Radiation and nitrogen use at the leaf and canopy level by wheat and oilseed rape during the critical period for grain number definition

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Abstract. During the critical period for grain number definition, the amount of biomass produced per unit absorbed radiation is more sensitive to nitrogen (N) supply in oilseed rape than in wheat, and reaches a higher value at high N. This response was investigated by combining experimental and modelling work. Oilseed rape and wheat were grown at three levels of N supply, combined with two levels of plant density at high N supply. Canopy photosynthesis and daytime radiation use efficiency (RUE_A) were calculated with a model based on observed N-dependent leaf photosynthesis and observed canopy vertical distribution of light and leaf N. In oilseed rape, RUE_A was higher than in wheat and, in contrast to wheat, the sensitivity to canopy leaf N content increased from the start to the end of the critical period. These results were partly explained by the higher leaf photosynthesis in oilseed rape *vs* wheat. In addition, oilseed rape leaves were increasingly shaded by the inflorescence. Thus, RUE_A increased because more leaves were operating at non-saturating light levels. In both species, the vertical distribution of leaf N was close to that optimising canopy photosynthesis. The results are discussed in relation to possibilities for improvement of N productivity in these crops.

Keywords: leaf photosynthesis, nitrogen use efficiency, oilseed rape, period of grain number definition, radiation use efficiency, wheat.

Introduction

Knowledge of the causes of differences, within or between species, in the use of N for canopy photosynthesis could help to pinpoint desirable characteristics for the design of more efficient crop types, and improve timing of N supply. Radiation use efficiency (RUE_A), defined as the amount of canopy photosynthesis (or biomass produced) per unit absorbed radiation (Table 1), can be used as an estimate of crop productivity. RUE_A is affected by N supply via effects at the leaf and the canopy levels (Sinclair and Horie 1989; Hall *et al.* 1995). At the leaf level, photosynthesis is strongly dependent on leaf N, about three-quarters of the leaf N being invested in proteins with different photosynthetic functions (Evans 1989). The partitioning of N between these fractions regulates the photosynthetic capacity at high light (A_{max}) and the photosynthetic efficiency at low light (EFF) (Evans 1989). At the canopy level, N shortage can change the light environment within the canopy, by restricting leaf area expansion and branching (Fischer 1993).

RUE_A increases with a low level of radiation (due to a more efficient use of light by sunlit leaves) and with a high proportion of diffuse radiation (Hammer and Wright 1994; Bange *et al.* 1997). The impact of the light environment on RUE_A depends also on the vertical distribution of leaf N (Shiraiwa and Sinclair 1993). The leaf N profile affects canopy photosynthesis because processes such as light absorption and regeneration of the activity of enzymes determining CO_2 fixation (e.g. Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase) are dependent on both light and leaf N (Evans 1989). In fact, for a given light distribution in the canopy, a matching leaf N profile can be calculated that maximises canopy photosynthesis (Hirose and Werger 1987; see Dreccer *et al.* 1998 for review).

Wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.) are alternative crops in rotations in temperate

Abbreviations used: DAS, days after sowing; DC, decimal code; I_0, *PAR* at the top of the canopy on a horizontal plane; *PAR*, photosynthetically active radiation; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; RUE_A. See also Table 1.

Table 1. List of acronyms

Acronym	Description	Unit
$A_{max,n}$	Leaf net photosynthesis at light saturation	μmol CO_2 m^{-2} s^{-1}
$A_{max,g}$	Leaf gross photosynthesis at light saturation	μmol CO_2 m^{-2} s^{-1}
Canopy-PNUE	Canopy photosynthetic N use efficiency	mol CO_2 mol N d^{-1}
DTNA	Daytime total net CO_2 assimilation by the canopy	mol CO_2 m^{-2} d^{-1}
EFF_I	Photosynthetic efficiency, on incident light	mol CO_2 mol^{-1} quanta
EFF_A	Photosynthetic efficiency, on absorbed light	mol CO_2 mol^{-1} quanta
I_{AL}	PAR absorbed per unit leaf area	μmol quanta m^{-2} leaf
k	Coefficient of light extinction	m^2 m^{-2}
LAI	Leaf area index	m^2 m^{-2}
Leaf N	Leaf N content	mmol N m^{-2} leaf
Leaf-PNUE	Leaf photosynthetic N use efficiency	μmol CO_2 mmol N s^{-1}
P_n	Leaf net photosynthesis rate	μmol CO_2 m^{-2} s^{-1}
R_d	Respiration in the dark	μmol CO_2 m^{-2} s^{-1}
RUE_A	Canopy radiation use efficiency on absorbed *PAR*	mol CO_2 mol quanta^{-1}
t	Light transmission coefficient	—

regions. In both crops, biomass accumulation during the critical period for grain number definition, i.e. around flowering, is crucial for the final yield (Fischer 1985; Habekotté 1993). In a previous paper reporting growth analysis during this period, we showed that RUE_A (on a total biomass basis) in oilseed rape was more sensitive to N shortage than in wheat, and tended to be higher at high N (Dreccer *et al.* 2000). For instance, when the N concentration in the leaves and inflorescence tissues was 1.5%, the calculated RUE_A (g glucose MJ^{-1}) was 2.4% higher in oilseed rape than wheat, whereas at the N concentration of 3%, the difference in RUE_A climbed to 20.6%. The present study aims to elucidate the physiological principles behind the differential response of RUE_A to N among species. Experimental and modelling work were combined to distinguish between process dynamics at the leaf and canopy levels. Canopy photosynthesis and daytime RUE_A were calculated with a model based on N-dependent leaf photosynthesis and intra-canopy light and leaf N distribution. The experimental set up included N treatments and density levels in order to create a range of light interception and leaf N contents.

Materials and methods

Experimental set up, treatments and statistical analysis

Spring wheat (*Triticum aestivum* L. var. Minaret) and spring oilseed rape (*Brassica napus* L. var. Aries) were sown on 16 April 1997 in Wageningen, The Netherlands (51°58′ N, 5°40′ E), in containers [0.7 (W) × 0.9 (L) × 0.4 (H) m], under an open-sided shelter of transparent plastic roof (3.5 m high). Soil moisture was kept at 80–90% field capacity. A detailed description of the experimental set up is given in Dreccer *et al.* (2000). The treatments consisted in adding 2 (N1), 5 (N2) or 14 (N3) g N m^{-2} to a soil originally containing 3 g mineral N m^{-2}. Plant density was 300 plants m^{-2} for wheat and 150 plants m^{-2} for oilseed rape (D2). At the highest N level, an additional density was tested (D1), 150 plants m^{-2} in wheat and 75 plants m^{-2} in oilseed rape. The distance between crop rows was 0.12 m. Nitrogen was added as Ca(NO$_3$)$_2$.4H$_2$O

in the irrigation water, and split in three additions evenly timed during vegetative growth to prevent depletion effects. Macronutrients other than N and micronutrients were supplied in non-limiting amounts. Treatments were replicated three times and arranged in a completely randomised block design. Two containers were available per replicate. Each container was surrounded by a net to minimise light penetration from the sides; sampling areas were bordered on either side by an intact row, and on either end by at least 0.25 m of crop row. Treatment effects were tested by ANOVA. Differences between regressions and regression parameters were assessed with a *t*-test (Genstat 5 1987).

