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Optimal Nitrogen Content and Photosynthesis in Cauliflower (*Brassica oleracea* L. *botrytis*). Scaling up from a Leaf to the Whole Plant

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A simple model of photosynthesis is described which is dependent on leaf area, organic nitrogen content and distribution within the canopy as well as on the light and temperature environments. The model is parameterized using a cauliflower crop as an example. The optimized protein-N profile within the canopy is calculated with respect to daily growth rate. By comparison with measured protein-N contents, the amount of super-optimal N-uptake, i.e. the N-uptake which does not increase productivity, is assessed for two different nitrogen and light treatments. The amount of super-optimal N accumulated in cauliflower depends on N-supply and can exceed 80 kg N ha^{-1}.

Key words: *Brassica oleracea* L. *botrytis*, cauliflower, nitrogen, photosynthesis, respiration, model, optimization.

INTRODUCTION

Since more than 50% of leaf-N is in components associated with photosynthesis (Evans, 1989), close relationships have been found for different species between photosynthetic capacity and leaf-N content, expressed either in terms of leaf area or leaf dry matter (Field and Mooney, 1986; Reich *et al*., 1994). The experimental results have in common a minimal N-content, n_s, below which gross assimilation does not occur. Following Caloin and Yu (1984), this photosynthetically-inactive part can be regarded as a component of leaf structure (structural N-pool). With large N-supply the content of N in leaves increases, but the rate of photosynthesis often does not, thus there is excess N which is of no benefit for carbon accumulation, either by the individual leaf or for the whole plant or crop. Assessment of the excess N in crops has been difficult because the information available for single leaves does not necessarily apply to a complete crop canopy. The N-content of leaves of dense crops with almost homogeneously distributed leaf area follows the decrease in radiation within the canopy (Lemaire *et al*., 1991; Anten *et al*., 1995). The adaptation of the N-profile increases whole-plant assimilation capacity compared to a homogeneous N-distribution within the canopy (Sinclair and Shiraiwa, 1993; Leuning *et al*., 1995), by 5–8% in *Cucumis sativus*, *Phaseolus vulgaris* (Evans, 1989) and *Medicago sativa* (Evans, 1993), and between 23–48% in *Solidago altissima* (Hirose and Werger, 1987). Therefore, there is a need to assess the effects of N-availability on the dry matter accumulation of crops and derive methods for optimizing the two, and to assess the accumulation of excess N in crops. This is best achieved by using simulation modelling methods which integrate over the range of scales from leaf to crop, and over time.

Not only is carbon assimilation affected by N-content, but respiratory losses of the crops are also affected. Theoretically, positive effects of higher leaf-N contents on productivity are accompanied by increased respiration (Penning de Vries *et al*., 1974; Penning de Vries, 1975) which offsets the gains from enhanced photosynthesis. The dependency of maintenance respiration on protein-N content is mainly attributed to protein turnover and is linear for *Chenopodium album*, *Amaranthus retroflexus* (Byrd *et al*., 1992) and *Lolium perenne* (Jones *et al*., 1978). Specific growth respiration is a major contributor to total CO_2 efflux, and has been proposed, empirically and theoretically, to be proportional to the organ's relative growth rate (McCree, 1970; Thornley, 1970). This has been confirmed in many species, e.g. herbaceous monocotyledonous plants (Van der Werf *et al*., 1994) and Mediterranean shrubs (Merino *et al*., 1982). Therefore, the effect of N on respiration has to be included in models of the relation between N and production.

The impact of canopy N-distribution on photosynthesis is evaluated here, to quantify the minimal nitrogen content of a plant required for maximal CO_2 uptake. Based on this knowledge it can be determined if plants restrict their uptake under non-limiting N-supply or show super-optimal consumption. This study presents a model of photosynthesis and respiration to predict crop CO_2 uptake from leaf area, leaf N-content and distribution, as well as light and temperature environments. The model is parameterized and validated using cauliflower as an example crop. In the second part of this study, measured leaf N-contents and distribution within the canopy are compared to the optimized values with respect to daily growth rate to assess super-optimal N-accumulation by the plant.

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MODEL

Single-leaf and canopy photosynthesis

The photosynthesis-light response of a single leaf is described by a non-rectangular hyperbola:

$$P_g = \frac{\alpha I + P_m - \sqrt{(\alpha I + P_m)^2 - 4\Theta\alpha I P_m}}{2\Theta} \quad (1)$$

where P_g is the rate of gross photosynthesis on a leaf area basis, and α and Θ are two parameters describing the initial slope and curvature of the response curve, respectively (for a full list of abbreviations, see Appendix). I is the incident photosynthetically active photon flux, and P_m denotes the gross photosynthetic capacity which is a linear function of the protein-N content on leaf area basis, n_a (Anten *et al.*, 1995):

$$P_m = m \times n_a + b \quad (2)$$

The respiration rate of a single leaf, R, has contributions from both maintenance and growth respiration, which are assumed to be proportional to leaf protein-N content and relative leaf growth rate, rgr, respectively:

$$R = m_m \times n_a + m_g \times \mathrm{rgr} \quad (3)$$

with m_m and m_g denoting the proportionality coefficients of maintenance and growth respiration, respectively. It is further assumed that the specific leaf area does not vary significantly during the time period considered.

