaf N; thin lines are DTNA for $A_{max,n}$ as in the optimum profile and EFF_A as a function of leaf N; dotted lines are for $A_{max,n}$ as a function of leaf N and EFF_A as in optimum profile. C and D show the sensitivity (S) of DTNA to $\pm 1\%$ change of $b_{N,LAi}$ around the optimum (see Materials and Methods) and when $A_{max,n}$ or EFF_A is a function of leaf N.

leaves, which was nearly twice that of the difference in their rates of photosynthesis. This is an indication that at high N supply, N was not limiting photosynthesis and the development of leaf N gradients was not a response to N deficiency but to the light gradient.

Other shortcomings of the present optimization approach are the absence of an assessment of the metabolic costs associated with N acquisition, remobilization and leaf construction (Mooney and Gulmon, 1979) and the assumption that leaf N and photosynthesis react without delay to changes in light gradients. A major improvement compared with most other studies is the description of N-dependent change in photosynthetic efficiency at low light. Most studies assume that only the photosynthesis rate at light saturation depends on leaf N, while the photosynthetic efficiency at low light is constant (e.g. Anten et al., 1995). In that case, N can be allocated to the top of the canopy without loss of photosynthesis in the less-illuminated layers. We propose that N-effects on photosynthetic efficiency are important and that acknowledgement of this aspect is necessary to understand N-optimization within a canopy.

Optimal leaf N profiles and trade-offs between photosynthetic parameters

As an alternative to the optimization theory of N allocation in the canopy, Chen et al. (1993) proposed that plants allocate N between leaf layers in order to maintain the balance between two processes that may limit the photosynthetic rate: the Rubisco-limited rate of carboxy-

lation and the electron transport limited rate of carboxylation. This theory is based on the photosynthesis model of Farquhar et al. (1980). However, a major constraint in the Farquhar model is that the efficiency of electron transport is fixed. Thus, changes in the photosynthetic efficiency at low light with leaf N cannot be simulated. This is an important consideration in the light of our sensitivity analysis, which showed that plants responded to changes in the light environment by keeping a leaf N distribution that balanced the capacity for photosynthesis at high light and low light (Fig. 6). This trade-off between maximum photosynthesis rates and photosynthetic efficiency at low light, throughout the canopy, resembles, on a larger spatial scale, the findings of Hikosaka and Terashima (1995) with respect to N partitioning within individual leaves.

CONCLUSIONS

In a vegetative wheat canopy, leaf N distribution closely followed the light gradient and thus maximized canopy photosynthesis. The association between light absorption by leaves and their N content was more stable under high than under low N supply. At low N supply, restricted canopy expansion and N remobilization had an effect on the vertical distribution of leaf N. This indicates that although the local light environment plays a major role in dictating leaf N distribution (Drouet and Bonhomme, 1999), the ultimate regulation of resource allocation is coordinated at the plant level.

In a canopy growing at high N availability, the optimal leaf N distribution can be calculated from observed light gradients provided that the dependency of photosynthesis on leaf N content is taken into account for both photosynthesis at high light and photosynthetic efficiency at low light.

ACKNOWLEDGEMENTS

We thank R. van Loo and D. Rodríguez for discussions and help during the experiment and A. de Vos for technical assistance. MFD is on leave from Departamento de Producción Vegetal, Facultad de Agronomía, Universidad de Buenos Aires (FAUBA) and acknowledges the support of CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina) and FAUBA.

