

Elevated CO₂ and nitrogen availability have interactive effects on canopy carbon gain in rice

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Summary

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Received: 14 July 2003

Accepted: 7 October 2003

doi: 10.1046/j.1469-8137.2003.00943.x

- Here we analysed the effects of CO₂ (C_a) elevation and nitrogen availability on canopy structure, leaf area index (LAI) and canopy photosynthesis of rice (*Oryza sativa*).
- Rice was grown at ambient and elevated C_a (c. 200 μmol mol⁻¹ above ambient, using the free-air CO₂ enrichment, FACE) and at two N availabilities. We measured leaf area, area-based leaf N contents and leaf photosynthesis, and calculated net daily canopy photosynthesis.
- FACE plants had higher light-saturated rates of photosynthesis (P_{max}) and apparent quantum yields than ambient plants, when measured at their own growth CO₂. C_a elevation reduced the total leaf N in the canopy (N_{leaf}) but had no effect on LAI, and the average leaf N content (N_{leaf}/LAI) was therefore reduced by 8%. This reduction corresponded well with our model predictions. Leaf area index increased strongly with N availability, which was also consistent with our model.
- Calculated canopy photosynthesis increased more strongly with N_{leaf} under elevated than under ambient C_a. This indicates that there is an N × C_a interactive effect on canopy carbon gain. This interaction was caused by the increase in LAI with N availability, which enhanced the positive effect of the higher quantum yield under C_a elevation.

Key words: canopy structure, free-air CO₂ enrichment (FACE), global change, leaf area index, model, nitrogen, photosynthesis, rice (*Oryza sativa*).

© *New Phytologist* (2003) **161**: 459–471

Introduction

The stimulation of plant growth by elevated atmospheric CO₂ concentrations (C_a) is generally found to increase with increasing nitrogen availability from the soil (Ziska *et al.*, 1996; Poorter, 1998; Zak *et al.*, 2000; Kim *et al.*, 2001; and see Stitt & Krapp, 1999), although some exceptions exist (Wong & Osmond, 1991; Aben *et al.*, 1999). A further knowledge of this possible interactive effect of C_a and N will be important for the assessment of stands of vegetation as potential carbon sinks, because natural soil fertility varies widely between different sites. It will also be important for crop production because it implies that maximization of yields under elevated C_a requires changes in the application of N fertilizers.

Photosynthesis provides the structural substrates for growth, and effects of C_a and N availability on growth are therefore largely mediated through their effects on whole-plant canopy net photosynthesis. Short-term exposure to elevated C_a can considerably enhance leaf photosynthesis per unit area as well as per unit N (leaf PNUE). Yet this initial stimulation of leaf photosynthesis is often reduced under long-term exposure to elevated C_a. This is believed to be because carbohydrates tend to be accumulated in leaves, which probably leads directly to a reduction in the activity of photosynthetic enzymes, and consequently lower rates of leaf photosynthesis (Stitt, 1991; Rogers *et al.*, 1996; Stitt & Krapp, 1999). This negative feedback appears to become more severe with decreasing N availability and this, in turn, has been suggested as one of the

mechanisms that could explain the interactive effect of C_a and N on growth (Rogers *et al.*, 1996).

However, to analyse adequately the effects of C_a elevation and N availability in whole-plant photosynthesis, it is essential to scale from leaf to canopy. Changes in whole-canopy carbon gain are strongly related to changes in the leaf area index (LAI) of a stand (Lambers *et al.*, 1990; Anten *et al.*, 1995b; Hirose *et al.*, 1996). But our knowledge of the effects of elevated C_a on canopy development and LAI, and how this may interact with N availability, is limited (DeLucia *et al.*, 2002). In principle, it might be expected that LAI will increase under elevated C_a because the quantum yield of leaf photosynthesis is increased, which means leaves have lower light compensation points and more leaves can therefore be maintained at lower light levels in the canopy (Saeki, 1960; Long, 1991). Yet previous studies have yielded contradictory results: in some cases LAI increased with C_a elevation (Nijs *et al.*, 1988; Hättenschwiler *et al.*, 1997; Luo *et al.*, 2001), but in other cases it remained unchanged (Körner & Arnone, 1992; Norby *et al.*, 2001; DeLucia *et al.*, 2002) or even decreased (Lutz & Gifford, 1998). A number of studies found that C_a elevation stimulated leaf area growth early in the season, but that this initial advantage was gradually lost and maximum LAI did not differ between CO_2 treatments (Harz-Rubin & DeLucia, 2001; Kim *et al.*, 2001; Kobayashi *et al.*, 2001; DeLucia *et al.*, 2002).

The effects of CO_2 on LAI are probably modified by N availability, which strongly controls leaf area growth (Lambers *et al.*, 1990; Anten *et al.*, 1995b). In a study with two species of annuals, Hirose *et al.* (1996) found a strong correlation between LAI and N uptake, and found that the C_a level did not significantly alter this relationship. They consequently argued that LAI would increase under elevated C_a only if N uptake from the soil is simultaneously increased. However, Harz-Rubin & DeLucia (2001) found that vegetation stands under elevated C_a had greater LAIs even when compared at the same N uptake. Kim *et al.* (2001) also found LAI for a given N uptake to be greater for plants under elevated C_a , but only when N uptake itself was high, and not when it was low.

Apparently the data regarding the interactive effects of C_a and N availability on canopy development, LAI and canopy photosynthesis are, as yet, inconclusive. To further our understanding in this respect, we have applied the model for optimal LAI developed by Anten *et al.* (1995b) and Goudriaan (1995). At a given total amount of leaf N in the canopy (N_{leaf}), an increase in leaf area implies that more light is intercepted, but that consequently N contents per unit leaf area (n_{area}) and associated leaf photosynthetic capacities (Field & Mooney, 1986; Hirose & Werger, 1987; Evans, 1993) will also be lower. It was thus shown that there is an optimal LAI (and n_{area}) beyond which a further increase in light capture no longer compensates for the lower photosynthetic capacities, and canopy photosynthesis is maximized. This model was used successfully to predict differences in LAI and n_{area} values between stands of species with different physiological and

structural characteristics, and between stands growing at different N availabilities (Anten *et al.*, 1995b; Schieving & Poorter, 1999; Anten, 2002). As such we use it here to predict, at least qualitatively, the effects of C_a and N elevation on LAI and canopy photosynthesis.

We address the following questions: (i) what are the effects of C_a elevation and N availability on leaf photosynthesis, N distribution between leaves and canopy structure; (ii) does the relationship between LAI and N uptake change under elevated C_a ; and (iii) is the stimulation of canopy photosynthesis by C_a elevation stronger at high than at low N availability? To this end, we conducted an experiment where rice (*Oryza sativa*) was grown at ambient or elevated C_a (ambient plus 200 $\mu\text{mol mol}^{-1}$) and at two N availabilities. C_a elevation was applied using the free-air CO_2 enrichment (FACE) technique, which allows C_a levels to be manipulated with minimal disturbance (Okada *et al.*, 2001). The data are used to calculate net daily canopy carbon gain as a function of N availability. We focused on leaf area growth and leaf and canopy photosynthesis during the heading stage of the crop, because at this stage the stands had achieved their maximum LAI (Kobayashi *et al.*, 2001), and because canopy carbon gain during this stage strongly determines final grain yields (Cock & Yoshida, 1972). In other studies (Kim *et al.*, 2001, 2003) we have investigated the dynamics of growth and N uptake over the course of the growing season.

Model

The model presented here is based on previous canopy photosynthesis models (Goudriaan, 1977; Spitters, 1986; Anten, 1997; De Pury & Farquhar, 1997), which have proven to give accurate estimates of canopy photosynthesis (De Pury & Farquhar, 1997). In the present study plants had started to produce panicles, and we observed that these significantly affected the light climate in the canopy. The model divides the canopy into horizontal layers, and within each layer estimates light interception by leaves and panicles. Photosynthesis is calculated as a function of absorbed photon flux and leaf N content per unit area (n_{area}). Calculations are made for the date at which we harvested plants, 8 August (Julian date 220).

Photosynthesis is calculated by assuming two classes of leaves: shaded and sunlit. The procedure for calculating the photosynthetically active photon flux density (PFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$; 400–700 nm) that is absorbed by shaded and sunlit leaves in layer i ($I_{sh,i(j=1)}$ and $I_{sl,i(j=1)}$, respectively) is given in the Appendix.