From single-leaf respiration, instantaneous canopy respiration, R_{leaf}, can be calculated as the integral over total leaf area, A_l, summing up the contributions of every leaf layer, l:

$$\begin{aligned} R_{leaf} &= \int_0^{A_l} [m_m n_a(l) + m_g \mathrm{rgr}(l)]\,\mathrm{d}l \\ &= m_m N_{P,leaf} + m_g \frac{\mathrm{d}A_l}{\mathrm{d}t} \end{aligned} \quad (4)$$

where $N_{P,leaf}$ is the total amount of leaf protein-nitrogen, and $\mathrm{d}A_a/\mathrm{d}t$ is the increase in leaf area per unit canopy. Similar expressions for canopy respiration were proposed by McCree (1970) and Thornley (1970), except that they associated maintenance with standing dry matter rather than with the amount of nitrogen. In the case of a constant nitrogen concentration both expressions can be transformed into each other.

CO_2 efflux from stem, R_{stem}, and root, R_{root}, are treated similarly to canopy respiration. Maintenance is proportional to the amount of protein-N in the compartment, $N_{p,stem}$, with the same coefficient m_m as in eqn (4). Stem growth respiration is calculated as a fraction of canopy growth respiration. This share is determined by the fraction of the compartment in leaf dry matter:

$$R_{stem} = m_m N_{P,stem} + m_g \frac{\mathrm{d}A_l}{\mathrm{d}t} \frac{W_{stem}}{W_{leaf}} \quad (5)$$

where W_{stem} and W_{leaf} denote the dry weight of the different organs. R_{root} is calculated analogously, using the fraction W_{root}/W_{leaf} determined by the final harvest.

During the exponential growth phase of the inflorescence, i.e. $\mathrm{d}W_{in}/\mathrm{d}t = \mathrm{rgr}_{in} W_{in}$, with relative growth rate, rgr_{in}, total inflorescence respiration, R_{in}, can be simplified to be proportional to its protein-N content, with m_{in} describing the rate of CO_2 production per unit protein-N (Alt, 1999):

$$\begin{aligned} R_{in} &= m_m N_{P,in} + m_{l,in} \frac{\mathrm{d}W_{in}}{\mathrm{d}t} \\ &= \left(m_m + m_{l,in} \frac{\mathrm{rgr}_{in}}{n_{in}}\right) N_{P,in} = m_{in} N_{P,in} \end{aligned} \quad (6)$$

where $m_{l,in}$ denotes the CO_2 production per unit increase in dry weight, and n_{in} is the protein-nitrogen concentration.

The whole-plant respiration per unit time is given by R_{leaf}, R_{stem}, R_{root} and R_{in}:

$$R_{plant} = R_{leaf} + R_{stem} + R_{root} + R_{in} \quad (7)$$

The instantaneous rate of whole-plant net CO_2 uptake for a certain light environment is the integral of single-leaf photosynthesis [eqn (1)] over the whole leaf area, A_l, with total plant respiration subtracted:

$$P_{plant} = \int_0^{A_l} \mathrm{d}l P_g(I, P_m) - R_{plant} \quad (8)$$

The single-leaf gross photosynthetic capacity, P_m, varies within the canopy. It is calculated for the different leaf layers by eqn (2) from the measured nitrogen profile.

Environmental influences

Since the radiation environment of the greenhouse experiments was predominantly diffusive, the distinction between direct and diffuse radiation was neglected for simplicity. Thus, the PPF upon a leaf layer, $I(l_c)$, with cumulative leaf area index l_c and light extinction coefficient k is related to the radiation above the canopy, I_0, by:

$$I(l_c) = kI_0 e^{-kl_c} \quad (9)$$

with light extinction coefficient for diffuse radiation and spherical leaf-angle distribution approximated by $k = 0{\cdot}72$ (Goudriaan, 1977).

The dependence of maintenance respiration and light-saturated photosynthetic capacity, P_m on air temperature is incorporated. Maintenance respiration resulting from all compartments at temperature T is corrected for by the factor $2^{(T-20)/10}$, i.e. using a Q_{10}-value of 2 (Penning de Vries *et al.*, 1989) and a reference temperature of 20°C. The temperature dependence of P_m is described by an asymptotic function for many species (Penning de Vries *et al.*, 1989; Walcroft *et al.*, 1997). Independent measurements showed no reduction in P_m of cauliflower leaves between 15 and 25°C. For every degree Celsius below 15°C or above 25°C, a

reduction in P_m of 1/15 is assumed. Thus, P_m was corrected for temperature by the factor f_T, given by:

$$f_T = \begin{cases} 0 & T \leqslant 0 \\ \dfrac{T}{15} & 0 < T \leqslant 15 \\ 1 & 15 < T \leqslant 25 \\ 1 - \dfrac{T-25}{15} & 25 < T \leqslant 40 \\ 0 & T > 40 \end{cases} \quad (10)$$

MATERIALS AND METHODS

Plant culture

In 1997 and 1998, cauliflower (*Brassica oleracea* L. *botrytis botrytis* L. 'Fremont') was grown in a glasshouse in Kick-Brauckmann (Kick and Große-Brauckmann, 1961) pots with approx. 8 dm^3 volume, filled with sand (particle size up to 2 mm). Seeds were germinated in planting plates filled with peat and transplanted, by hand, after about 7–9 d into cubes of rock wool (Grodan, Grodania A/S) with 4 cm edge length. Plants with three visible leaves were moved to the glasshouse (Table 1) with a minimum temperature of 14°C during the day and 10°C during the night.