LITERATURE CITED

- Anten NPR, Schieving F, Werger MJA. 1995.** Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C₃ and C₄ mono- and dicotyledonous species. *Oecologia* **101**: 504–513.
- Chen JL, Reynolds JF, Harley PC, Tenhunen JD. 1993.** Coordination theory of leaf nitrogen distribution in a canopy. *Oecologia* **93**: 63–69.
- Connor DJ, Sadras VO, Hall AJ. 1995.** Canopy nitrogen distribution and the photosynthetic performance of sunflower crops during grain filling—a quantitative analysis. *Oecologia* **101**: 274–281.
- DeJong TM, Doyle JF. 1985.** Seasonal relationships between leaf nitrogen content (photosynthetic capacity) and leaf canopy light exposure in peach (*Prunus persica*). *Plant, Cell and Environment* **8**: 701–706.
- 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, Schapendonk AHCM, van Oijen M, Pot CS, Rabbinge R. 2000.** Radiation and nitrogen use at the leaf and canopy level by wheat and oilseed rape during the critical period for grain number definition. *Australian Journal of Plant Physiology* **27**: (in press).
- Drouet JL, Bonhomme R. 1999.** Do variations in local leaf irradiance explain changes to leaf nitrogen within row maize canopies? *Annals of Botany* **84**: 61–69.
- Evans JR. 1993.** Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy. II. Stability through time and comparison with a theoretical optimum. *Australian Journal of Plant Physiology* **20**: 55–67.
- Evans JR. 1996.** Developmental constraints on photosynthesis: effects of light and nutrition. In: Baker NR, ed. *Photosynthesis and the environment*. Dordrecht: Kluwer Academic Publishers, 281–304.
- Farquhar GD, von Caemmerer S, Berry JA. 1980.** A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* **149**: 78–90.
- Field C. 1983.** Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control of the allocation program. *Oecologia* **56**: 341–347.
- Fischer RA. 1993.** Irrigated spring wheat and timing and amount of nitrogen fertiliser. II. Physiology of grain yield response. *Field Crops Research* **33**: 57–80.
- Foyer CH, Valadier MH, Ferrario S. 1995.** Co-regulation of nitrogen and carbon assimilation in leaves. In: Smirnoff N, ed. *Environment and plant metabolism, flexibility and acclimation*. Oxford: BIOS Scientific Publishers Limited, 17–33.
- Gastal F, Belanger G, Lemaire G. 1992.** A model of the leaf extension rate of tall fescue in response to nitrogen and temperature. *Annals of Botany* **70**: 437–442.
- Genstat 5 Committee. 1987.** *Genstat 5 reference manual*. Oxford: Clarendon Press.
- Goudriaan J, van Laar HH. 1994.** *Modelling potential crop growth processes. Textbook with exercises*. Dordrecht: Kluwer Academic Publishers.
- Hikosaka K, Terashima I. 1995.** A model of the acclimation of photosynthesis in the leaves of C₃ plants to sun and shade with respect to nitrogen use. *Plant, Cell and Environment* **18**: 605–618.
- Hikosaka K, Terashima I, Katoh S. 1994.** Effects of leaf age, nitrogen nutrition and photo 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. 1987a.** Nitrogen use efficiency in instantaneous and daily photosynthesis of leaves in the canopy of a *Solidago altissima* stand. *Physiologia Plantarum* **70**: 215–222.
- Hirose T, Werger MJA. 1987b.** Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oecologia* **72**: 520–526.
- Hollinger DY. 1996.** Optimality and nitrogen allocation in a tree canopy. *Tree Physiology* **16**: 627–634.
- Inskeep WP, Bloom PR. 1985.** Extinction coefficients of chlorophyll a and b in N,N-dimethylformamide and 80% acetone. *Plant Physiology* **77**: 483–485.
- Lemaire G, Gastal F. 1997.** N uptake and distribution in plant canopies. In: Lemaire G, ed. *Diagnosis of the nitrogen status in crops*. Berlin: Springer-Verlag, 3–43.
- Leuning R, Kelliher FM, De Pury GG, Schulze ED. 1997.** Leaf nitrogen, photosynthesis, conductance and transpiration: scaling from leaves to canopies. *Plant, Cell and Environment* **18**: 1183–1200.
- Meinke H. 1996.** *Improving wheat simulation capabilities in Australia from a cropping systems perspective*. PhD Thesis, Wageningen Agricultural University, The Netherlands.
- Mooney HA, Gulmon SL. 1979.** Environmental and evolutionary constraints on the photosynthetic characteristics of higher plants. In: Solbrig OT, ed. *Topics in plant population biology*. New York: Columbia University Press, 316–337.
- Mooney HA, Field C, Gulmon SL, Bazzaz FA. 1981.** Photosynthetic capacity in relation to leaf position in desert vs. old-field annuals. *Oecologia* **50**: 109–112.
- Ort DR, Baker NR. 1988.** Consideration of photosynthetic efficiency at low light as a major determinant of crop photosynthetic performance. *Plant Physiology and Biochemistry* **26**: 555–565.
- 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.
- Schapendonk AHCM, Spitters CJT, de Vos ALF. 1990.** Comparison of nitrogen utilisation of diploid and tetraploid perennial ryegrass genotypes using a hydroponic system. In: El Bassam N, ed. *Genetic aspects of plant mineral nutrition*. Dordrecht: Kluwer Academic Publishers, 299–306.
- Schieving F, Werger MJA, Hirose T. 1992.** Canopy structure, nitrogen distribution and whole canopy photosynthetic carbon gain in growing and flowering stands of tall herbs. *Vegetatio* **102**: 173–181.
- Shiraiwa T, Sinclair TR. 1993.** Distribution of nitrogen among leaves in soybean canopies. *Crop Science* **33**: 804–808.
- Steiner AA. 1984.** The universal nutrient solution. *Proceedings of the VI International Congress on Soilless Culture*. Lunerren: International Society for Soilless Culture, 633–650.
- Thornley JHM, Johnson IR. 1990.** *Plant and crop modelling: a mathematical approach to plant and crop physiology*. Oxford: Oxford University Press.
- Vos J, van der Putten PEL. 1998.** Effect of nitrogen on leaf growth, leaf nitrogen economy and photosynthetic capacity. *Field Crops Research* **59**: 63–72.
- Zadoks JC, Chang TT, Konzak CF. 1974.** A decimal code for the growth of cereals. *Weed Research* **14**: 415–421.