The light response of net leaf photosynthesis (P_L , $\mu\text{mol m}^{-2} \text{s}^{-1}$) was calculated using a nonrectangular hyperbola:

$$P_L = \frac{(P_{\max} + \phi I_L) - [(P_{\max} + \phi I_L)^2 - 4\phi\theta P_{\max} I_L]^{0.5}}{(2\theta)} - R_d \quad \text{Eqn 1}$$

(I_L , absorbed PFD; P_{\max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$), light-saturated rate of gross photosynthesis; R_d , dark respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$); ϕ and θ , quantum yield (mol mol^{-1}) and curvature factor, respectively; Marshall & Biscoe, 1980). P_{\max} and R_d were calculated as a linearly increasing function of the leaf N content per unit leaf area (n_{area}):

$$P_{\max} = a_p(n_{\text{area}} - n_b) \quad \text{Eqn 2a}$$

$$R_d = a_r(n_{\text{area}} - n_b) + b_r \quad \text{Eqn 2b}$$

(a_p and n_b , slope and x -intercept of the $P_{\max} - n_{\text{area}}$ relationship; a_r , slope of the $R_d - n_{\text{area}}$ relationship; and b_r , R_d for a leaf with $n_{\text{area}} = n_b$; Anten *et al.*, 1995a). θ was set to 0.69 (see Results). To estimate the quantum yield ϕ we considered the following relationship: $\phi_{\text{app}} = \alpha\phi$, where ϕ_{app} is the apparent quantum yield estimated from the measured light response of leaf photosynthesis (see Materials and Methods, Results) and α the leaf absorbance, which was assumed to be 0.8.

The photosynthetic rate of shaded and sunlit leaf area in canopy layer i ($P_{sh,i}$ and $P_{sl,i}$, respectively) can be found by directly substituting I_L in equation 1 by $I_{sh,i(j=1)}$ (equation A1) or $I_{sl,i(j=1)}$ (equation A6), respectively (Spitters, 1986). Note that for photosynthesis we leave out the subscript ($j = 1$). For sunlit leaves photosynthesis is subsequently integrated over the leaf-angle distribution using equations 16 and 17 of Goudriaan (1988). Instantaneous rates of net canopy photosynthesis (P_c , $\text{mol m}^{-2} \text{s}^{-1}$) are then calculated by integrating leaf photosynthesis over canopy depth for each layer separately ($P_{c,i}$) and subsequent summation over the layers:

$$P_{c,i} = \sum_j \frac{L_{ij(j=1)}}{L_{ij}} \int_{\zeta_{T,i+1}}^{\zeta_{T,i}} [f_{sl,i} P_{sl,i} + (1 - f_{sl,i}) P_{sh,i}] d\zeta \quad \text{Eqn 4a}$$

$$P_c = \sum_i P_{c,i} \quad \text{Eqn 4b}$$

(L_{ij} , total area of leaves ($j = 1$) or panicles ($j = 2$) in layer i ; $\zeta_{T,i}$, sum of total cumulated area of leaves and panicles above layer i ; $f_{sl,i}$ the fraction of sunlit leaf area; see Appendix).

The daily course of direct and diffuse PFD above the canopy (I_{ob} and I_{od}) was assumed to be sinusoidal:

$$I_{ob} = I_{ob,noon} \sin(\beta) \text{ and } I_{od} = I_{od,noon} \sin(\beta) \quad \text{Eqn 5}$$

($I_{ob,noon}$ and $I_{od,noon}$ direct and diffuse PFD above the canopy at noon; β , solar inclination angle). P_c values are then integrated over the day, from sunrise to sunset, to obtain daily values (P_D , $\text{mol m}^{-2} \text{d}^{-1}$). β is calculated as a function of latitude and date (equation 6.6 of Gates, 1980) and day length (equation 17 of Spitters *et al.*, 1986). We intended to estimate canopy carbon gain assuming light conditions that

are reasonably representative for the conditions at our site around the harvesting date. Thus we chose the values of $I_{ob,noon}$ and $I_{od,noon}$ to be 1050 and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (average noon PFD of 1450 $\mu\text{mol m}^{-2} \text{s}^{-1}$). These values yield a total daily irradiance of 38.6 $\text{mol m}^{-2} \text{d}^{-1}$ calculated for 8 August at a latitude of 39° N, which was equivalent to the average daily irradiance between 3 and 13 August (K.K., unpublished). Night respiration is estimated by integrating equation 2b over canopy depth and then over the period from sunset to sunrise.

Materials and Methods

Experimental setup and crop history

A detailed description of the FACE facility used for this study is given by Okada *et al.* (2001), and we describe it only briefly here. The experiment was located in Shizukuishi in the northern part of Honshu (39° N, 141° E). This area is typical for the agro-ecological zone in which a large part of the Japanese rice crop is grown. The monthly mean max/min temperatures were June, 24.6/13.5°C; July, 28.7/18.8°C; August, 30.6/19.2°C; and mean annual precipitation is c. 1200 mm.

Elevated C_a was supplied by placing octagonal 12 m diameter emission structures (rings) in the paddy. Within these rings, pure CO_2 is emitted at pressure towards the centre from peripheral emission tubes that were raised gradually such that they were always c. 0.5 m above the canopy. A unique feature of the current FACE system was that the injected CO_2 was adequately mixed but, unlike previous FACE systems, without using blowers, which can have significant effects on the microclimate within the ring (Okada *et al.*, 2001). There were four rings, each placed in a different paddy. The target C_a at the centre of the rings was 200 $\mu\text{mol mol}^{-1}$ above ambient. In four other paddies, plants were grown without ring structures under ambient CO_2 . To avoid CO_2 contamination, ambient plots were placed at least 90 m from the FACE plots. In the FACE plots, daily average C_a levels varied between 553 and 609 $\mu\text{mol mol}^{-1}$ with a seasonal mean of 571 $\mu\text{mol mol}^{-1}$, and in the ambient plots they varied between 337 and 393 $\mu\text{mol mol}^{-1}$ with a seasonal mean of 366 $\mu\text{mol mol}^{-1}$. The experiment was arranged in four blocks, each containing a FACE and an ambient plot.

Rice (*Oryza sativa* L. cv. Akitakomachi) plants were grown mostly following the agronomic techniques typical of the local area. The crop was grown as described by Kim *et al.* (2001). On 22 May 2000, 24 d after seed emergence, seedlings were transplanted from trays in groups of three (hereafter 'hills') into the FACE and ambient plots. Hills and rows were 17.5 and 30 cm apart, respectively, which is equivalent to 19.1 hills m^{-2} . Two levels of N fertilizer were applied: a standard application (9 g N m^{-2} , standard N) and a high application (15 g N m^{-2} , high N). The timing and technique,

as well as rates of phosphorus and potassium applications, are as described by Kim *et al.* (2001) (procedure for the year 1999).

Harvest and sampling

Light distribution in the canopy was measured on 6 August with a sunflecks ceptometer (Model SF-80; Decagon Devices, Pullman, WA, USA). Within each plot we measured the diffuse PFD at height increments of 10 cm under an overcast sky. The reference PFD above the canopy was measured immediately after each individual measurement. Leaf and panicle inclination angles were measured with a handheld protractor.

On 8 August (76 d after transplanting), six hills were harvested from each plot. This date coincided with the heading stage of the crop. Plants from five of the six hills were divided into leaves, stems (leaf sheaths included in the stem fraction), roots and panicles. Leaf area was measured with a leaf-area meter (LI3100, LiCor, Lincoln, NE, USA) and leaf mass was determined after oven-drying for 3 d at 70°C. The three plants from every sixth hill were used for stratified clipping to determine the vertical distribution of mass leaf area and leaf N. All plants were clipped at every 10 cm from the base, and clippings were divided into leaves, stems and panicles. Leaf and panicle area and the dry mass of individual parts were measured as described above. Nitrogen contents were determined with a gas chromatograph after combustion with circulating O₂ (NC-analyser, Sumigraph NC-80, Kyoto, Japan).