Treatments

Upon transplanting, two different light environments and N-fertilizer applications were imposed in 1997 (Table 1). Supplementary light was used in half of the glasshouse and a shading net which absorbed 14% of the photosynthetically active radiation (PAR) in the other. The supplementary light consisted of sodium high pressure lamps (SON-T 400, Philips) arranged to ensure a uniform light distribution of 200 µmol PAR m^{-2} s^{-1} at pot height, and operating from 1 h after sunrise until 1 h before sunset. The average totals of daily PAR during the cultivation period were 2·6 and 4·6 MJ m^{-2} d^{-1} in the low-light and high-light treatments, respectively. Assuming a period of 12 h of daily sunlight, these light environments correspond to an average of 275 and 490 µmol m^{-2} s^{-1} during daytime. Plants were irrigated five–20 times each day, depending on accumulated radiation and plant size. Each pot was irrigated with about 300 cm^3 of nutrient solution on each occasion. The low N nutrient solution consisted of 0·7 kg m^{-3} N-free basic fertilizer (Flory Basisdünger 1, Euflor GmbH, Germany), 0·3 kg m^{-3} $Ca(Cl)_2$ and 0·3 kg m^{-3} $Ca(NO_3)_2$ in 1997. The high N-solution was the same as the low N-composition plus 0·3 kg m^{-3} $NH_4(NO_3)$. In 1998, the N-concentrations of the nutrient solutions were reduced [low N-treatment: 0·7 kg m^{-3} N-free basic fertilizer, 0·5 kg m^{-3} $Ca(Cl)_2$ and 0·1 kg m^{-3} $Ca(NO_3)_2$; high N-treatment: 0·7 kg m^{-3} N-free basic fertilizer, 0·3 kg m^{-3} $Ca(Cl)_2$ and 0·4 kg m^{-3} $Ca(NO_3)_2$] and the irrigation frequency was increased. Depending on plant size about 300 cm^3 of nutrient solution were given on each occasion two–five times per hour during daytime to ensure uniform nitrogen concentration in the rooting zone. In both years, total N-supply varied between 9 to 30 g N per plant for the low and high nitrogen treatments, respectively, and exceeded the amount of nitrogen taken up by the plant by 50 to 300%, depending on treatment.

Physiological measurements in 1997

The experiments in 1997 served for the parameterization of the photosynthesis model at the single-leaf level and for evaluation of the whole plant. The respiration model was parameterized for single leaves with data from the glasshouse experiment in 1998 and evaluated on whole plants with data from both glasshouse experiments.

At five intermediate harvests in 1997, approx. every 2 weeks, the rate of CO_2 uptake by single leaves and whole plants was determined. Single-leaf measurements were made using a mini-cuvette with 2·5 cm^2 measuring area (Ciras-1, PP Systems, UK) on leaves 3, 5, 8, 11 and 14. All leaves were exposed to a range of PPF, from 0–2100 µmol m^{-2} s^{-1}. After measurements had been completed, leaf sections were collected and their total nitrogen and nitrate-nitrogen contents determined by the micro-Kjeldahl method and a nitrate-sensitive electrode, respectively. For whole-plant measurements, pots were transferred to a cuvette with a chamber volume of 1 m^3 (Krug *et al.*, 1977) around the middle of the day. CO_2 uptake rates were recorded for various PPF up to 1500 µmol m^{-2} s^{-1}. All gas-exchange measurements were carried out at the ambient CO_2 concentration (360 ± 20 µmol mol^{-1}), relative humidity between 70–90% and air temperature between 14–24°C. Two to four plants per treatment and harvest were chosen for gas-exchange analysis depending on plant size. Following CO_2 exchange measurements, four plants per treatment and harvest were separated into stem, leaves including petioles, and inflorescence. At final harvest root dry matter was also determined by cutting the soil into quarters vertically and washing the soil from the roots. The stems were cut 1 cm below the surface of the sand and at the onset of inflorescence. The foliage was subdivided into groups of three consecutive leaves. Leaf area of each group was determined with a Licor 3100 leaf area meter (LI-COR Inc., Lincoln,

TABLE 1. *Planting dates and treatments of the greenhouse experiments*

Year	Sowing date	Transplanting date	Row spacing (m × m)	Nitrogen levels (mol N m^{-3})	Light environment
1997	28 Jan.	28 Feb.	0·60 × 0·50	2·5, 10·4	Shading net, suppl. light
1998	11 Feb.	12 Mar.	0·60 × 0·55	0·9, 3·2	No variation

NE, USA). Plant parts were then oven dried to constant weight, and dry matter determined. Dry matter samples were analysed for total and nitrate-N as described previously.

Physiological measurements in 1998

In 1998, gas-exchange was measured during the last 6 weeks before final harvest. Efflux of CO_2 from single leaves was measured in darkness twice a week on leaves 3, 5, 8, 11, and 13, depending on plant size, of plants of both treatments. In order to calculate the relative growth rates, length and width of the same leaves were determined on three consecutive days with gas exchange measurements in between. After measurements were completed the leaves were collected and leaf area, total nitrogen and nitrate-N were determined as above. Once a week, plants of both treatments were transferred to the whole-plant cuvette and total plant respiration was measured under darkness in the evening and next morning. Following the respiration measurements, plants were separated into organs for dry matter determination and analysis of total and nitrate-N (see above). On four intermediate harvests three plants per treatment and replication were collected and separated into organs as described above. Their dry mass and nitrogen contents were analysed.