Measurements

Crop phenology was described with the decimal codes (DC) proposed by Zadoks *et al.* (1974) for wheat and by Sylvester-Bradley and Makepeace (1984) for oilseed rape. Biomass was harvested at 50 and 69 days after sowing (DAS) (0.30 m row per replicate). Plants were dissected into canopy layers of 0.05 m depth, and green and senescent leaves were separated. Leaf area was measured with a planimeter (LI-3100, LI-COR Inc., Lincoln, NE, USA). Samples were oven-dried at 70°C for 48 h, weighed and analysed for total N content with a CHN-O analyser (Fissons Instruments, Interscience BV, Breda, The Netherlands).

Photosynthetic rates were measured on detached leaves at five levels of photosynthetically active radiation (*PAR*), starting at 1500 down to 0 μmol m^{-2} s^{-1}, supplied by a halogen lamp (Philips type 6423, 15V/150W) with an infrared filter. Leaves were cut under water to prevent airlock and stomatal closure. Differential measurements of CO_2 and H_2O were made in an open gas exchange system (model 225 MK3, The Analytical Development Co. Ltd, UK). The concentration of CO_2 was adjusted to 350 ppm and relative humidity to 70%. Measurements were made at 50 and 69 DAS on leaf numbers 5–8 in wheat and 2–8 in oilseed rape, when present.

From the light response curve, the net photosynthetic rate at light saturation ($A_{max,n}$, μmol CO_2 m^{-2} s^{-1}), the initial slope at low light (between 0 and 60 μmol m^{-2} s^{-1}) or photosynthetic efficiency based on incident light (EFF_I, mol CO_2 mol^{-1} quanta) and the respiration in the dark (R_d, μmol CO_2 m^{-2} s^{-1}) were derived. The gross rate of photosynthesis at light saturation ($A_{max,g}$) was calculated by adding R_d to $A_{max,n}$. From the light response curves, the level of *PAR* at which leaves were light-saturated was taken as that for 95% of maximum observed leaf photosynthesis. Samples were taken from test leaves, for determination of N and chlorophyll *a* and *b* (Inskeep and Bloom 1985). On a separate set of leaves, reflectance and transmittance were measured

between 400 and 700 nm wavelength, with a spectroradiometer connected to a Taylor-type integrating sphere (LI-COR Inc.) to calculate absorptance; chlorophyll was determined following Inskeep and Bloom (1985).

PAR was measured twice a week (within 1 h of solar noon) above the canopy, below the inflorescences and lower in the canopy at every 0.05 m height, down to the layer of senescent leaves and at ground level. Data were obtained with a linear ceptometer (SF-80, DELTA-T Devices LTD, Cambridge, England), placed parallel to the rows and are the average of measurements in the middle of the interrow and against the rows. Global radiation data were obtained from a nearby station and *PAR* taken as 50%. Roof transmissivity was 0.85. *PAR* followed an exponential extinction through the canopy (Monsi and Saeki 1953). The level of *PAR* absorbed per unit leaf area at any height (I_{AL}, μmol quanta m^{-2} leaf) was calculated as:

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

where I_0 is *PAR* at the top of the canopy on a horizontal plane (μmol quanta m^{-2}); k is the coefficient of light extinction in the canopy; LAI is the leaf area index (m^2 m^{-2}) and t is the transmission coefficient.

Model for calculation of actual and optimal canopy photosynthesis

A static explanatory model was developed to calculate daytime total net CO_2 assimilation by the canopy (DTNA, mol CO_2 m^{-2} d^{-1}). Green leaves were the only organs considered for contribution to photosynthesis. Incident *PAR* on sunlit and shaded leaves, light absorption and photosynthetic response to radiation level and leaf N are calculated for 10 layers of equal LAI, and integrated five times during the day to yield DTNA, by modification of subroutines of the model SUCROS (Goudriaan and van Laar 1994). According to observed data, radiation is exponentially extinguished with cumulative LAI, while leaf N declines linearly with cumulative LAI (*cf.* Fig. 7). The leaf net photosynthetic response to radiation (P_n, μmol CO_2 m^{-2} s^{-1}) is described assuming an asymptotic negative exponential function, in terms of $A_{max,g}$, photosynthetic efficiency based on absorbed radiation (EFF_A) and R_d (Goudriaan and van Laar 1994):

$$P_n = A_{max,g} \{1 - \exp[-EFF_A \, I_{AL} \, /(A_{max,g})]\} - R_d. \qquad (2)$$

Our experimental data on $A_{max,g}$, R_d and EFF_I correlated with leaf N content per unit leaf area (mmol N m^{-2} leaf). $A_{max,g}$ and R_d followed a linear equation, and EFF_I an asymptotic curve. EFF_A was calculated as the ratio of EFF_I and the corresponding absorptance value.

The average radiation level of the 4 days preceding the harvest, the observed LAI, k, and the total amount of N in the canopy were used as model inputs to calculate DTNA. The model was run for a wide range of vertical leaf N distributions. Within this range of leaf N distributions, one corresponded to the observed profile and the optimal distribution, i.e. that maximising DTNA, could be identified. In the model, leaf layers could lose N until N content became equal to that at which $A_{max,g} = 0$. This minimum leaf N content and the observed LAI and total N in the canopy set the extremes for the leaf N distributions tested, i.e. those where either the bottom or the top of the canopy reached the minimum leaf N value.

Calculation of radiation and N use efficiency

RUE_A (mol CO_2 mol quanta^{-1}) is defined as the ratio of calculated daytime net canopy photosynthesis and the daily absorbed *PAR*, both on a ground area basis. Leaf photosynthetic N use efficiency [leaf-PNUE, μmol CO_2 (mmol N s)$^{-1}$] is the ratio between P_n and leaf N content (mmol N m^{-2} leaf). Canopy photosynthetic N use efficiency [canopy-PNUE, mol CO_2 (mol N d)$^{-1}$] is the ratio between daytime canopy photosynthesis and N content in green leaves, both on a ground area basis.
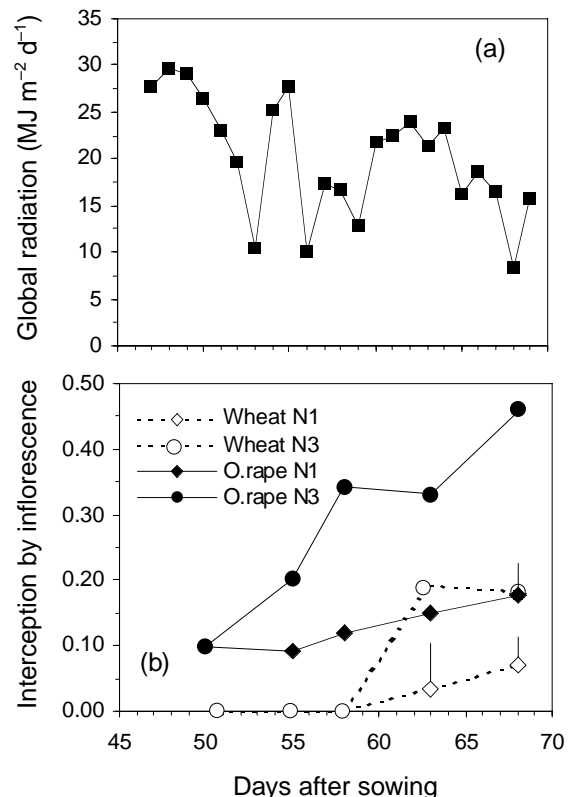
Results