Photosynthesis and respiration were measured with a portable open gas-exchange system (LI-6400, LiCor) on attached leaves. The system was equipped with an LED light source (LI-6400-02B, LiCor) and a dual Peltier device to regulate the temperature in the chamber. To estimate light-saturated rates of photosynthesis (P_{\max}) and the curvature factor (θ , equation 1), we measured light-response curves on at least 25 attached leaves from each CO₂ treatment by reducing the PFD in the chamber stepwise from 2000 to 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Leaves were chosen from different heights in the canopy. C_a in the chamber was set at values close to the growth C_a (370 and 570 $\mu\text{mol mol}^{-1}$ for the ambient and FACE plots, respectively). To determine whether P_{\max} values change with prolonged exposure to C_a elevation, we measured light-response curves on an additional 10 leaves from the ambient plots, but setting C_a in the chamber at 570 $\mu\text{mol mol}^{-1}$.

Most previous studies have used light-response curves to estimate the apparent quantum yield, ϕ_{app} . However, using measurements that extend beyond the linear part of the curve may lead to substantial underestimation of ϕ_{app} (Singsaas *et al.*, 2001). On a separate set of leaves (15 from each CO₂ treatment) we therefore estimated ϕ_{app} by reducing the PFD level in the chamber stepwise from 100 to 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (light-response curves were always linear between these PFD levels). Dark respiration (R_d) was measured after leaves had

been in the dark for 1 h. Leaf temperature during all measurements was maintained at 25°C. After the gas-exchange measurements, all leaves were dried and their N content determined as described.

Statistical analysis

Differences between treatment means were determined using ANOVA (split-plot) with CO₂ levels as the main plot and N level as the subplots ($n = 4$). Significance of the difference in N distribution in the canopy was determined by testing for among-slope effects in an ANCOVA with log-transformed n_{area} and relative PFD values as the dependent variable and covariate, respectively, and N and CO₂ as factors as in the split-plot ANOVA. The effects of CO₂ elevation on photosynthetic capacity and dark respiration were also analysed using an ANCOVA with n_{area} as the covariate and CO₂ as a fixed factor (in the case of photosynthetic capacity, with an added third level, ambient measured at 550 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

Results

Leaf characteristics and light and nitrogen distribution

There was a strong positive correlation between light-saturated photosynthesis (P_{\max}) and leaf N content per unit area (n_{area}) for both CO₂ treatments (ambient and FACE measured 370 and 570 $\mu\text{mol mol}^{-1}$, respectively, Fig. 1). The relationships were linear in both cases: second-order polynomial terms were not significantly different from zero ($P > 0.05$). ANCOVA showed a significant CO₂ effect on the slope of this relationship ($P = 0.0341$). A pairwise comparison between individual slopes further revealed that slopes of the $P_{\max} - n_{\text{area}}$ relationship was significantly ($P = 0.0421$) and considerably (35%) greater for FACE than for ambient plants (Table 1). By contrast, the slope value for the ambient plants measured at 570 $\mu\text{mol mol}^{-1}$ was not significantly different from that measured for the FACE plants ($P = 0.459$), indicating that prolonged exposure to elevated C_a had no effect on this relationship (Fig. 1b; Table 1). The x -intercept (n_b value, equation 2a) was not affected by C_a elevation (Table 1). Dark respiration R_d increased linearly with n_{area} , and this relationship was not significantly affected by CO₂ (Fig. 1c; Table 1, $P = 0.453$). The apparent quantum yield (ϕ_{app}) was, on average, considerably higher in FACE than in ambient plants (Fig. 1d). ϕ_{app} was not significantly correlated with n_{area} ($P = 0.241$ and 0.604, in the coefficient in linear regression, ambient and FACE, respectively). The curvature factor (θ) was not significantly affected by either C_a or n_{area} (Table 1). The combined average 0.69 was used for the calculations (see Model).

Leaf angles were predominantly vertical; 75% of leaves had inclination angles $> 60^\circ$, and c. 1% had an angle smaller than 30° . Practically all the panicles were inclined at $> 60^\circ$ (see Appendix). Correspondence between the measured and

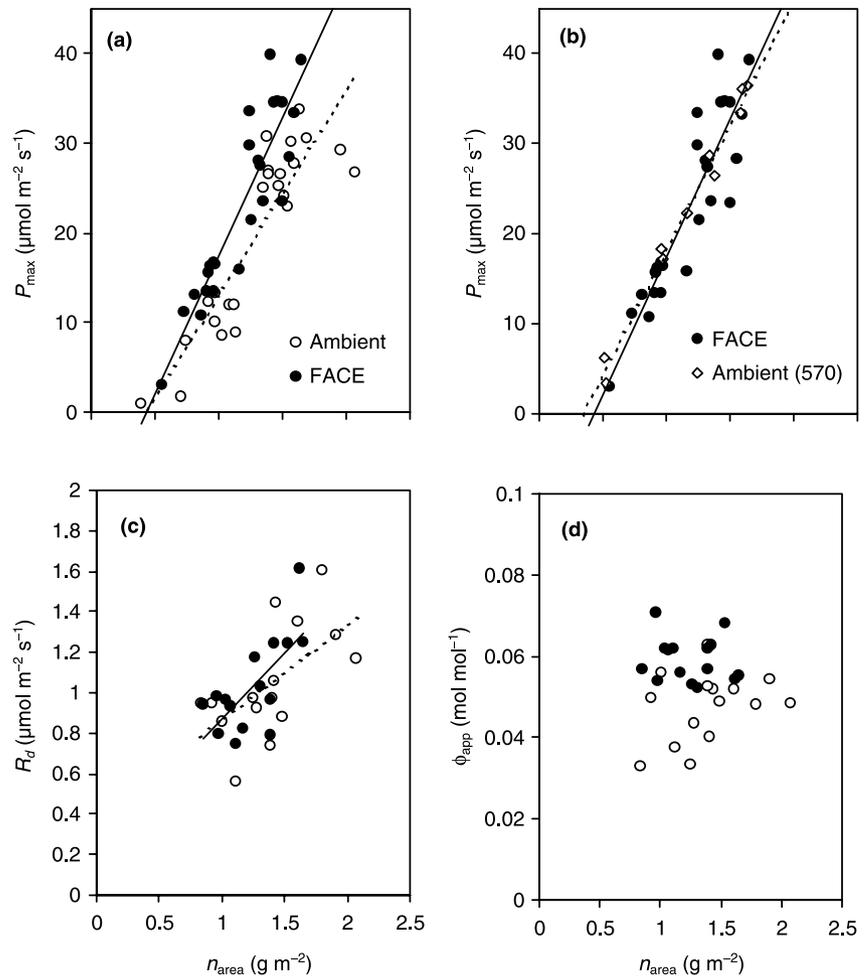


Fig. 1 Leaf gas exchange in relation to CO₂ and nitrogen availability. (a,b) Light-saturated photosynthesis (P_{\max} , equation 2a); (c) dark respiration (R_d , equation 2b); (d) apparent quantum yield (ϕ_{app}) as a function of leaf nitrogen content per unit leaf area (n_{area}) of rice (*Oryza sativa*). (a,c,d) Measurements made at growth C_a , ambient (370 $\mu\text{mol mol}^{-1}$) and FACE (570 $\mu\text{mol mol}^{-1}$). (b) P_{\max} for ambient plants measured at 570 $\mu\text{mol mol}^{-1}$ compared with P_{\max} for FACE plants also measured at this C_a (taken from (a)). Solid lines, FACE; dotted lines, ambient.