Field experiment 1997

In order to include typical growth data, single-leaf photosynthesis was measured on plants in a field experiment in 1997. The same cauliflower cultivar 'Fremont', was grown on the institute's experimental farm 15 km south of Hannover, Germany. Different nitrogen fertilization was given as ammonium nitrate at the time of transplanting to obtain 0, 150, 300 and 450 kg N ha^{-1}, allowing for soil nitrate content of 15 kg N ha^{-1} between 0–60 cm. At three intermediate harvests, single measurements were made on leaves 5, 8 and 11 of randomly chosen plants from different N-treatments under various PPF up to 2100 µmol m^{-2} s^{-1}.

Calculation of optimal nitrogen distribution

In order to calculate the optimal nitrogen distribution with respect of daily net CO_2-gain, i.e. to determine the N-profile which maximizes the daily integral of P_{plant} [eqn (8)], the model described above was programmed in Turbo Pascal, implementing the downhill simplex optimization method (Press *et al.*, 1986). Integration over time was by the Euler-algorithm with a time-step of 1 h. The optimal N-contents of different leaf layers were calculated for every treatment and harvest in 1997. Input parameters were the measured leaf area and daily increase in leaf area [eqn (4)] determined by interpolating the measured data using a logistic growth function. Variation in PPF above the canopy, I_0, with time, t, was assumed to follow a sine-squared function:

$$I_0(t) = I_{noon} \sin^2\left(\pi \frac{t}{d}\right), \qquad (11)$$

where d denotes the daylight period and I_{noon} is the maximum PPF at solar noon calculated from the daily total, I_{tot}, by $I_{noon} = 2I_{tot}d^{-1}$. I_{noon} was averaged over a period of 2 weeks before each harvest and ranged from 500 to 800 µmol m^{-1} s^{-1}, and from 700 to 1100 µmol m^{-1} s^{-1} for the low and high light treatments, respectively, in 1997. The assumed temperature regime was set to 18°C during the daylight period and 14°C during night. The average daylight period varied for the different harvests according to the season from 12 to 14 h.

Statistical analysis

All statistical analyses were carried out using the procedures NLIN and REG of the SAS software package (SAS Institute, 1988). Significance was calculated with an error probability $<0{\cdot}05$.

RESULTS

Parameterization of the single-leaf photosynthesis model

Separate estimates of the parameters α and Θ of the non-rectangular hyperbola [eqn (1)] for every leaf showed no statistically significant relationship with light-saturated gross photosynthesis, P_m (data not shown). The parameter estimates $\alpha = 0{\cdot}056 \pm 0{\cdot}002$ µmol CO_2 μmol^{-1} PAR and $\Theta = 0{\cdot}899 \pm 0{\cdot}012$ resulted in a good description of the measured data of all leaves under different light intensities up to 2100 µmol PAR m^{-2} s^{-1} (Table 2).

P_m was determined as the sum of maximum net photosynthesis and respiration measured for each leaf and varied from 2 to 50 µmol PAR m^{-2} s^{-1} within the canopy. Seventy-five percent of the observed variation in P_m could be attributed to differences in leaf protein-N content, n_a (Fig. 1). If P_m was related to total leaf N-content including nitrate, the variability increased ($r^2 = 0{\cdot}70$). The structural

TABLE 2. *Results of the regression analysis*, slope and* r^2, *between measured and calculated single-leaf gross photosynthesis,* P_g, *whole-plant photosynthesis,* P_{plant}, *and whole-plant respiration,* R_{plant}

Symbol	Equation	Experiment	Unit	Slope (±s.e.)	r^2	n
P_g	1	1997	µmol CO_2 m^{-2} s^{-1}	1·01 (±0·01)	0·97	275
P_{plant}	8	1997	µmol CO_2 s^{-1}	0·99 (±0·02)	0·95	55
R_{plant}	7	1997, 1998	µmol CO_2 s^{-1}	0·94 (±0·03)	0·87	18

* Intercepts were, in all cases, not significantly different from zero ($\alpha = 0{\cdot}05$).
Number of observations (n).

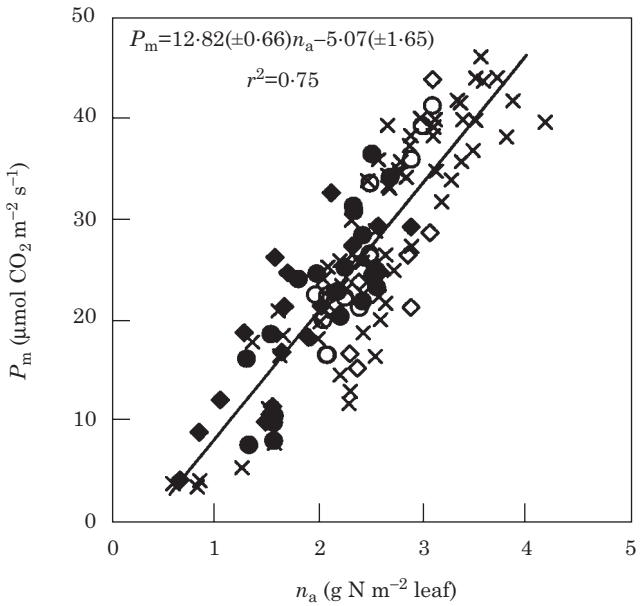


FIG. 1. Relationship between gross photosynthetic capacity, P_m, and protein-N content per unit leaf area, n_a [eqn (2)]; glasshouse experiment 1997, field experiment 1997; (○, 145 g N l^{-1}, light; ●, 35 g N l^{-1}, light; ◇, 145 g N l^{-1}, net; ◆, 35 g N l^{-1}, net; x, field).