General observations

The observations were concentrated between 50 and 69 DAS. At 50 DAS the ligule of the flag leaf of wheat was just visible (DC39) and in oilseed rape the first flower was fully open (DC41). At 69 DAS, wheat was in the beginning of grain filling (DC71), and in oilseed rape the lower pods were longer than 2 cm (DC51). Therefore in both crops, the period between 50 and 69 DAS coincided with the period of grain number definition as defined by Fischer (1985) for wheat and by Habekotté (1993) for oilseed rape. During this period, weekly averages of daily minimum temperature were between 9.6 and 12.1°C, with maximum temperature between 19.4 and 25.1°C. Daily global radiation between 50 and 69 DAS is presented in Fig. 1a (*cf.* Dreccer *et al.* 2000 for pattern during the whole season).

Leaf photosynthesis vs leaf N

The components of leaf photosynthesis, i.e. $A_{max,g}$, R_d and photosynthetic efficiency based on incident light (EFF_I) were related to leaf N and were higher in oilseed rape than wheat. The regressions between leaf N and either $A_{max,g}$ (Fig. 2a) or R_d (Fig. 2b) were linear ($P < 0.001$). The slopes

Fig. 1. Daily pattern of global radiation (*a*) and fraction of *PAR* intercepted by the inflorescence in plants growing at D2 (*b*) during the critical period for grain number definition (from 50 to 69 DAS). Bars are standard errors.

of the $A_{max,g}$ – leaf N regression were not significantly different between species (although oilseed rape tended to a higher slope), but the intercept on the abscissa was lower in oilseed rape than in wheat ($P < 0.001$). R_d was more responsive to leaf N in oilseed rape than wheat, as shown by the higher slope value ($P < 0.001$). EFF_I (Fig. 2c) and EFF_A were asymptotic functions of leaf N. The relations for EFF_A were, for oilseed rape: $EFF_A = 0.068 \times [1 - \exp(-(\text{Leaf N} - 7.6)/30.3)]$, $R^2 = 0.49$, $P < 0.001$, $n = 19$; and for wheat: $EFF_A = 0.048 \times [1 - \exp(-(\text{Leaf N} - 22.7)/43.4)]$, $R^2 = 0.60$, $P < 0.001$, $n = 17$).
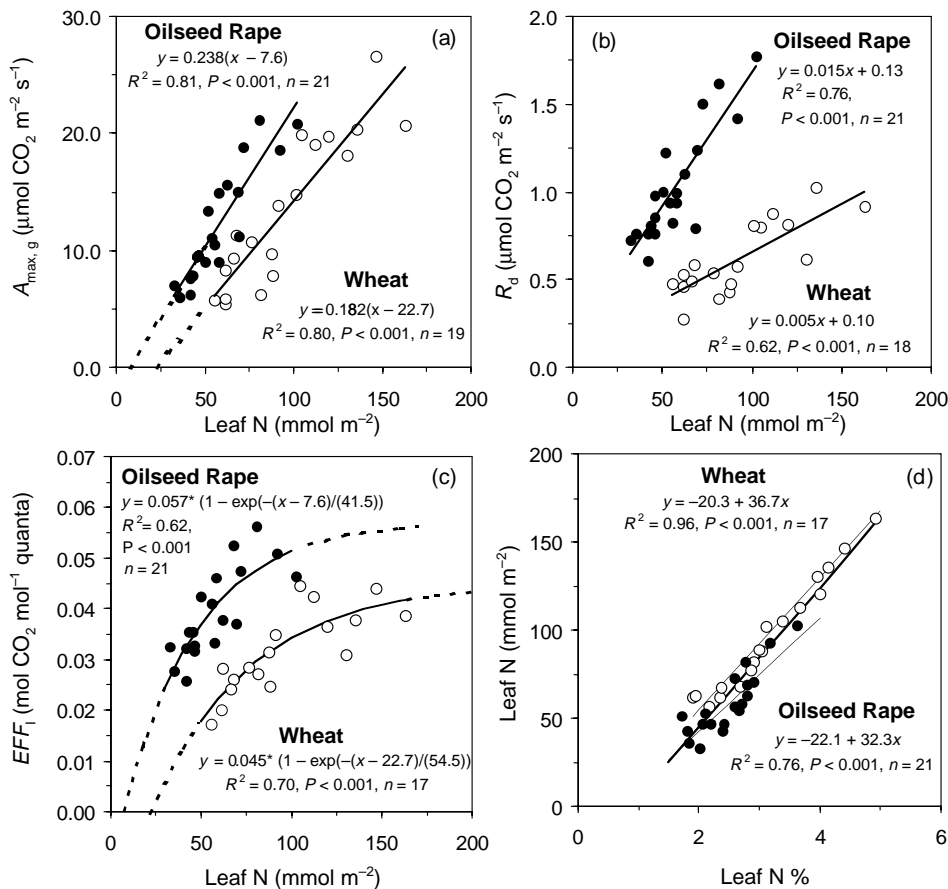
For consistency when modelling canopy photosynthesis, the EFF_I *vs* leaf N regression was forced through the abscissa intercept determined for $A_{max,g}$ *vs* leaf N, after checking that the models were not significantly different when this parameter was free. EFF_I in oilseed rape was significantly higher than in wheat over the whole leaf N range ($P < 0.001$). In both species, the variation in leaf N on an area basis was caused by concomitant changes in leaf N concentration (Fig. 2d) and could be explained by a unique regression. Finally, wheat had a higher proportion of leaf N invested in chlorophyll compared to oilseed rape (Fig. 3a) and tended to a slightly higher maximum absorptance of light per unit chlorophyll ($P < 0.05$) (Fig. 3b).

Oilseed rape had a higher leaf net photosynthesis rate than wheat (calculated with Eq. 2) over a wide range of leaf N both at non-saturating and saturating levels of *PAR* (300 and 1000 µmol quanta m^{-2} s^{-1}, respectively) (Fig. 4a). Thus, N use efficiency at the leaf level (leaf-PNUE) was higher in oilseed rape than in wheat (Fig. 4b). This advantage for oilseed rape became smaller with higher leaf N contents and lower radiation levels.

