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Changes in source–sink relations during development influence photosynthetic acclimation of rice to free air CO_2 enrichment (FACE)

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Abstract. Relationships between photosynthetic acclimation and changes in the balance between source–sink supply and demand of carbon (C) and nitrogen (N) were tested using rice (*Oryza sativa* L. cv. Akitakomachi). Plants were field-grown in northern Japan at ambient CO_2 partial pressure [$p(CO_2)$] or free air CO_2 enrichment (FACE; $p(CO_2)$ ~ 26–32 Pa above ambient) with low, medium or high N supplies. Leaf CO_2 assimilation rates (A) and biochemical parameters were measured at 32–36 (eighth leaf) and 76–80 (flag leaf) d after transplanting, representing stages with a contrasting balance between C and N supply and demand in sources and sinks. Acclimation due to FACE was pronounced in flag leaves at each N supply. This was not fully accounted for by reductions in leaf N concentrations, because A/N and V_{cmax}/N were lower in FACE-grown flag leaves. Acclimation did not occur in the eighth leaf, and A/N and V_{cmax}/N was not significantly increased in FACE-grown leaves. Soluble protein/sucrose and amino acid/sucrose concentrations decreased under FACE, whereas sucrose phosphate synthase protein levels increased. At flag leaf stage, there was a discrepancy between the demand and supply of N, which was resolved by enhanced leaf N remobilization, associated with the lower Rubisco concentrations under FACE. In contrast to the early growth stage, enhanced growth of rice plants was accompanied by increased plant N uptake in FACE. We conclude that photosynthetic acclimation in flag leaves occurs under FACE because there is a large demand for N for reproductive development, relative to supply of N from root uptake and remobilization from leaves.

Introduction

Photosynthetic acclimation has intrigued researchers in the area of plant responses to elevated $p(CO_2)$ over the last decade, as evidenced by two recent reviews (Moore *et al.* 1999; Stitt and Krapp 1999). Acclimation, which occurs only after prolonged exposure of plants to elevated $p(CO_2)$, is characterized by lower leaf photosynthetic capacity (Drake *et al.* 1997). The reduction in photosynthetic capacity after growth at high $p(CO_2)$ is attributed to lower concentrations of Rubisco, and is more pronounced at low N supplies (e.g. Rogers *et al.* 1996). Whether the reduction in Rubisco concentration is caused by accumulation of soluble carbohydrates is still a matter for debate (Moore *et al.* 1999; Stitt and Krapp 1999). Despite the occurrence of acclimation later in plant development, an increase in A at elevated $p(CO_2)$ observed soon after seedling emergence, or after transfer of plants to high $p(CO_2)$, accelerates development in the shoot apex and enhances leaf area growth (Jitla *et al.* 1997; Makino *et al.* 1997). These changes in A, soon after exposure to high $p(CO_2)$, are reflected in greater net assimilation and relative growth rates at the whole plant level, and are the forerunners to greater dry mass in the vegetative and reproductive organs later in development (Seneweera *et al.* 1994; Makino *et al.* 1997; Tjoelker *et al.* 1998). Rice is no exception, and increases in grain yield due to CO_2 enrichment have been demonstrated for plants grown

Abbreviations used: A, CO_2 assimilation rate; DAT, days after transplanting; FACE, free air CO_2 enrichment; J_{max}, photosynthetic electron transport rate estimated from gas exchange; $p(CO_2)$, CO_2 partial pressure; P_i, intercellular CO_2 partial pressure; SPS, sucrose phosphate synthase; V_{cmax}, maximal Rubisco activity estimated from gas exchange; VPD, vapour pressure deficit.

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in controlled environments, despite photosynthetic acclimation (Baker *et al.* 1990; Rowland-Bamford *et al.* 1991; Ziska and Teramura 1992; Seneweera *et al.* 1994).