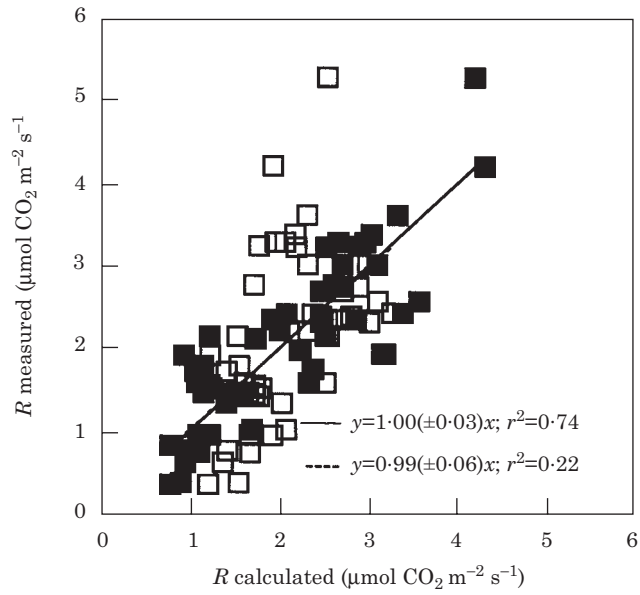


FIG. 2. Relation between measured and calculated single-leaf respiration, R, based on protein-N content per unit leaf area, n_a, and relative growth rate, rgr, [eqn (3)]: $R = 0{\cdot}68(\pm 0{\cdot}05)n_a + 1{\cdot}31(\pm 0{\cdot}17)$rgr, $r^2 = 0{\cdot}74$, (closed symbols, solid regression line); neglecting rgr shows the importance of growth respiration, $R = 0{\cdot}99(\pm 0{\cdot}06)n_a$, $r^2 = 0{\cdot}22$, (open symbols, almost identical regression line); experiment 1998.

protein-N pool, n_s, defined as $P_m(n_s) = 0$, was 0·40 g N m^{-2} calculated from the linear regression equation.

The respiration rate of single leaves was well described by eqn (3) with growth respiration included (Fig. 2). The respiration rate of the inflorescence, measured immediately after cutting, was linearly related to the protein-N content of the inflorescence. The proportionality coefficient between R_{in} and $N_{P,in}$ [eqn (6)] was $m_{in} = 1{\cdot}61 \pm 0{\cdot}10$ mg CO_2 min^{-1} g N^{-1}, $r^2 = 0{\cdot}75$, $n = 9$.

Independent measurements showed a linear decrease in whole-plant respiration with time, t_n, during the first 10 to 16 h of darkness (data not shown):

$$R_{plant,a}(t_n) = R_{plant,a}(0) - m_R t_n \qquad (12)$$

where m_R denotes the rate of decrease in respiration per unit leaf area and $R_{plant,a}(0)$ is the initial whole-plant respiration per unit leaf area at the beginning of the night. In 1997 and 1998 the rate constant, m_R, depended on $R_{plant,a}(0)$ (Fig. 3).

Evaluation of the single-leaf photosynthesis model

There was good agreement between canopy photosynthesis measured with the whole-plant cuvette and the predicted values based on the single-leaf model described above (Table 2). The model was evaluated with plants at various growth stages, differing in leaf area, leaf growth rate, total and inflorescence dry mass as well as under varying PPF up to 1500 μmol m^{-2} s^{-1}. The measurements of instantaneous whole-plant respiration were evaluated separately with plants from all growth stages (Table 2). In both years the CO_2 efflux of randomly chosen plants differing in leaf area, leaf growth rate, nitrogen content and inflorescence dry weight was determined under darkness during the day.

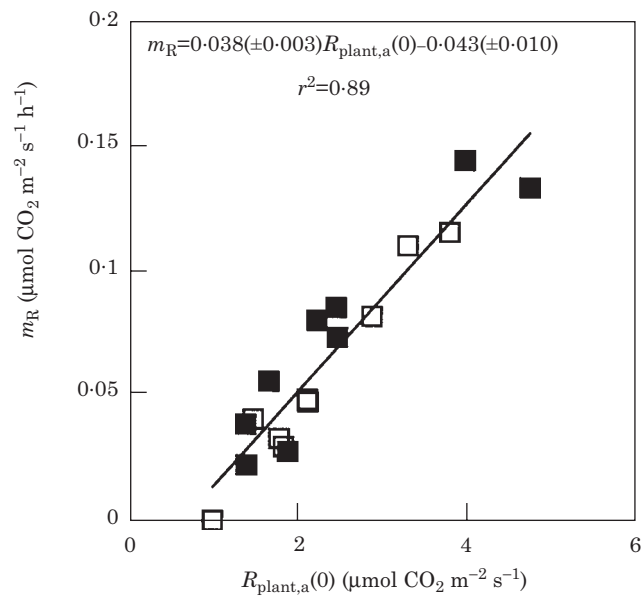
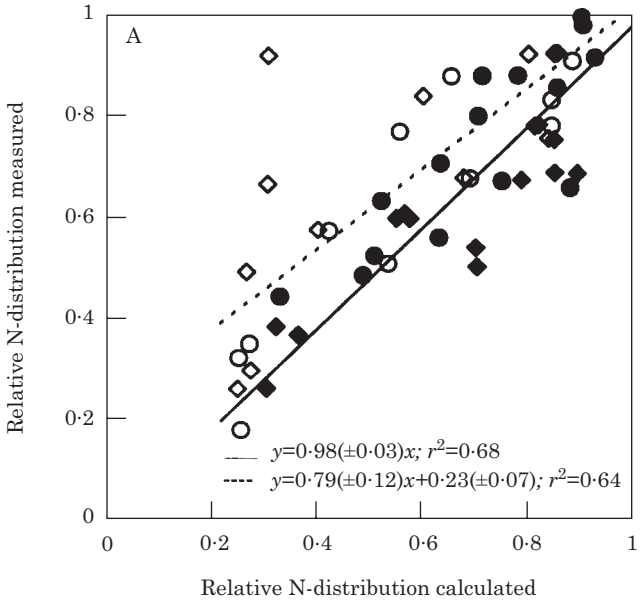