Canopy photosynthesis

At 50 DAS, DTNA was higher in oilseed rape than in wheat, due to a higher RUE_A, since the amount of *PAR* absorbed by each crop canopy was quite similar per N level (Table 2). By

Fig. 2. Variation in light-saturated gross photosynthesis rate (*a*), respiration in the dark (*b*), photosynthetic efficiency on incident radiation (*c*) and leaf N on area *vs* dry matter basis for oilseed rape (●) and wheat (○). Each point is the mean of three replicates. In (*d*), thin lines indicate individual crop regressions, and thick line is combined regression for both crops (Leaf N [mmol N m^{-2}] = –35.1+39.6 × Leaf N [%]; $R^2 = 0.90$, $P < 0.001$, $n = 39$).
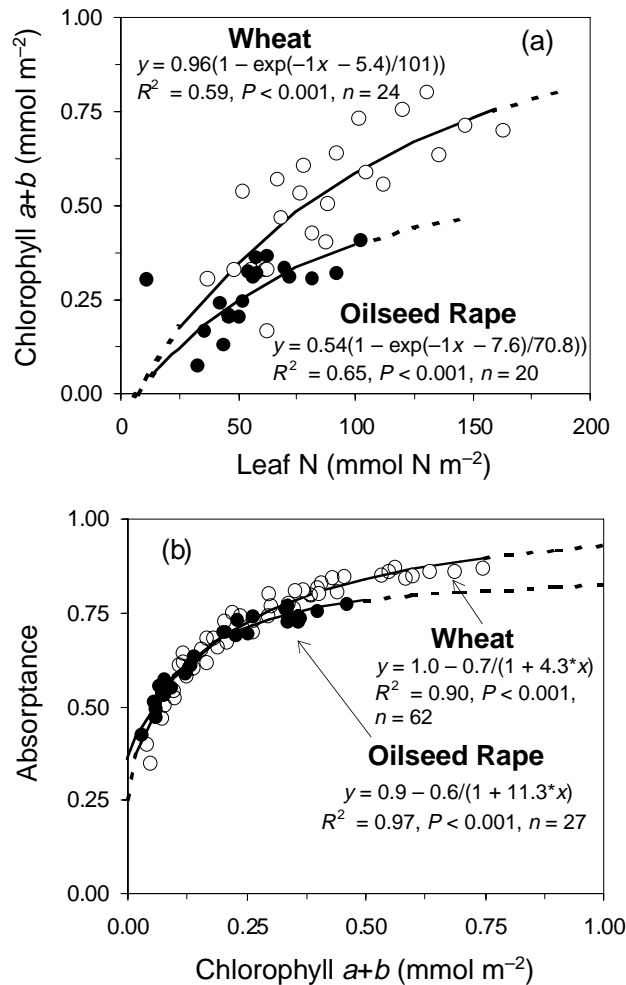
contrast, at 69 DAS, DTNA in oilseed rape was about half of that in wheat, due to the decrease in the total amount of absorbed *PAR*, since RUE_A remained higher in oilseed rape than wheat (Table 2). The N productivity of the canopy, expressed as canopy-PNUE, was higher in oilseed rape than in wheat at equivalent levels of N supply (Table 2).

RUE_A increased with N supply (Table 2) and was linearly related to the average leaf N content of the canopy for both species and harvesting dates (Fig. 5). At 50 DAS, the N-dependent changes of RUE_A as evaluated through the slopes of the linear regressions show that oilseed rape tended to be slightly more responsive than wheat (ratio of slopes of oilseed rape *vs* wheat was *ca* 1.5). Instead, at 69 DAS, oilseed rape had not only higher values of RUE_A but was also much more sensitive to N (ratio of slopes of oilseed rape *vs* wheat was *ca* 3). Why was RUE_A higher in oilseed rape than wheat, and why did the relationship with canopy N change over time? One of the reasons, interspecific differences in the leaf
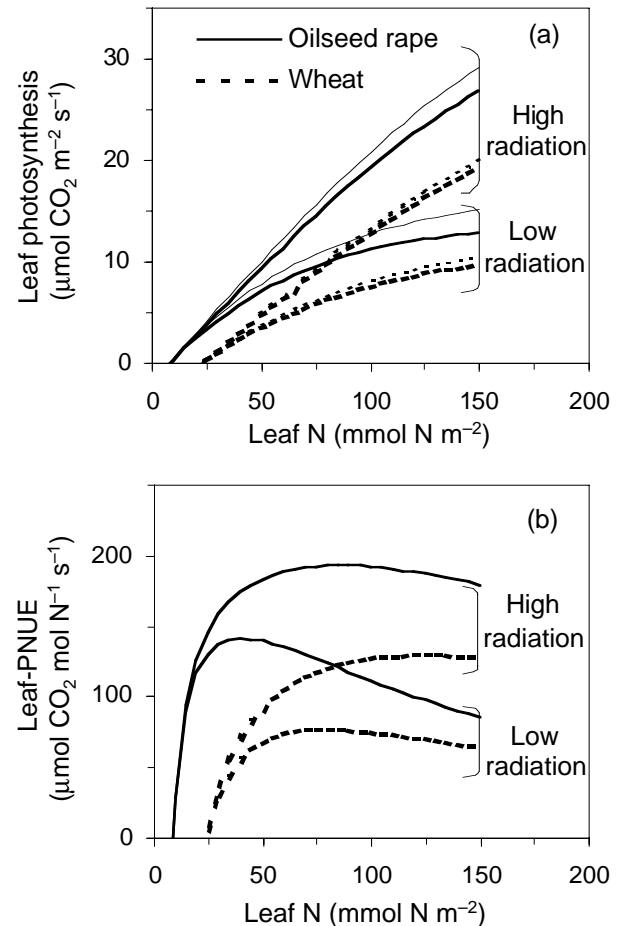
photosynthesis response to N, has been treated in the previous section. In the following sections, two other relevant aspects are explored, i.e. the degree of canopy saturation by radiation and the vertical distribution of leaf N.