The debate about photosynthetic acclimation to high $p(CO_2)$ has recently touched on the relationship between acclimation and changes in relationships between C source and sink activity in the plant. For example, Rogers *et al.* (1998) investigated photosynthetic acclimation to high $p(CO_2)$ in the Swiss FACE experiment with *Lolium perenne*. They used a defoliant to remove 89% of the canopy, thereby dramatically increasing the ratio of C sink to source activity. There was no acclimation to high $p(CO_2)$ immediately after defoliation, but it was observed later in regrowth when the ratio of sink to source activity had declined (Rogers *et al.* 1998). In another study in the same FACE experiment, the balance between the source and sink was estimated from the ratio of free amino acid to free hexose and sucrose concentrations in leaves (Isopp *et al.* 2000). This ratio declined during regrowth when sink demand decreased relative to leaf area (Isopp *et al.* 2000). It was also demonstrated that there is a high correlation between source/sink ratios and amounts of sucrose phosphate synthase (SPS), a key enzyme in the pathway of sucrose synthesis. Xu *et al.* (1994) altered source–sink balance in *Pisum sativum* by removing pods, and concluded that photosynthetic acclimation resulted from optimization of N deployment within the plant.

While the relationship between the C source and sink is important, the supply of N from the source to the sink is also likely to be a key consideration in photosynthetic acclimation, because Rubisco makes up such a large proportion of the total shoot N. Consequently, Paul and Driscoll (1997) showed that repression of *A* in *Nicotiana tabacum* due to C sink–source imbalances critically depends on the C to N ratio, rather than carbohydrate concentrations alone. In rice, there are dramatic shifts in the capacity of the source to supply C and N during plant development. In terms of C and N metabolism, the development of the rice plant can be roughly divided into two phases. The first phase, from seed germination to panicle initiation, is characterized by rapid tillering and leaf area development. The leaves are a large sink for both C (amino acid backbones) and N (for synthesis of photosynthetic proteins such as Rubisco). However, the demand for C for structural carbohydrates and starch is relatively small (Mae 1997). Production of amino acids in leaves increases ahead of protein synthesis (Arima 1995), and little soluble carbohydrates accumulate. Consequently, it can be expected that the amino acid to carbohydrate ratio would be high. Uptake of N is rapid, meeting the demand for protein synthesis. Therefore, N concentrations in the source leaves are high, and photosynthetic capacity is large.

The transition to the second phase of rice development, panicle initiation to grain maturity, is characterized by dramatic shifts in C and N metabolism. The panicles rather than the leaves are now the major sinks for C and N, which are used primarily for production of starch, structural C, and storage protein. Photosynthate is supplied from the leaves, which contribute 60–90% of the C in the developing grain (Mae 1997). Usually, the flag leaf, which reaches full expansion after flowering and senesces during grain filling, supplies a large proportion of the photosynthate to the grain. Plant N uptake is greatly reduced after panicle initiation, and the leaves, including the flag leaf, are the major source of N, contributing 60% of the N to grain filling (Mae 1997). The remarkable feature of the flag leaf is that it supplies photosynthate to the grain for starch production and remobilized N for synthesis of storage proteins. These are conflicting roles, because Rubisco is a source of N and also the first enzyme in the pathway of CO_2 fixation (Mae 1997).

At elevated $p(CO_2)$, rice leaf N concentrations are generally reduced during vegetative and reproductive phases due to the greater carboxylation efficiency of Rubisco (Makino *et al.* 1997). Grain yield is enhanced by high $p(CO_2)$, increasing the demand for N for grain filling (Baker *et al.* 1990; Seneweera *et al.* 1994; Ziska *et al.* 1996). Consequently, the dramatic shifts after panicle initiation in the balance between C and N supply from the source and sink demand for C and N are likely to be accentuated at high $p(CO_2)$. This change in balance will undoubtedly influence photosynthetic acclimation during development, and may explain why Aben *et al.* (1999) found no acclimation prior to panicle initiation, whereas acclimation was demonstrated after panicle initiation in wild-type rice (Nakano *et al.* 1997) and Rubisco antisense plants (Makino *et al.* 2000)

Establishment of the first rice FACE experiment, in northern Japan provided an excellent opportunity to explore photosynthetic acclimation during development without complications arising from restricted root growth due to small pot size (Arp 1991). Given the importance of rice as a staple food for much of the world's population, this FACE experiment was established with the core objective of investigating the effects of elevated $p(CO_2)$ on rice growth, yield and ecosystem processes. The $p(CO_2)$ in the FACE treatments was targeted to be 20 Pa above ambient (~ 59 Pa), because increases of this magnitude are expected during the first half of this century. In contrast, in most controlled environment experiments studying acclimation, $p(CO_2)$ is increased to about 66–100 Pa (Poorter 1993).