FIG. 3. Dependence of the rate constant m_R [eqn (12)], denoting the decrease in whole-plant respiration per hour of darkness, on whole-plant respiration during daytime, $R_{plant,a}(0)$; glasshouse experiments 1997 (□) and 1998 (■).

Calculating the optimum nitrogen contents

Comparison between measured and calculated protein-N distributions within the canopy shows that the low and high N-treatments behaved differently. Whereas the high N-treatments are well above the 1:1 line, the low N-treatments lie closer to the calculated optima when expressed relative to the top of the canopy (Fig. 4A) or



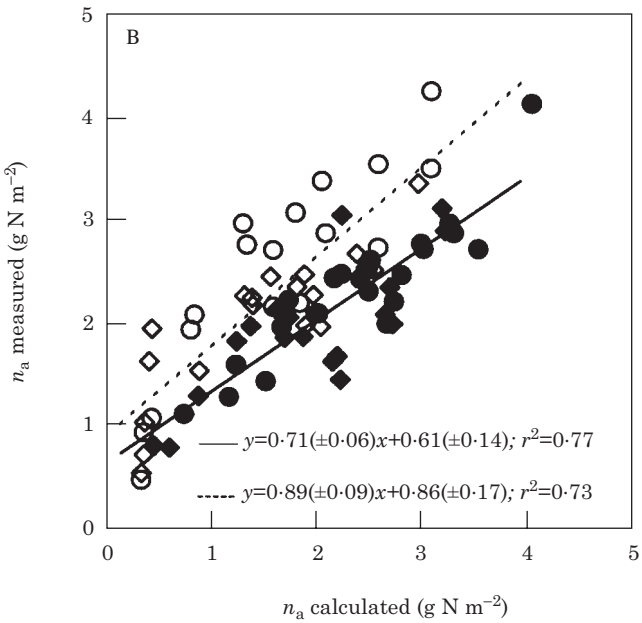
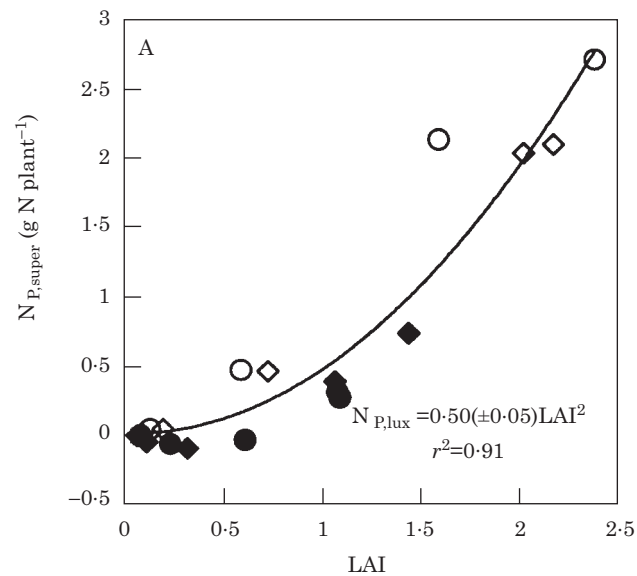


FIG. 4. Measured *vs.* calculated protein-N contents per unit leaf area, n_a, of leaf layers in different positions in the canopy, relative to the value at the top of the canopy (A) and in absolute terms (B), for all treatments and harvests; glasshouse experiment 1997; symbols as in Fig. 1; dashed and closed lines denote regression lines for open and closed symbols, respectively.



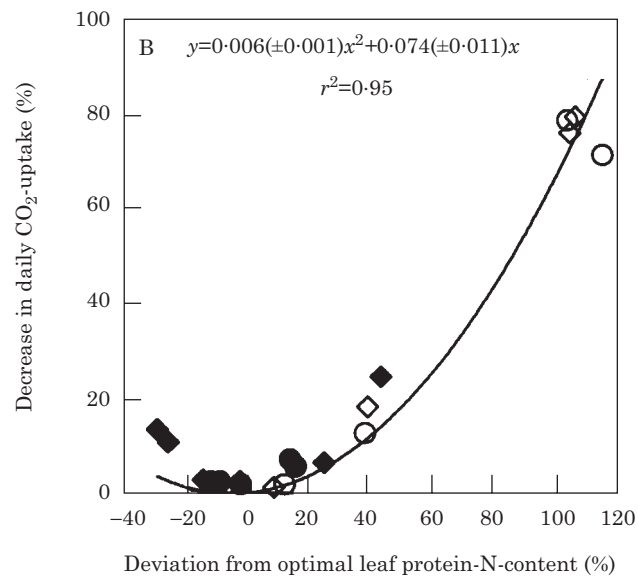


FIG. 5. Amount of protein-N in the canopy above the predicted optimum, i.e. super-optimal N-uptake, $N_{P,super}$, *vs.* leaf area index, LAI (A). Decrease in daily net CO_2-uptake relative to the optimized value *vs.* relative deviation from the optimized canopy protein-N content (B); glasshouse experiment 1997; symbols as in Fig. 1.