Degree of canopy saturation by light

To evaluate the extent to which oilseed rape and wheat canopies were light-saturated, we compared the *PAR* level at which photosynthesis was saturated (see 'Materials and methods') *vs* the *PAR* absorbed per unit leaf area per leaf layer (I_{AL}) at noon of an average day at the beginning and end of the period under study (Fig. 6, data points above the $x = y$ line indicate saturation). The *PAR* saturation level (µmol quanta $m^{-2} s^{-1}$) varied with leaf N (mmol N m^{-2}) in both species (oilseed rape: *PAR* saturation $= 1568 - 1459 \times 0.992^{Leaf N}$, $R^2 = 0.99$; wheat: PAR saturation $= 1548 - 1142 \times 0.993^{Leaf N}$, $R^2 = 0.99$). At 50 DAS and low N supply, photosynthesis was light-saturated in all leaf layers, particularly in

Fig. 3. Leaf chlorophyll *vs* leaf N (*a*); light absorptance *vs* leaf chlorophyll content (*b*) for oilseed rape (●) and wheat (○). Each point is the mean of three replicates.
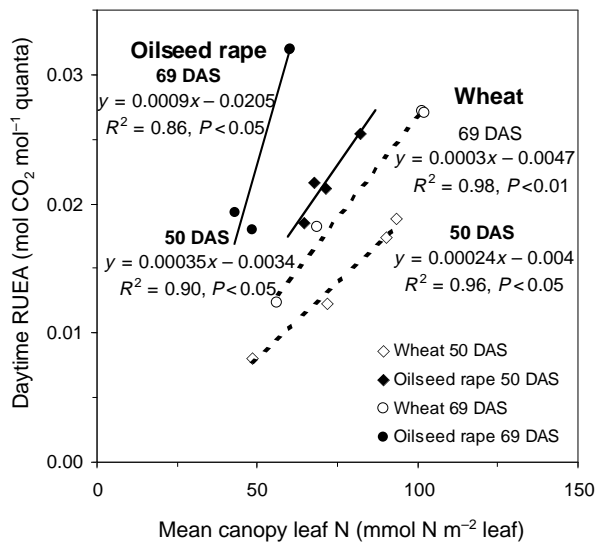
Fig. 4. Model calculated leaf photosynthesis (*a*) and leaf photosynthesis per unit leaf N (*b*). In (*a*), thin lines represent gross photosynthesis and thick net photosynthesis. Low radiation was 300 µmol $m^{-2} s^{-1}$, high radiation was 1000 µmol $m^{-2} s^{-1}$.

Table 2. Canopy photosynthesis and related characteristics in wheat and oilseed rape at the beginning and end of the critical period for grain number definition

Leaf area index (LAI), model calculated daytime total net canopy photosynthesis (DTNA), ratio between DTNA of wheat and oilseed rape, canopy daily absorption of *PAR*, canopy radiation use efficiency (RUE_A), ratio between canopy radiation use efficiency and the maximum leaf photosynthetic efficiency (RUE_A/EFF_A), daily canopy photosynthetic N use efficiency (canopy-PNUE). Treatments are combinations of species, N level (N1 = low, N2 = intermediate, N3 = high) and plant density (D1 = low, D2 = high). LAI values taken from Dreccer *et al.* (2000), with standard error of the difference between means (*n* = 3) among nitrogen (N) or density (D) levels

Treatment		LAI (m^2 m^{-2})	DTNA (mol CO_2 m^{-2} d^{-1})	Ratio DTNA Wheat/o.rape	Canopy absorbed *PAR* (mol m^{-2} d^{-1})	RUE_A (mol mol^{-1})	RUE_A/EFF_A	Canopy-PNUE (mol CO_2 mol^{-1} N d^{-1})
50 DAS								
Wheat	N1D2	0.8	0.246	0.56	20.1	0.0122	0.38	4.33
	N2D2	1.5	0.188	0.36	23.5	0.0080	0.34	2.67
	N3D2	2.7	0.664	0.70	35.3	0.0188	0.50	2.60
	N3D1	2.0	0.591	0.77	33.9	0.0174	0.48	3.25
O.rape	N1D2	0.9	0.442		23.9	0.0185	0.35	7.69
	N2D2	1.0	0.524		24.7	0.0212	0.37	6.96
	N3D2	2.2	0.954		37.6	0.0254	0.42	5.36
	N3D1	2.2	0.767		35.4	0.0216	0.37	5.03
	s.e.d. N	0.3						
	s.e.d. D	0.4						
69 DAS								
Wheat	N1D2	0.6	0.109	2.53	8.8	0.0123	0.48	3.37
	N2D2	0.9	0.146	2.31	8.1	0.0181	0.58	2.44
	N3D2	2.5	0.415	2.11	15.3	0.0271	0.68	1.66
	N3D1	2.2	0.383	1.91	14.2	0.0270	0.68	1.72
O.rape	N1D2	0.1	0.043		2.4	0.0180	0.35	7.75
	N2D2	0.2	0.063		3.3	0.0193	0.40	6.62
	N3D2	1.2	0.197		6.1	0.0322	0.57	2.84
	N3D1	0.8	0.201		6.2	0.0321	0.59	3.56
	s.e.d. N	0.23						
	s.e.d. D	0.23						

Fig. 5. Daytime calculated radiation use efficiency as a function of average leaf N content in the canopy. Wheat identified by open symbols and dotted lines, oilseed rape by closed symbols and solid lines. All regressions with *n* = 4.

oilseed rape, whereas at high N, only the top layers of oilseed rape were light-saturated at noon (Fig. 6*a*). Later in time, the crops were not light-saturated, except for oilseed rape at low N supply (Fig. 6*b*).

The decline in the light saturation of the canopies with time was a consequence of shading by the inflorescences and the decline in incident radiation. At 69 DAS, the fraction intercepted by the inflorescence was between 0.07 and 0.18 in wheat, and between 0.18 and 0.46 in oilseed rape, for the lowest and highest N treatments, respectively (Fig. 1*b* and Dreccer *et al.* 2000). To test how much the N-dependent changes in RUE_A (Fig. 5) observed at 69 DAS were due to less light reaching the leaves because of the growth of the inflorescence, and how much was attributable to less global radiation, we ran the model keeping everything as observed at 69 DAS except the global radiation, which was set similar to 50 DAS. The regressions obtained were for oilseed rape: RUE_A (mol CO_2 mol^{-1} quanta^{-1}) = 0.0011 mean canopy leaf N (mmol N m^{-2} leaf) –0.0352, R^2 = 0.89, $P < 0.05$, *n* = 4; for wheat: RUE_A = 0.0003 mean canopy leaf N –0.0088, R^2 = 0.99, $P < 0.005$, *n* = 4. This shows that the trends between species did not change and the role of the inflores-

cence in defining the light environment over the leaves is very important in oilseed rape.

Furthermore, we compared the RUE_A with EFF_A, as calculated for the average leaf N content of the canopy (following Figs 3 and 2c) (Table 2). The ratio between RUE_A and EFF_A is 1 when crop photosynthesis operates at its maximum efficiency. This ratio was higher at 69 DAS, confirming that both crops were closer to maximum efficiency than at 50 DAS. Over the whole period analysed, the wheat canopy operated nearer to its maximum efficiency than did oilseed rape.

Vertical distribution of leaf N in relation to absorbed radiation

Leaf N was preferentially partitioned to the upper, more illuminated leaf layers. The distribution pattern of leaf N was proportional to the logarithm of the ratio between the absorbed radiation at a given leaf level (I_{AL}) and at the top (I_0) of the canopy (Table 3; Fig. 7). Leaf N distribution, as judged by the slope of the leaf N partitioning along the light gradient, became steeper from 50 to 69 DAS in most treatments, particularly in oilseed rape. The effect of N level on this slope was transient. In general, steeper leaf N profiles, which correspond to higher remobilisation of N from bottom

to top canopy layers, were observed at low (N1D2) compared to high N supply (N3D2) (Table 3). Both crops had similar leaf N distributions with respect to the absorbed radiation in the lowest or the highest N treatments (Table 3). No differences were found between the N distribution at high (D2) or low density (D1) (Table 3).