Table 1 Parameter values for relationships between light-saturated photosynthesis (P_{\max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$, equation 2a); slope a_p , x-intercept (n_b) and dark respiration (R_d , $\mu\text{mol m}^{-2} \text{s}^{-1}$, equation 2b); a_r slope and b_r R_d value at $n_{\text{area}} = (n_b)$ and leaf nitrogen content per unit area (n_{area} , g m^{-2}); average values of apparent quantum yield (ϕ_{app} , mol mol^{-1}) and curvature factor (θ)

	FACE	Ambient	Ambient-570
$P_{\max} = a_p(n_{\text{area}} - n_b)$			
a_p	30.7 (2.42) ^a	22.6 (2.07) ^b	27.8 (1.14) ^{ab}
n_b	0.421	0.413	0.333
$R_d = a_r(n_{\text{area}} - n_b) + b_r$			
a_r	0.614 (0.100)	0.521 (0.128)	–
b_r	0.524	0.531	–
ϕ_{app}	0.0591 (0.0014) ^a	0.0476 (0.0015) ^b	–
ϕ	0.074	0.059	–
θ	0.657 (0.034)	0.724 (0.034)	–

Ambient-570 denotes measurements for plants from ambient plots measured at elevated C_a (570 $\mu\text{mol mol}^{-1}$). FACE and ambient were measured at growth C_a (370 and 570 $\mu\text{mol mol}^{-1}$). SE in parentheses ($n = 25$ for ambient and FACE; 10 for ambient-570). Different superscripts denote significant differences in a pairwise comparison of slopes based on a t -test ($P < 0.05$) following ANCOVA (see Statistical analysis).

calculated relative diffuse PFD on a horizontal plane was very good ($r^2 = 0.962$). The regression line was $y = 0.968x + 0.0215$ ($y =$ calculated and $x =$ measured), suggesting that the model slightly overestimated light availability in the lowest layers of the canopy.

Within each stand, leaf N content per unit area (n_{area}) decreased with decreasing relative PFD from the top towards the bottom of the canopy (Fig. 2). This distribution pattern was steeper (greater slope of the n_{area} vs relative PFD relationship) in the standard-N than in the high-N plots ($P < 0.0001$). This difference can also be seen from the k values in the power function used to fit the N distribution, which were 0.32–0.37 for the standard-N and 0.21–0.25 in the high-N plots (Fig. 2). N distribution appeared to be unaffected by CO₂ ($P = 0.341$).

Whole-stand characteristics

Neither leaf mass nor LAI was significantly affected by elevated C_a , but both increased significantly with N availability (Tables 2, 3). Specific leaf area (SLA) did not differ between CO₂ treatments, but was significantly higher in high-N than

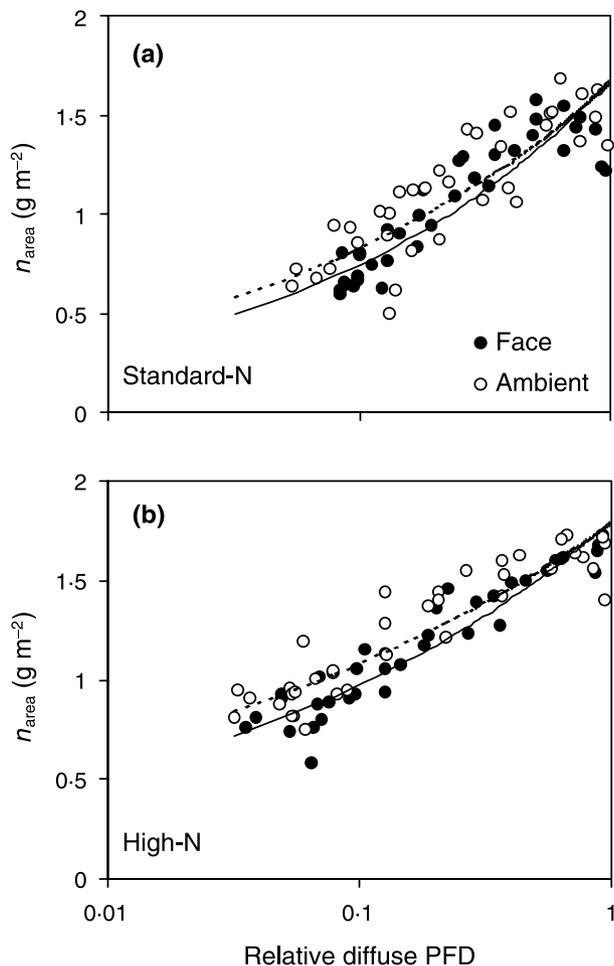


Fig. 2 Nitrogen contents per unit leaf area (n_{area}) as a function of measured relative diffuse PFD in the canopy of rice (*Oryza sativa*) stands grown in either ambient or FACE (ambient plus $200 \mu\text{mol mol}^{-1}$) plots at two N availabilities: (a) standard N, 9 g N m^{-2} ; (b) high N, 15 g N m^{-2} . Lines (broken lines, ambient; solid lines, FACE) represent fits made with a power function, $n_{\text{area}} = n_o (I/I_o)^k$ with I/I_o the relative diffuse PFD; n_o the n_{area} at the top of the canopy; and k a parameter that determines the steepness of the n_{area} distribution (Anten *et al.*, 1995a). Regression equations: FACE (standard N), $n_{\text{area}} = 1.68(I/I_o)^{0.355}$, $r^2 = 0.680$; FACE (high N), $n_{\text{area}} = 1.79(I/I_o)^{0.265}$, $r^2 = 0.864$; ambient (standard N), $n_{\text{area}} = 1.67(I/I_o)^{0.304}$, $r^2 = 0.680$; ambient (high N), $n_{\text{area}} = 1.79(I/I_o)^{0.218}$, $r^2 = 0.832$ (solid lines, FACE; dotted lines, ambient).

in standard-N plants. There was no interactive effect of C_a elevation and N availability on any of these mass or area values (Tables 2, 3).

The total amount of N in the plant did not differ between CO_2 treatments (Table 2). FACE plants, however, allocated a significantly lower fraction of their total N to leaves (Table 2). As a result, the total amount of N in the leaves (N_{leaf}) was *c.* 8–14% lower in the FACE plots (Fig. 3), although this difference was not significant ($P = 0.076$; Table 2). A greater N availability resulted in increased total N, canopy N and

Table 2 Results of ANOVA (split-plot) with CO_2 treatment as main effect and nitrogen treatment as subplots

	CO_2 effect	N effect	$\text{CO}_2 \times \text{N}$ interaction
Leaf mass and area			
Leaves (g m^{-2})	0.585	0.0131*	0.425
LAI ($\text{m}^2 \text{ m}^{-2}$) ^{log}	0.505	0.0138*	0.660
SLA ($\text{m}^2 \text{ g}^{-1}$) ^{log}	0.706	0.0659	0.905
Nitrogen			
Total N (g m^{-2} soil)	0.774	0.004**	0.263
Total leaf N (g m^{-2} soil)	0.076	0.0061	0.448
Leaf N fraction (g g^{-1})	0.0395*	0.0475*	0.614
Average n_{area} (g m^{-2})	0.0212*	0.0072**	0.507
Average n_{mass} (mg g^{-1})	0.0432*	0.0135*	0.795

Values presented are *P* for each effect. Significance levels: *, $P < 0.05$; **, $P < 0.01$. ^{log}denotes log-transformation of values.

LAI, leaf area index; SLA, specific leaf area; n_{area} , n_{mass} , nitrogen content per unit leaf area and per unit mass.

Table 3 Measured and predicted optimal leaf area indices (LAI) and average area-based leaf N contents for rice (*Oryza sativa*) stands grown in either ambient or FACE (ambient plus $200 \mu\text{mol mol}^{-1}$) plots at two N availabilities (standard N, 9; high N, 15 g N m^{-2})

	Standard N		<i>F</i> : <i>A</i>	High N		<i>F</i> : <i>A</i>
	FACE	Ambient		FACE	Ambient	
LAI ($\text{m}^2 \text{ m}^{-2}$)						
Measured	4.40	4.47	0.98	6.18	6.06	1.02
Predicted optimal	3.31	3.36	0.98	4.80	4.39	1.09
Leaf N content (g m^{-2})						
Measured	1.03	1.10	0.94	1.14	1.23	0.91
Predicted optimal	1.38	1.47	0.94	1.46	1.71	0.86

F : *A*, ratio between values for FACE and ambient.

fractional N allocation to leaves. Average leaf N per unit mass (average $n_{\text{mass}} = N_{\text{leaf}}/\text{leaf mass}$) and per unit leaf area (average $n_{\text{area}} = N_{\text{leaf}}/\text{LAI}$) were 6–8% lower in FACE than in ambient, and considerably higher in high-N than in standard-N plots (Tables 2, 3; Fig. 3). As with LAI, there was no interactive effect of CO_2 and N availability on either the amounts of N or the tissue N contents (Table 2).

Canopy net daily photosynthesis was calculated to increase considerably under C_a elevation, and this enhancement was greater at high N (31%) than at standard N (24%). Nitrogen availability, on the other hand, was estimated to have a relatively small effect on carbon gain (Fig. 3). This N effect was larger for the FACE plots (10%) than for the ambient plots (5%). Photosynthesis per unit canopy N was considerably enhanced at high C_a .