slightly below the predicted values for the upper leaf layers when expressed in absolute terms (Fig. 4B).

During growth the total amount of nitrogen taken up in excess of the calculated optimum was more than 2·5 g N per plant (Fig. 5A). As before, both light treatments at the same N-supply behaved similarly. The super-optimal N-uptake of the high N-treatments was accumulated almost from the time of transplanting. For the low N-treatments, with a LAI between 0·5 and 1, N-uptake did not follow demand. At the end of the growth phase the plant accumulated less nitrogen than the high N-treatments. The influence of N-uptake on the assimilation capacity of a plant was the same for all treatments and depended on the deviation from the calculated N-content (Fig. 5B). A relative deviation from the calculated canopy N-content of about 10% had little consequence for CO_2-assimilation capacity.

DISCUSSION

Regulation of N-accumulation and distribution in the leaves of cauliflower plants was tested, to see if plants

control their N-accumulation and distribution such that they maximize net photosynthesis under the conditions given. A simple model was developed to predict the amount and distribution of nitrogen necessary for potential CO_2-fixation. First, the instantaneous plant CO_2-assimilation, and its dependence on leaf area, protein-N content and distribution in leaves as well as on light and temperature, were quantified. Then measured leaf protein-N content and distribution within the canopy were compared to the calculated optimal values with respect to daily growth rate, to assess the plant's potential for super-optimal N-consumption with respect to CO_2 fixation. Maximizing daily net photosynthesis does not necessarily imply potential plant growth, e.g. the amount of super-optimal N-uptake may maximize leaf expansion rate which in turn positively influences plant growth.

The non-rectangular hyperbola [eqn (1)] fitted the light response data of photosynthesis for all treatments well. The estimated initial slope, $\alpha = 0{\cdot}056 \pm 0{\cdot}002$ μmol CO_2 μmol^{-1} PAR, and the curvature factor, $\Theta = 0{\cdot}899 \pm 0{\cdot}012$, are similar to the values for $\alpha = 0{\cdot}052$ μmol CO_2 μmol^{-1} PAR and Θ in the range of 0·7 to 0·9 cited by Boote and Loomis (1991) for C_3 species. An almost constant value of $\alpha = 0{\cdot}04$ μmol CO_2 μmol^{-1} PAR and the same range of Θ were observed in maize (Stirling *et al*., 1994). The single-leaf photosynthesis model agreed well with measured data from plants of all growth stages differing in leaf area, growth rate and protein-N content when scaling up to canopy photosynthesis. The linear relationship between P_m and leaf protein-N content provided an estimate of the structural protein-N pool, $n_s = 0{\cdot}40$ g N m^{-2}, within the range of 0·2 to 0·4 g N m^{-2} measured for *Oryza sativa*, *Glycine max*, *Sorghum bicolor*, *Amaranthus cruentus* and *Tetrorchidium rubrivenium* (Anten *et al*., 1995).

The specific respiration of a single leaf during daytime was linearly related to its protein-N content and its relative growth rate. Maintenance respiration, defined by the first term in eqn (3), ranges from 0·27 to 2·72 μmol CO_2 m^{-2} s^{-1} in leaves with protein-N contents between 0·4 and 4 g N m^{-2}. Converting these values from area to mass, using a specific leaf area of 0·01 m^2 g^{-1} for cauliflower, leads to 0·01 to 0·10 g CO_2 g^{-1} d.wt d^{-1}, a larger range than the 0·03 to 0·08 g g^{-1} d^{-1} cited by Penning de Vries *et al*. (1989) for field-grown leaves of different species at 20°C. If the coefficient of the growth contribution, i.e. the second term in eqn (3), is converted using a specific leaf area of 0·01 m^2 g^{-1} and corrected for the decrease in growth respiration during the night [eqn (12)] due to the exhaustion of carbohydrate stores (Azcon-Bieto and Osmond, 1983), a CO_2 efflux of 0·48 g per g leaf dry matter produced is predicted. Penning de Vries *et al*. (1989) reported a theoretical value of 0·46 g CO_2 g^{-1} for leaves of non-leguminous and non-rice crops. The same specific respiratory energy was assumed for all plant organs, although it varies with the biochemical composition of the biomass (Penning de Vries *et al*., 1989). Following Penning de Vries *et al*. (1989), the specific respiratory energy for roots is 10–15% less than that for leaves. If it is assumed that the fraction of root to total dry matter lies in the range of 0·1 to 0·3 during most of the growth period, and if growth respiration contributes about 50% to the total respiration of the plant, then the overestimation of root growth respiration is less than 3% of the total CO_2-efflux of the plant. The respiratory costs involved in ion uptake of roots were also neglected. Van der Werf *et al*. (1994) cite a value of 0·58 g C lost per g N taken up as the average for *Briza media* and *Dactylis glomerata*. Based on this value and a C/N ratio of 12, root respiration is underestimated by less than 5% of the plant's total C gain. The underestimation of root respiration due to neglecting ion uptake may offset the overestimation of root growth respiration. With the methods used, the difference in respiratory costs for various organs, the energy involved in ion uptake, and the exudation of assimilates could not be resolved.