In general, leaf N distribution was within 1–2% of the value that would maximise canopy photosynthesis, as calculated with the model (Fig. 7). Remarkably, this occurred in canopies of different LAI (Table 2) and extinction coefficients for PAR (k). At 50 DAS, k in wheat was 0.65 at low and 0.44 at high N; in oilseed rape the values were 0.73 at low and 0.63 at high N (s.e.d. = 0.07). In both crops, leaf N decreased linearly with cumulative LAI (some examples in Fig. 8). All regressions between these variables were significant at $P < 0.01$, except for those treatments with very low LAI (i.e. oilseed rape at 69 DAS at N1D1 and N2D2).
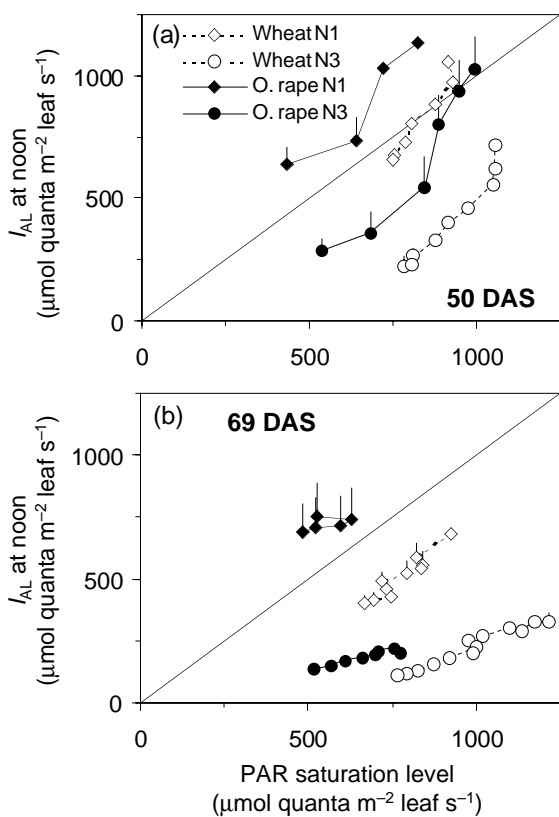
Discussion

Oilseed rape was more efficient than wheat in converting radiation or N into canopy photosynthesis, and it was also more responsive to changes in N nutrition during the critical period for grain number definition, confirming the results from growth analysis (Dreccer *et al.* 2000). Our analysis of photosynthesis from the leaf to the canopy level helped to explore the causes of differences in crop responses to N. These were mainly interspecific differences in leaf photosynthesis and differences between species in N effects on canopy architecture (via the growth of the inflorescence), with consequent changes in light distribution.

RUE, leaf photosynthesis and leaf photosynthetic N use efficiency

Oilseed rape had higher leaf photosynthesis than wheat, with consequences for the canopy photosynthesis and hence RUE_A (Figs 2 and 4). Calculations with the model indicated that transposing the photosynthesis of oilseed rape to wheat could increase wheat canopy photosynthesis and the associated RUE_A by 50% (Option 1, Table 4). This effect is expected to decline when the N content of the canopy increases (Sinclair and Horie 1989).

Leaf-PNUE was higher in oilseed rape than in wheat. Higher leaf-PNUE is generally associated with thinner leaves (Poorter and Evans 1998), but in our study, interspecific variation in leaf mass per unit area was minimal. A number of differences in the chain of events leading to CO_2 assimilation may explain the differences in leaf-PNUE between species (Fig. 4b) (Poorter and Evans 1998), such as the capacity for light absorption (Vogelmann *et al.* 1996), stomatal conductance (Henson *et al.* 1990) and the activation state of Rubisco (Makino *et al.* 1988). Jensen *et al.* (1996) concluded that in rape, leaf photosynthesis and leaf conductance are high compared to other dicots. We observed that oilseed rape and wheat partitioned N differently to certain

Fig. 6. *PAR* level absorbed per leaf layer at noon *vs PAR* level of light-saturated photosynthesis, at 50 DAS (*a*) or 69 DAS (*b*). Bars are standard errors.
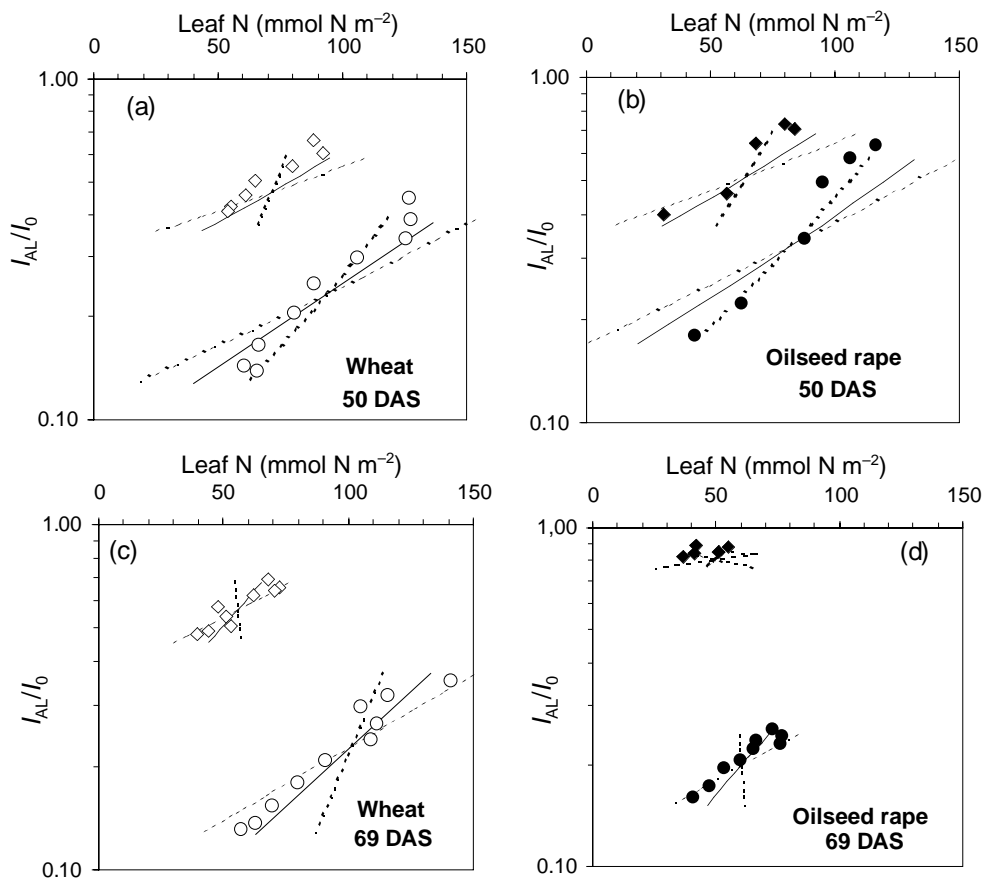
photosynthetic functions. In the range of leaf N where photosynthesis was responsive, oilseed rape had *ca* 70% of the chlorophyll content of wheat and 90% of its absorptance. The higher chlorophyll to leaf N ratio in wheat could be an adaptation to the relatively low levels of radiation (Evans 1996) normally incident on erectophile leaves. The higher absorptivity per unit chlorophyll of oilseed rape compared to wheat could be related to the spongy mesophyll present in dicots leaves, which enhances light scattering (Vogelmann *et al.* 1996). If in wheat the higher investment of leaf N in pigments occurs at the expense of electron transport carriers and Rubisco, a negative impact on the leaf-PNUE could be expected, particularly at high radiation levels.