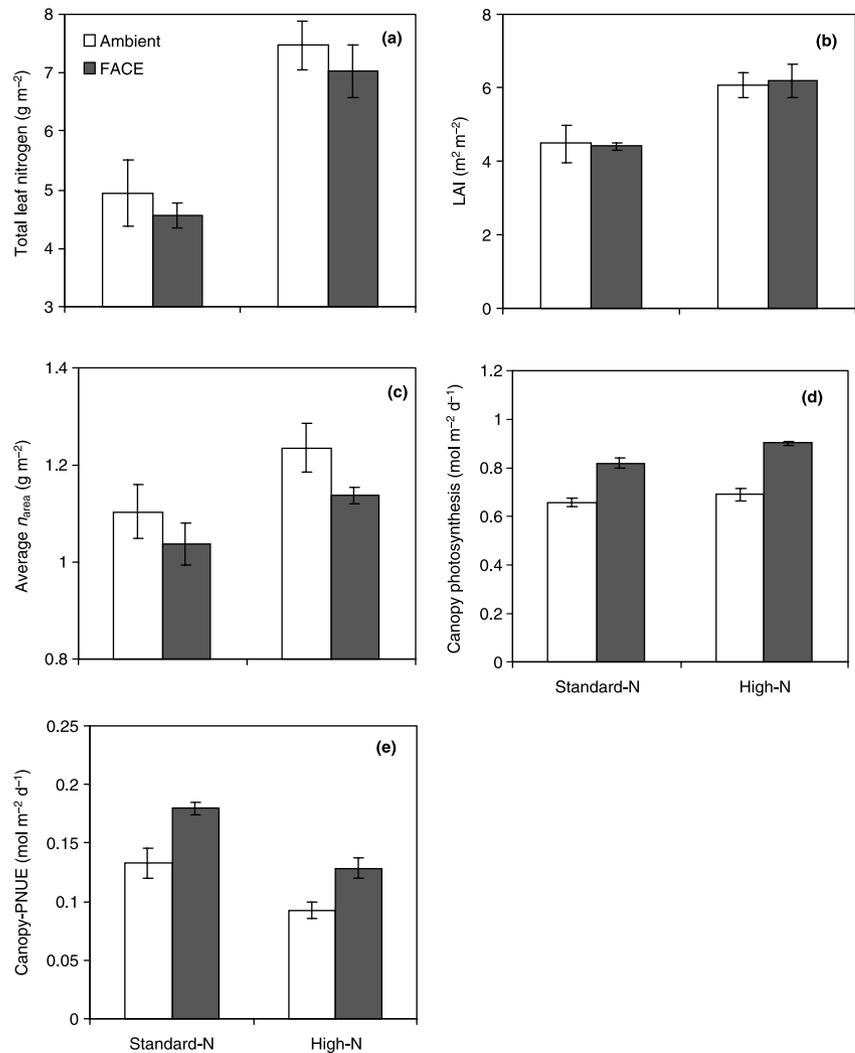


Fig. 3 Canopy structure, nitrogen contents and canopy photosynthesis in relation to CO₂ and N availability. (a) Total leaf nitrogen (expressed per unit soil area); (b) leaf area index; (c) average N content per unit leaf area (n_{area}); (d) canopy net daily photosynthesis; (e) canopy photosynthesis per unit N (canopy PNUE) of rice (*Oryza sativa*) stands grown in either ambient (open bars) or FACE (ambient plus 200 $\mu\text{mol mol}^{-1}$; closed bars) plots at two N availabilities (standard N, 9; high N, 15 g N m⁻²). Bars, SE ($n = 4$). Statistical analysis given in Table 2.

Sensitivity analysis

We conducted a sensitivity analysis of our model to further analyse the relationship between N availability, leaf area growth and canopy photosynthesis at different C_a levels. In Fig. 4, canopy photosynthesis was calculated as a function of LAI, keeping total leaf N (N_{leaf}) constant. This implied that an increase in LAI was associated with a proportional reduction in N content per unit leaf area (n_{area}). In all stands, canopy photosynthesis was calculated first to increase and then to decrease with increasing LAI, indicating that there was an optimal LAI and therefore an optimal average n_{area} ($N_{leaf}/\text{optimal LAI}$) at which canopy photosynthesis for a given N_{leaf} is maximized. When LAI was increased beyond this optimum, the resulting increase in light capture did not compensate for the reduction in n_{area} and associated reduction in leaf photosynthetic capacities (P_{max} , see Introduction).

In absolute terms, the model for optimal LAI underestimated the measured LAIs and therefore overestimated average

leaf N contents n_{area} (Fig. 4). Consequently, at actual LAI, canopy photosynthesis was 6–10% lower than at optimal LAI. On the other hand, the model made surprisingly good qualitative predictions of the effects of elevated C_a and N availability on LAI and n_{area} . It predicted the FACE plots to have approximately the same LAI as ambient plots, as we observed (Fig. 3). It therefore also predicted the FACE plants to have 6 and 14% lower n_{area} at low and high N, respectively, which compares well with the 6–9% reduction that we observed (Table 3). The regression of predicted vs measured LAIs showed no difference between CO₂ treatments, further indicating that the CO₂ effect on LAI was well predicted (Fig. 4c). The calculated effect of high N availability on LAI (30 and 45% increase for the ambient and FACE plots) was also consistent with the measured value (35 and 40% increase; Fig. 3; Table 3).

In Fig. 5, canopy photosynthesis was calculated for different values of total leaf N (N_{leaf}). To predict canopy development as a function of N availability, we calculated the

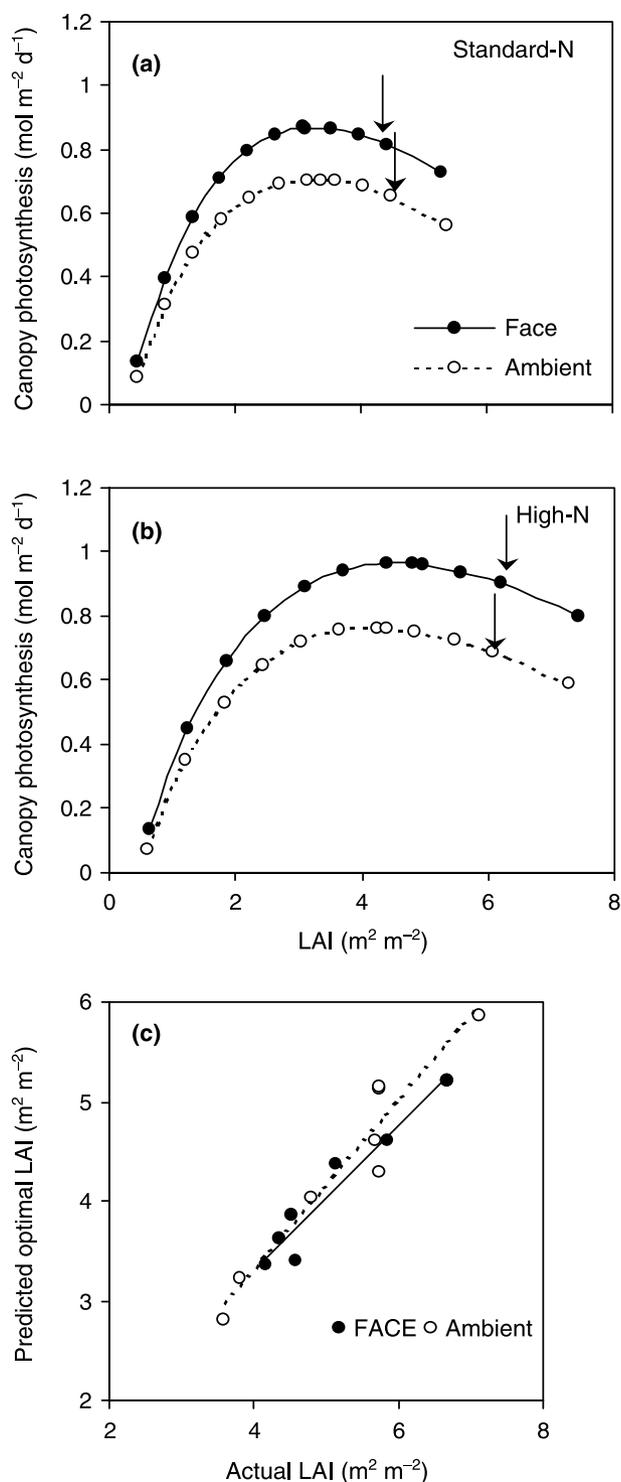


Fig. 4 Net daily canopy photosynthesis of rice (*Oryza sativa*) as a function of leaf area index, LAI (arrows indicate carbon gain at actual LAI) for (a) standard N (9 g N m^{-2}); (b) high N (15 g N m^{-2}) plots. ((a,b) Show only calculations made with canopy characteristics averaged for the four replicate plots). In this analysis LAI is changed, keeping total leaf N in the canopy (N_{leaf}) constant; an increase in LAI therefore reduces leaf N content per unit area (n_{area}). (c) Optimal LAI values calculated for each replicate plot against measured LAI (regression lines: solid, FACE; dashed, ambient).