The photosynthesis model was used to calculate the optimal N-distributions and N-contents for plants grown under different light environments and N-supply with respect to daily net CO_2 gain. In the high N-treatments, nitrogen was more evenly distributed within the canopy than predicted by the model to be optimal, whereas the low N-treatments were close to the optimized distribution. Also, the absolute N-contents of the high N-treatments were above the optimized values. It was, therefore, observed that the closer the total amount of nitrogen in the canopy is to its optimum, the closer the relative N-distribution within the canopy follows the optimized distribution. The absolute N-contents of the lower leaf layers were above the predicted values in all treatments, indicating insufficient N-translocation into upper layers. The limited rate of N-remobilization within the canopy may be due to low rates of leaf protein-recycling, or to the additional costs of N-translocation that are not taken into account by the model. Incorporating the acclimation of single-leaf N-contents to changing light environment (Hikosaka and Terashima, 1995; Thornley, 1998) may explain the dynamical N-distribution within the canopy.

Super-optimal N-accumulation in leaves, with respect to CO_2-fixation, can reach about 2·5 g N per plant, resulting in more than 80 kg N ha^{-1} with 33 000 plants ha^{-1}. This neither includes nitrate-nitrogen nor contributions from stem or curd. Light environment has no significant influence on the calculated amount of super-optimal N-consumption. During most of the growing period, the N-contents of the low N-treatments were within ±15% of the optimized values. This is about the range within which the assimilation capacity of the plant is little affected under average environmental conditions (Fig. 5B). At the end of the growing period the high N-treatments deviate by more than 100% from the calculated N-content. This may be due to the fact that either the plant has no mechanism to down-regulate N-content sufficiently, or that super-optimal N-contents are advantageous from a different perspective, e.g. leaf expansion or inter-plant competition.

In conclusion the model predicted whole-plant assimilation from single-leaf photosynthesis in cauliflower under different environmental conditions. Nitrogen uptake of cauliflower mainly depends on N-supply and can exceed the optimal amount with respect to potential growth rate. This super-optimal consumption can amount to more than 100% of the leaf-nitrogen requirement or 80 kg N ha^{-1} in

cauliflower. The closer the total amount of nitrogen in the canopy is to its calculated optimum, the closer the relative N-distribution within the canopy follows the predicted distribution.

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APPENDIX

Symbols, definitions and units

Symbols	Definition	Unit
A_l	Leaf area	m^2 per plant
d	Daylight period	h
f_T	Temperature factor of photosynthesis	–
k	Light extinction coefficient	$m^2\ m^{-2}$
LAI	Leaf area index	$m^2\ m^{-2}$
l_c	Cumulative leaf area index	$m^2\ m^{-2}$
m_g	Coefficient of growth respiration	mol $CO_2\ m^{-2}$
m_m	Coefficient of maintenance respiration	µmol $CO_2\ s^{-1}\ g^{-1}$ N
m_R	Rate of decrease in respiration during the night per unit leaf area	µmol $CO_2\ m^{-2}\ s^{-1}\ h^{-1}$
n_a	Protein-N content per unit leaf area	g N m^{-2}
$N_{P,x}$*	Total of protein-N	g protein-N per plant
n_s	Structural leaf N-content per unit leaf area	g N m^{-2}
P_g	Rate of gross photosynthesis	µmol $CO_2\ m^{-2}\ s^{-1}$
P_m	Rate of gross photosynthetic capacity	µmol $CO_2\ m^{-2}\ s^{-1}$
P_{net}	Rate of single-leaf net photosynthesis	µmol $CO_2\ m^{-2}\ s^{-1}$
P_{plant}	Rate of whole-plant net CO_2-uptake	µmol CO_2 $plant^{-1}\ s^{-1}$
R	Rate of single-leaf respiration	µmol $CO_2\ m^{-2}\ s^{-1}$
rgr	Relative growth rate	d^{-1}
rgr_{in}	Relative growth rate of inflorescence	d^{-1}
R_{plant}	Rate of whole-plant respiration	µmol CO_2 per plant s^{-1}
$R_{plant,a}$	Rate of whole-plant respiration per unit leaf area	µmol $CO_2\ m^{-2}\ s^{-1}$
R_x*	Respiration rate	µmol CO_2 per plant s^{-1}
T	Temperature	°C
t	Time	h
t_n	Duration in darkness	h
W_x*	Dry weight	g per plant
I	Photosynthetically active photon flux (PPF)	µmol $m^{-2}\ s^{-1}$
I_0	PPF above the canopy	µmol $m^{-2}\ s^{-1}$
I_{noon}	Maximum PPF at solar noon	µmol $m^{-2}\ s^{-1}$
I_{tot}	Daily total of PPF	µmol m^{-2}
α	Initial slope of the light-response curve	µmol CO_2 μmol^{-1} PPF
θ	Curvature factor of the light-response curve	–

* index x: in, inflorescence; leaf, leaf; root, root; stem, stem.