Finally, variability in maximum photosynthesis or respiration at similar leaf N content has been observed between species (Evans 1989; Reich *et al.* 1998). Instead, interspecific variability and changes in the photosynthetic efficiency based on incident or absorbed light (EFF_I or EFF_A) in response to N are not frequently reported. It is generally accepted that N affects EFF_I through the chlorophyll content and thus through leaf absorptance (Evans 1996). The finding of a decrease in EFF_A with leaf N could be linked to a relative increase in photorespiration at low N nutrition (Schapendonk *et al.* 1999). At low N availability, leaves have a more compact mesophyll and lower proportion of intercellular spaces (van Arendonk *et al.* 1997); this structural change could hamper CO_2 more than O_2 diffusion to the sites of carboxylation, and thus increase photorespiration. This hypothesis needs to be investigated further, given the quantitative importance of the photosynthetic efficiency for canopy *RUE* and biomass production (Ort and Baker 1988).

RUE and canopy characteristics

Daytime RUE_A was related to canopy N as calculated for other crops (e.g. Sinclair and Horie 1989). At the beginning of the critical period for grain number definition, the response of RUE_A to canopy N was slightly steeper in oilseed rape than in wheat (Fig. 5). This was not anticipated since in broad-leaf crops, N deficiency severely restricts leaf expan-

Fig. 7. Leaf N *vs* absorbed relative to incoming *PAR* level (I_{AL}/I_0). Wheat at 50 (*a*) or 69 DAS (*c*), oilseed rape at 50 (*b*) or 69 DAS (*d*). Diamonds are low N (N1D2) and circles are high N supply (N3D2). Solid lines represent model-calculated optimal leaf N distribution; dotted lines represent a 1% deviation from optimal. I_{AL}/I_0 is pictured on the *y*-axis to facilitate the analogy with the vertical extinction of light in a canopy.
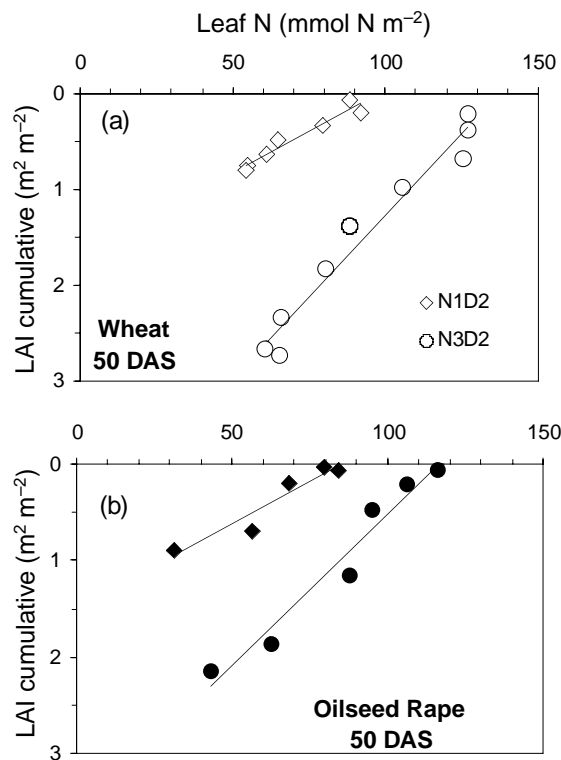
sion, thus minimising the effects on the photosynthetic capacity (Vos and van der Putten 1998). Indeed we observed that the range over which leaf N contents and LAI varied was narrower for oilseed rape than for wheat. Towards the end of the period, RUE_A increased in both crops, and the sensitivity of RUE_A to canopy N increased in oilseed rape more than in wheat. The lower and more diffuse radiation incident on the leaves, due to the decrease in global radiation but mainly due to the development of inflorescences, are likely causes for the observed increase in RUE_A (Fig. 5) (Bange et al. 1997). However, at low leaf N, the size of the antennae and electron transport chain limit the capacity for processing incoming radiant energy (Evans 1996), drifting the canopy towards saturation (Hirose and Werger 1987). Thus, in oilseed rape at high N supply, the shading by the inflorescence partly counteracted the effect of the decrease in canopy N, and RUE_A increased from 50 to 69 DAS. Instead, at low N supply, inflorescence growth only compensated for the N loss by leaves, and RUE_A was maintained from 50 to 69 DAS (Table 2, Fig. 6b).

In wheat and oilseed rape, vertical leaf N distribution was close to the theoretical optimal for canopy photosynthesis (Hirose and Werger 1987) and RUE_A. A steeper leaf N distribution in dicots vs monocots, observed when leaf N is related to cumulative leaf area (Anten et al. 1995), was not a marked feature in our study. By relating the leaf N content to the gradient of absorbed irradiance, the differences in leaf area and orientation are combined, forming a more straightforward basis for comparison between species. The distribution of leaf N with absorbed radiation changed dynamically and tended to steeper values at lower radiation level and low N supply. This phenomenon is associated with increasing N remobilisation, in this case from the leaves to the growing inflorescences (Dreccer et al. 2000), but others have observed a similar trend during vegetative stages (Hikosaka et al. 1994). Finally, in other studies comparing crop canopies, the calculated optimal distribution was found to be steeper than the actual distribution (e.g. Anten et al. 1995). This difference with our work could be related to their assumption that the photosynthetic efficiency is independent

Table 3. Linear regressions between leaf N content per layer (mmol N m^{-2}) and *PAR* absorbed per unit leaf area relative to above-canopy incident *PAR* (I_{AL}/I_0, after logarithmic transformation)

Treatments are combinations of species, N level (N1 = low, N2 = intermediate, N3 = high) and plant density (D1 = low, D2 = high). Each point in the regression is average of three values. Significance of *F*-probability values: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, NS = non-significant ($P \geq 0.05$). Leaf N = Intercept + Slope × ln(I_{AL}/I_0)