corresponding optimal LAI for each N_{leaf} value in the same way as in Fig. 4. The LAI and average leaf N content (n_{area}) were predicted to increase with increasing N_{leaf} (Fig. 5a,b). But at every N_{leaf} , the stands under elevated C_a were predicted to have slightly (6–8%) larger LAIs and lower n_{area} than the stands under ambient C_a (Fig. 5a,b).

The predicted effect of elevated C_a on LAI growth was the result of its effects on leaf photosynthetic characteristics. To analyse this further, we calculated the optimal LAI values assuming all characteristics of the stands in the ambient plots and then separately increasing the $P_{\text{max}} : n_{\text{area}}$ ratio (changing a_p and n_b in equation 2a; Fig. 1; Table 2) or the quantum yield (ϕ ; Table 1) to the values found for the FACE plants. The two changes had opposite effects. Increasing the $P_{\text{max}} : n_{\text{area}}$ ratio yielded an increase of *c.* 11% in optimal LAI at any given N_{leaf} , while an increase in ϕ resulted in a 5% reduction (Fig. 5a,b).

Calculated canopy photosynthesis first increased with N_{leaf} , then saturated to a maximum value (Fig. 5c). At elevated C_a , however, canopy photosynthesis saturated at a higher N_{leaf} value than at ambient C_a . As a result, the ratio of canopy photosynthesis at elevated to that at ambient C_a increased strongly with N_{leaf} (Fig. 5d). In other words, the model predicted that the stimulation of canopy photosynthesis by C_a elevation increases with the amount of N taken up and allocated to the canopy. As in Fig. 5b, we also calculated canopy photosynthesis assuming all characteristics of the stands in the ambient plots, and then separately increasing the $P_{\text{max}} : n_{\text{area}}$ ratio or the quantum yield (ϕ) to that of the FACE plants, and divided this by the canopy photosynthesis of the ambient stand (Fig. 5e). This ratio also increased with N_{leaf} if ϕ was increased, but tended to decline when the $P_{\text{max}} : n_{\text{area}}$ ratio was increased. Thus the increase in ϕ appears to drive the simulated interactive effect of C_a and N on canopy photosynthesis.

Discussion

Effects of CO_2 on leaf photosynthesis and canopy nitrogen distribution

Both light-saturated photosynthesis (P_{max}) and P_{max} per unit leaf N ($P_{\text{max}} : n_{\text{area}}$) were *c.* 35% higher in FACE than in ambient plants, when measured at their own growth CO_2 level (Fig. 1a). This enhancement is consistent with the range of values reported in other studies (Hirose *et al.*, 1997; Norby *et al.*, 1999), including those on rice (Nakano *et al.*, 1997; Aben *et al.*, 1999). Long-term exposure to elevated C_a can lead to reductions in P_{max} . Two factors appear to contribute to this reduction. First, growth under elevated C_a can lead to accumulation of carbohydrates in leaves, and this may directly or indirectly inhibit photosynthesis (Stitt, 1991; Makino & Mae, 1999). Second, reductions in P_{max} can result directly from reductions in n_{area} (Nakano *et al.*, 1997). In the present study the $P_{\text{max}} - n_{\text{area}}$ relationship was not changed by

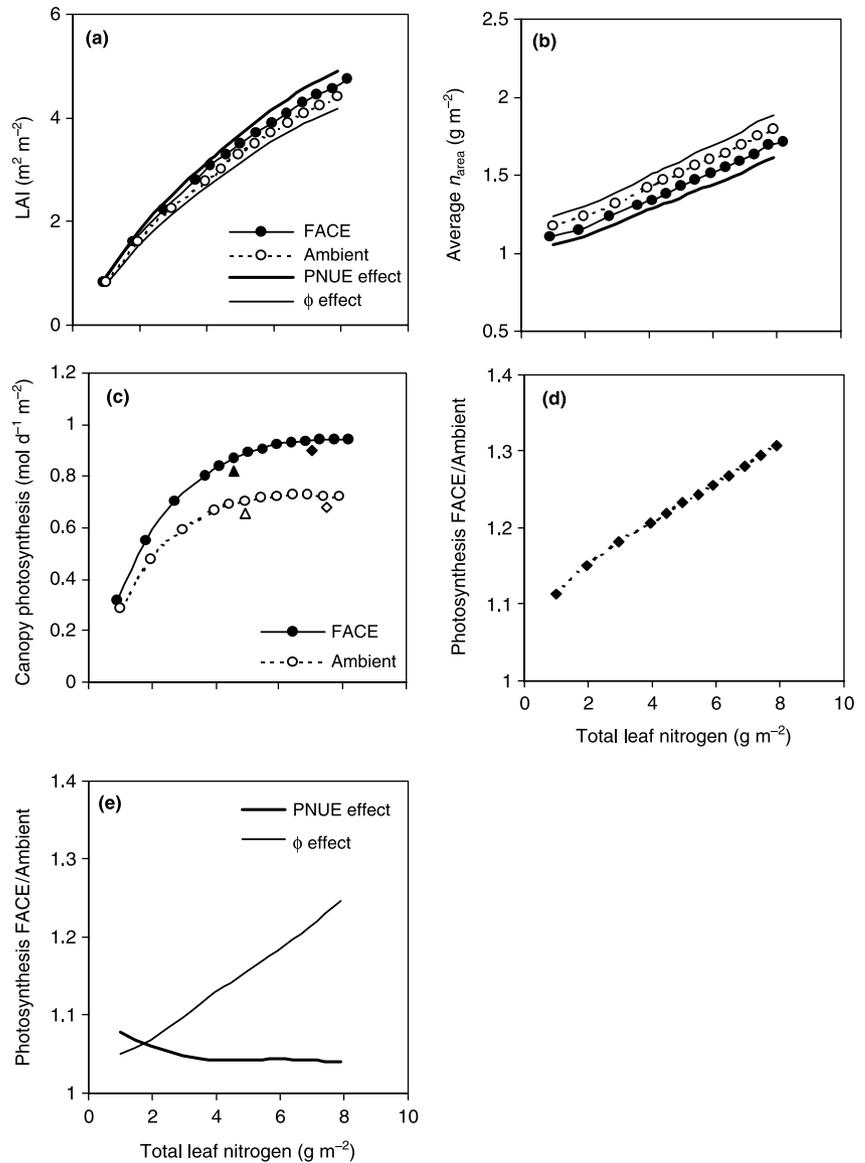


Fig. 5 Effects of Ca elevation on the relationship between LAI, canopy photosynthesis and leaf nitrogen. (a) Optimal LAI for maximum carbon gain as a function of total amount of leaf nitrogen in the canopy (N_{leaf}) and associated (b) optimal average leaf N content (optimal $n_{\text{area}} = N_{\text{leaf}}/\text{optimal LAI}$) and (c) net daily canopy carbon gain. Optimal LAI values are calculated as in Figure 4. Canopy photosynthesis at actual N_{leaf} and LAI values are also given in (c) (closed symbols, FACE; open symbols, ambient; triangles, standard N (9 g N m^{-2}); diamonds, high N (15 g N m^{-2}), from Fig. 3d). (d) Ratio between canopy photosynthesis in FACE (ambient plus $200 \mu\text{mol mol}^{-1}$) to that in ambient plots (FACE : ambient). (a,b) Optimal LAI values also calculated; (e) FACE : ambient calculated assuming all characteristics from ambient plots, then separately changing either the $P_{\text{max}} - n_{\text{area}}$ relationship (equation 2a, P NUE effect) or the quantum yield (ϕ , ϕ effect) to the values found for FACE plants. Calculations were made with canopy characteristics averaged for the four replicate plots.

prolonged exposure to elevated C_a (Fig. 1b), suggesting that photosynthesis was not directly affected by carbohydrate accumulation. This result is consistent with other studies on rice (Nakano *et al.*, 1997; Aben *et al.*, 1999), and might be related to the fact that, in rice, accumulation of carbohydrates in elevated C_a is relatively small (Nakano *et al.*, 1997). However, n_{area} values were 5–10% lower in FACE than in ambient plants (Table 3), and it can be calculated that this resulted in a *c.* 10% average reduction in P_{max} .