Treatment		Intercept	Slope	N	R^2	P	Among Slopes	Among lines
Nitrogen comparison:								
50 DAS								
Wheat	N1D2	127.2	83.5	7	0.927	< 0.001	**	
	N2D2	106.0	47.9	6	0.958	< 0.001		
	N3D2	182.3	62.5	9	0.948	< 0.001		
Oilseed rape	N1D2	105.0	73.5	5	0.884	0.011		
	N2D2	134.5	87.1	6	0.920	0.002		
	N3D2	136.3	51.2	6	0.958	< 0.001		
69 DAS								
Wheat	N1D2	106.7	89.8	10	0.891	< 0.001	NS	***
	N2D2	184.4	135.8	11	0.902	< 0.001		
	N3D2	250.1	98.1	13	0.857	< 0.001		
Oilseed rape	N1D2	65.1	126.0	5	0.104	0.313		
	N2D2	106.4	112.0	6	0.488	0.074		
	N3D2	180.8	76.5	9	0.899	< 0.001		
Density comparison:								
50 DAS								
Wheat	N3D1	156.2	60.0	9	0.859	< 0.001	NS	**
	N3D2	182.3	62.5	9	0.948	< 0.001		
Oilseed rape	N3D1	122.4	47.5	8	0.889	< 0.001		
	N3D2	136.3	51.2	6	0.958	< 0.001		
69 DAS								
Wheat	N3D1	219.8	79.3	12	0.963	< 0.001	NS	**
	N3D2	250.1	98.1	13	0.857	< 0.001		
Oilseed rape	N3D1	57.2	125.5	8	0.764	0.003		
	N3D2	180.8	76.5	9	0.899	< 0.001		

Fig. 8. Leaf N as a function of LAI at 50 DAS in wheat (*a*) or oilseed rape (*b*). Diamonds are low N (N1D2) and circles are high N supply (N3D2). Regressions for each treatment, where *y* is leaf N and *x* is cumulative LAI, were as follows: wheat at low N, $y = 96.2-54.7x$, $R^2 = 0.93$, $P < 0.001$, $n = 8$; wheat at high N, $y = 135.3-28.2x$, $R^2 = 0.96$, $P < 0.001$, $n = 9$; oilseed rape at low N, $y = 83.3-51.6x$, $R^2 = 0.88$, $P < 0.05$, $n = 5$; oilseed rape at high N, $y = 115.2-30.4x$, $R^2 = 0.95$, $P < 0.001$, $n = 6$.

of leaf N content. In that case, N can be allocated to the top of the canopy without apparent loss for photosynthesis in the less-illuminated leaf layers.

Options for improving canopy photosynthesis per unit N

From this study we conclude that options for improving biomass production per unit canopy N during the critical period for grain number definition are different for wheat and oilseed rape. Leaf-PNUE has been recognised as a major determinant of the capacity for biomass production per unit plant N (Garnier *et al.* 1995) and was certainly a factor behind the higher canopy-PNUE of oilseed rape. However, the increase in RUE_A of oilseed rape by the end of the critical period did not translate into higher canopy photosynthesis because of the lower amount of *PAR* absorbed due to leaf shedding and the shading by the inflorescence. Maintaining the LAI and N from the beginning to the end of the critical period would be clearly advantageous (Option 2 in Table 4). At the observed LAI, oilseed rape could equal or duplicate the photosynthesis of wheat depending on the N supply, but smaller differences between species are expected at higher LAI values.

The sowing date chosen for this study in combination with a warm summer resulted in somewhat lower LAI values than those explored in other spring-sown crops. Management practices that ensure a high LAI during flowering and minimise leaf loss during grain filling will help to overcome the frequently source-limited yields in this crop (Rood and Major 1984; Dreccer *et al.* 2000). Making N available near the time of flowering, e.g. using slow release products supplied at rosette stage, could be an option to try to increase leaf area duration. Selecting for oilseed rape genotypes that can keep leaves active during grain filling is another possibility. Promising efforts in this area, e.g. delaying leaf senes-

Table 4. Model output of canopy daytime photosynthesis (mol m^{-2} d^{-1}) for 'optional' *vs* observed canopy characteristics

In each run with an 'optional' characteristic only the one indicated in the text is changed, the others remain as observed

Option	Wheat			Oilseed rape			Ratio oilseed rape with option/wheat observed
	Observed	Changed by option	Ratio option/observed	Observed	Changed by option	Ratio option/observed	
High N at 50 DAS							
1.							
Wheat with leaf photosynthesis-N relations of oilseed rape	0.667	1.012	1.52	—	—	—	—
69 DAS							
2a.							
At high N (N3), oilseed rape keeps the LAI and total leaf N in canopy from 50 until 69 DAS	0.412	—	—	0.197	0.393	2.0	0.95
2b.							
At low N (N1), oilseed rape keeps the LAI and total leaf N in canopy from 50 until 69 DAS	0.109	—	—	0.043	0.220	5.1	2.02

cence through enhanced cytokinin synthesis targeted to senescing leaves (Jordi *et al.* 2000), are on the way in other species, though integration to the crop scale is still rudimentary. Retention of leaves would also increase canopy-PNUE, particularly if we consider that pods, the main sink for N from the end of flowering to maturity, have *ca* three times lower maximum photosynthetic capacity per unit N than leaves (Gammelvind *et al.* 1996).

In wheat, the options for increasing potential biomass production will have to rely more on breakthroughs of photosynthesis at the leaf level, which can then be translated at the canopy level (e.g. Option 1 in Table 4), or improving the radiation distribution within the canopy (Slafer *et al.* 1999). One often mentioned alternative to improve leaf photosynthesis is to reduce respiration rates. Practical experiences in *Lolium* have had a variable degree of success (Wilson 1982 *vs* Kraus *et al.* 1993). Photorespiration and the glycolate pathway also represent a drain of assimilated CO_2 and energy. However, they also perform protective functions, decreasing the energy burden on photosystem II, sensitive to photoinhibition, and consuming reductant compounds, which may be important when photosynthesis is severely restricted (Lawlor 1995). Thus, selecting against these processes may lead to unstable yields if crops are grown at high light intensities, under severe water or N limitation, or highly fluctuating environments.

Higher stomatal conductance seems to be another distinctive feature associated with higher yields in cultivars released by CIMMYT from 1962 to 1988 (Fischer *et al.* 1998). This characteristic did not translate into different growth rates, and its advantage has yet to be tested in a wider range of environments, particularly under water stress. Lawlor (1995) questions that increased photosynthesis will come from altering the potential for electron transport, on the grounds that it is already a largely flexible process, although this could have an enormous impact in environments with intermediate light levels (Watanabe *et al.* 1994). From our results we conclude that the photosynthetic efficiency may be the most promising target for improvement. However, there is evidence that in the last decades the opposite occurred. Watanabe *et al.* (1994) found lower electron transport capacities per unit leaf N, and thus lower photosynthetic efficiencies in modern *vs* old Australian wheat cultivars. Finally, the ultimate goal is that any increase in leaf photosynthesis is converted into biomass and yield. This can only be attained when the development and maintenance of the sink capacity keeps pace with a higher photosynthetic rate. This will in turn ensure that photosynthesis is not down-regulated and N use efficiency can be raised.

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