Dark respiration (R_d) increased linearly with n_{area} , but this relationship was not affected by C_a elevation (Fig. 1c). Plants growing in elevated C_a tend to accumulate carbohydrates in their leaves, which could lead to greater R_d , but the contribution of this mechanism appears to be small (Amthor, 1989). On the other hand, it is believed that elevated C_a can reduce R_d ,

either through inhibition of certain respiratory enzymes (Drake *et al.*, 1999) or because elevated C_a results in lower protein levels (Bunce & Caulfield, 1991). The latter appears consistent with our results; there was a small reduction in R_d (*c.* 5%) mediated by a reduction in n_{area} and associated protein levels.

The quantum yield (the measured apparent quantum yield, ϕ_{app} , divided by 0.8) was estimated to be 0.059 and $0.074 \text{ mol mol}^{-1}$ under ambient and elevated C_a , respectively, which is consistent with other results (Long, 1991). There was no significant correlation between ϕ_{app} and n_{area} , which was also found in some previous studies (Schieving *et al.*, 1992a; Anten *et al.*, 1995a; Hirose *et al.*, 1997). Others (Pons *et al.*, 1990; Schieving *et al.*, 1992b) found curvilinear relationships between ϕ_{app} and n_{area} , which is expected based on optimal allocation of N among photosynthetic proteins (see Anten

et al., 2000). However, these studies also found that above n_{area} values of 0.5–0.6 g m⁻², ϕ_{app} levels off and is practically independent of n_{area} . In any case, sensitivity analysis revealed that the assumption of constant ϕ_{app} had no implications for the conclusions of this paper.

There was no effect of C_a elevation on the average SLA of plants, in contrast to most previous studies (Wong, 1979; Poorter, 1998) which found a reduction in SLA. Those studies attributed this reduction in SLA to accumulation of carbohydrates in leaves. The discrepancy between their results and ours could be attributed to the fact that they grew isolated plants, while we grew them in dense stands where most leaves are shaded. It is unlikely that these shaded leaves would accumulate large amounts of carbohydrates (Hirose *et al.*, 1996).

The pattern of vertical N distribution in the canopy has a strong influence on canopy photosynthesis. A nonuniform distribution, with n_{area} decreasing with PFD from the top towards the bottom of the canopy, may confer up to 30% increase in whole-canopy carbon gain compared with the uniform distribution in which n_{area} of all the leaves equals the mean (Hirose & Werger, 1987; Werger & Hirose, 1991; Anten *et al.*, 2000). Consequently, there is much interest in the extent to which C_a elevation can affect N distribution (Hirose *et al.*, 1996, 1997). Here we found that N distribution was relatively unaffected by C_a (Fig. 2). With an optimization model, Anten *et al.* (1995a) derived that n_{area} distribution should be strictly determined by the light distribution in the canopy and by N availability, and that it is independent of physiological characteristics or environmental conditions which may determine the relationship between leaf photosynthesis and n_{area} . The absence of a C_a effect on N distribution appears to be consistent with this prediction. C_a elevation had a strong effect on the photosynthesis–N relationship of leaves, but a much smaller effect on canopy structure and canopy N. Nitrogen availability, on the other hand, had a strong effect on N distribution: the distribution was steeper in standard-N than in high-N plots (Fig. 2), which is consistent with Hikosaka *et al.* (1994).

Interactive effects of CO₂ and nitrogen on leaf area index development and canopy photosynthesis

The potential interactive effects of N and CO₂ on canopy photosynthesis are probably mediated through their effects on leaf photosynthesis and leaf area growth. Generally, leaf area growth increases with N uptake by plants, but it is unclear whether this relationship will change under elevated C_a (see Introduction). Here we found that N uptake and LAI increased with N availability, but not with C_a elevation. To analyse further the potential effects of C_a and N on LAI, we calculated the optimal LAI for maximal carbon gain as a function of the total leaf N in the canopy (N_{leaf}). This model predicted a strong correlation between N_{leaf} and the LAI of the stand. But it also predicted that, for a given N_{leaf} , stands under

elevated C_a should produce slightly larger (8%) LAIs than stands under ambient C_a , also resulting in 8% lower area-based leaf N contents (n_{area} ; $n_{\text{area}} = N_{\text{leaf}}/\text{LAI}$, Figs 4, 5a,b). These predicted effects were surprisingly close to the actual data obtained (Figs 3, 4c; Table 3) and are consistent with Harz-Rubin & DeLucia (2001) who also found that stands under elevated C_a produced larger LAIs for a given amount of canopy N than those under ambient C_a . But they appear to be in contrast with Hirose *et al.* (1996), who found that the relationship between LAI and total N uptake by plants did not change under C_a elevation. However, plants under elevated C_a allocated *c.* 10% lower fraction of the total N that was taken up to leaves. Makino & Mae (1999) obtained similar results in rice. This reduction counterbalances the increase in LAI produced for a given N_{leaf} and our results, when plotted against total N uptake, are consistent with Hirose *et al.* (1996). It can thus be argued that LAI will increase under elevated C_a only if N uptake from the soil is simultaneously increased.

An enhancement of N uptake under elevated C_a might come from an increase in root growth, arising from a higher growth rate and/or a shift in allocation, allowing plants to exploit a larger soil volume. Yet in a well developed mature stand where plants grow close together, such as the rice stands investigated here, most of the available soil volume is already exploited, and the potential for additional N uptake might be small. Added N uptake may occur only early in the season when the soil is not yet fully exploited. In the rice paddy studied by us, C_a elevation stimulated N uptake and LAI early but not late in the season (Kim *et al.*, 2001, 2003). This gradual increase in N limitation could explain further why many other studies found stimulation of leaf area growth by C_a elevation early in the season, but did not find an effect on maximum LAI (see Introduction).

It should be noted that the photosynthesis model and estimation of optimal LAI were based on a number of simplifying assumptions. First, extrapolation of a linear $P_{\text{max}} - n_{\text{area}}$ relationship (equation 2a) may lead to overestimation of carbon gain at high n_{area} (and thus to overestimation of optimal n_{area}) because at these values, this relationship tends to become curvilinear. Second, the present approach ignores competitive interactions between plants. Including such interactions results in higher predicted LAIs and lower n_{area} , and could explain the absolute difference between the actual and optimal LAI that we found (Schieving & Poorter, 1999; Anten, 2002). Third, we used dark respiration values measured after leaves had been placed in the dark for 1 h. But Noguchi & Terashima (1997) reported that during darkness, respiratory rates fall over a period of *c.* 6 h to 70–80% of the value at the onset of darkness. Thus our model may have overestimated night respiration by 10–15%. However, a sensitivity analysis indicated that this overestimation had no consequences for the main conclusions of the paper. Fourth, we did not include photosynthesis of panicles, but the gross

rate of panicle photosynthesis is, at most, twice as high as the rate of respiration (Imaizumi *et al.*, 1990; J. Goudriaan, pers. comm.). Consequently their net diurnal carbon gain will probably be close to zero. Finally, it should be noted that the concept of optimal LAI assumes that maximization of whole-canopy carbon gain drives the relationship between N allocation to leaves, leaf area growth and resulting n_{area} (Anten *et al.*, 1995b). In the case of responses to elevated C_a , this presumes that plants can adjust to conditions in which they did not evolve (Anten *et al.*, 2000). On the other hand, the strong correspondence between the predicted and measured differences may merit the use of this approach.

The next question is: does the effect of C_a elevation on canopy carbon gain depend on N availability? We did not find a significant $C_a \times N$ interactive effect on N uptake and LAI, which is not consistent with the idea that N uptake increases with C_a elevation only when N availability itself is high (Stitt & Krapp, 1999). However, we calculated that the stimulation of canopy photosynthesis was greater in the high-N than in the standard-N plots, suggesting that there might indeed be an interactive effect on canopy photosynthesis (Fig. 3). To further analyse this question, we used the model for optimal LAI to simulate canopy photosynthesis as a function of total leaf N (N_{leaf} ; Fig. 5). Canopy photosynthesis increased curvilinearly with N_{leaf} approaching a maximum value. Apparently increased N uptake and allocation to leaves cannot enhance carbon gain indefinitely, but saturates at very high N_{leaf} because of light limitations. Increasing LAI with N availability increases self-shading, inhibiting further enhancement in photosynthesis with increasing photosynthetic capacities that are associated with high n_{area} (equation 2a).

However, under elevated C_a canopy photosynthesis increased more with N_{leaf} , and saturated at a higher N_{leaf} value than under ambient C_a (Fig. 5c). Our model thus estimated that the stimulation of canopy photosynthesis by C_a elevation would increase from 11% at very low N availability to 31% at very high availability (Fig. 5d). Thus our calculations indicate a strong interactive effect of C_a and N on canopy photosynthesis. At our site, Kim *et al.* (2001) (their Fig. 3b) and Kobayashi *et al.* (2001) found curvilinear relationships between measured rice growth and N availability that were surprisingly similar in form to our photosynthesis – N_{leaf} curves, and they also found that under elevated C_a , growth saturated at a higher N availability than under ambient C_a .

So far the interactive effect of C_a and N on growth has mainly been attributed to two mechanisms (see Introduction). First, inhibition of leaf photosynthesis by carbohydrate accumulation after prolonged exposure to elevated C_a tends to be stronger under low than under high N availability (Rogers *et al.*, 1996). Second, N uptake increases under C_a elevation only when N availability is high, and not when it is low (Stitt & Krapp, 1999). While we do not discard the potential importance of these mechanisms, our model predicts an interactive effect of N and C_a on canopy photosynthesis that

is independent of either. The explanation for this interactive effect is relatively simple. As shown in this paper, LAI growth is strongly determined by N availability, more or less independently of C_a . When N availability is low, the canopy is relatively open and most leaves receive relatively high light. Under these conditions, the effect of C_a elevation on canopy photosynthesis will be predominantly through its effect on photosynthetic capacity. But as N availability increases, the canopy becomes denser and lower leaves become increasingly shaded. Under these conditions the enhanced quantum yield under elevated C_a will have an increasingly positive effect on canopy photosynthesis (Fig. 5e).

Acknowledgements

We thank Kouki Hikosaka and Thijs Pons for valuable comments on the manuscript and Alamaz, Sinjiro Ishizaki, Riichi Oguchi, Yuko Yasumura for technical assistance. This work was supported by the CREST (Core Research for Evolution Science and Technology) program of the Japan Science and Technology Cooperation (JTC).

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Appendix

Photon flux density (PFD, μmol m⁻² s⁻¹) absorbed by shaded leaf area at a given depth in canopy layer *i* (*I*_{sh,i(j=1)}, layers counted from top to bottom) is given as:

$$I_{sh,i(j=1)} = I_{d,i(j=1)} + I_{bs,i(j=1)} \quad \text{Eqn A1}$$

(*I*_{d,i(j=1)} and *I*_{bs,i(j=1)}, absorbed diffuse PFD and scattered direct PFD; subscript *j*, type of light-intercepting tissue, *j* = 1 for leaves, and *j* = 2 for panicles). *I*_{bs,i(j=1)} can be calculated from the distribution of direct PFD in the canopy. The average direct PFD above layer *i* in the canopy (*I*_{ob,i}) is found as:

$$I_{ob,i} = (1 - \gamma) I_{ob} \exp\left(\sum_{i=1} \sum_j K_{b,j} \alpha_{ij}^{0.5} L_{ij}\right) \quad \text{Eqn A2}$$

(*K*_{b,j}, extinction coefficient for direct light of 'black' non-scattering light-absorbing tissue, leaves or panicles; α_{ij} , absorbance of this tissue; γ , canopy reflectance which is set to 0.05; *L*_{ij}, area index of leaves or panicles in the layers above layer *i*; *I*_{ob}, direct beam irradiance above the canopy). The distribution of absorbed scattered direct PFD in the canopy (*I*_{bs,i(j=1)}) is:

$$I_{bs,i(j=1)} = I_{ob,i} K_{b,(j=1)} \left[\alpha_{i(j=1)}^{0.5} \exp\left(\sum_j - K_{b,j} \alpha_{ij}^{0.5} \zeta_{ij}\right) - \frac{\alpha_{i(j=1)}}{(1 - \gamma)} \exp\left(\sum_j - K_{b,j} \zeta_{ij}\right) \right] \quad \text{Eqn A3}$$

(ζ_{ij} , cumulative amount of area of class *j* within height class *i*; Anten, 1997. It has a value of zero at the top of each height class and accumulates from top to bottom within the layer, at the bottom of the layer $\zeta_{ij} = L_{ij}$).

The distribution of diffuse PFD was calculated by assuming that it can be represented by a summation of radiation components, each of which originates from a different ring zone of the sky, and has an angle of incidence equal to the centre angle of each zone (Goudriaan, 1977). We distinguish three 30° sky zones (0–30, 30–60, 60–90°, with elevation angles 15, 45 and 75°, respectively). Thus the diffuse PFD on

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a horizontal plain originating from sky elevation zone *k* above layer *i* (*I*_{od,ik}) is found as:

$$I_{od,ik} = Z_k (1 - \gamma) I_{od} \exp\left(\sum_i \sum_j - K_{d,jk} \alpha_{ij}^{0.5} L_{ij}\right) \quad \text{Eqn A4}$$

(*I*_{od}, diffuse PFD above the canopy originating from sky elevation *k*; *K*_{d,jk}, extinction coefficient of nonscattering tissue for this irradiance component; *Z*_k, fraction of the total diffuse PFD originating from elevation *k*, calculated according to the standard overcast sky; Goudriaan, 1977). The absorbed diffuse irradiance (*I*_{d,i(j=1)}) is now calculated as:

$$I_{d,i(j=1)k} = I_{od,ik} K_{d,(j=1)k} \alpha_{i(j=1)}^{0.5} \exp\left(\sum_j - K_{d,jk} \alpha_{ij}^{0.5} \zeta_{ij}\right) \quad \text{Eqn A5a}$$

$$I_{d,i(j=1)} = \sum_k - I_{d,i(j=1)k} \quad \text{Eqn A5b}$$

(*I*_{d,i(j=1)k}, absorbed diffuse PFD originating from elevation *k*). Sunlit leaves receive both direct beam and diffuse (sky and scattered) PFD. The PFD absorbed by a single sunlit leaf at given height in canopy layer *i* (*I*_{sl,i(j=1)}) is given as:

$$I_{sl,i(j=1)} = O_{i(j=1)} I_{ob} \alpha_{i(j=1)} / \sin \beta + I_{sh,i(j=1)} \quad \text{Eqn A6}$$

(*O*_{i(j=1)} is the projection of a single leaf into the direction of the sun, calculated according to Goudriaan (1988); α , solar elevation).

The extinction coefficient for direct light (*K*_{b,j}) can be estimated as a function of solar elevation and leaf angle distribution following the method of Goudriaan (1988) (equations 3–5 of Anten, 1997). The extinction coefficients for diffuse light (*K*_{d,j}) are calculated in the same way, with the solar elevation angle being replaced by the sky zone elevation angle. Leaf absorbance ($\alpha_{i(j=1)}$) is calculated as a function of leaf N and panicle absorbance ($\alpha_{i(j=1)}$) was assumed to be 1 (see text).

The fraction of sunlit leaf area (*f*_{sl,i}) is found as:

$$f_{sl,i} = \exp\left(\sum_i \sum_j - K_{b,j} L_{ij}\right) \exp\left(\sum_j - K_{b,j} \zeta_{ij}\right) \quad \text{Eqn A7}$$