In this study, we examine the hypothesis that there is no photosynthetic acclimation to high $p(CO_2)$ during the first phase of rice growth (seedlings to panicle initiation), and that acclimation occurs only in the second phase of development, after panicle initiation. The eighth leaf, which appears before panicle initiation during the rapid tillering stage, was used to represent the first phase, and the flag leaf, which expands after panicle initiation and senesces during grain filling, represents the second phase. We also report on plant growth responses to elevated $p(CO_2)$.

Materials and methods

FACE site and growth conditions

The rice FACE site is located in Shizukuishi, Iwate prefecture, in northern Honshu, Japan (39°38′ N, 140°57′ E). It is situated in a valley at an altitude of about 200 m. Over the year, daily average temperatures vary from –2.5 (January) to 23.3°C (August). Volcanic ash soils dominate in this area. This site was chosen because it is a typical agro-ecosystem in a region that produces the largest quantity of rice in Japan. The cultivar used, Akitakomachi, which is commonly grown in the region because of its superior grain quality, has a medium yield potential and is not highly responsive to N fertilization (Hatakeyama 1985). One crop is grown each season from May, when seedlings are transplanted into the field, until late September when the grain is harvested. Cultivation of rice in FACE followed agronomic techniques typical of the region.

The FACE experiment was carried out for 3 years from 1998 to 2000, of which the 1999 results are addressed in this paper. There were eight plots arranged in four replicates of two $p(CO_2)$ levels: ambient CO_2 and FACE. The FACE plots were maintained between 26 and 32 Pa $p(CO_2)$ above the ambient $p(CO_2)$ by injecting pure CO_2 into air from octagonal FACE rings 12 m across (Okada *et al.* 2001). The ambient plots had no CO_2 injection and, hence, had season-long average daylight hours $p(CO_2)$ of 37.3 Pa.

Rice seedlings were grown in trays in greenhouses for 23 d, commencing on 27 April. The seedlings to be planted in FACE plots were grown in a greenhouse with 20 Pa $p(CO_2)$ above ambient $p(CO_2)$, whereas the seedlings for the ambient plots were grown in another greenhouse under ambient $p(CO_2)$. The CO_2-enriched greenhouse had mean $p(CO_2)$ of 58 Pa and mean air temperature of 18.6°C, whereas that without CO_2 addition had mean $p(CO_2)$ of 38 Pa and mean air temperature of 18.3°C. Seedlings were hand-planted in groups of three plants per hill in the FACE and ambient plots on 20 May. There were approximately 19 hills m^{-2} (Kim *et al.* 2001). Each of the FACE and ambient plots had subplots of three N-supply levels. N was supplied as $(NH_4)_2SO_4$ at 4 (low), 9 (medium) and 15 (high) g N m^{-2}, with 63% of N applied as a basal dressing, 20% at mid tillering, and 17% at panicle initiation as top dressing. Phosphorus and potassium were applied at the rate of 48 P_2O_5 g m^{-2} and 15 K_2O g m^{-2} across the N-supply levels as a basal dressing. Polyvinyl chloride (PVC) barriers were used to minimize mixing of paddy water between N treatments. The soil was flooded prior to transplanting, and the water level was maintained at 3–4 cm above soil level throughout the experiment, except when it was drained for a week in mid July. CO_2 enrichment in the FACE plots commenced immediately after transplanting, and took place during the day and night.

Plant growth

Plant samples from medium-N plots were taken on 62 (panicle initiation) and 123 (maturity) d after transplanting (DAT). For the low-N treatment, sampling was at 61 and 124 DAT, and for high-N it was at 63 and 124 DAT. Three hills from low-N, 12 from medium-N, and 8 from high-N treatments were taken for estimation of dry mass. The shoots were separated from the roots, and tiller numbers per hill were counted. The leaf blades were separated from the leaf sheaths and stems, and green leaf area was measured using a leaf area meter (Model AAM-9; Hayashi Denko Co. Ltd, Tokyo, Japan). Each plant fraction was oven-dried for 72 h at 80°C prior to estimation of dry mass.

Gas exchange measurements

Measurements were made in the field at 32–36 and 76–80 DAT on the last fully expanded leaf (eighth leaf and flag leaf, respectively) on the main culm of three plants from the three N treatments from two FACE plots and two ambient plots (six plants from each CO_2 treatment). The leaves were carefully chosen to ensure that they had reached full expansion prior to measurement. Measurements were made first in the FACE plots, and then 2 d later in the ambient plots. This time gap should have accounted for the accelerated development of plants in the FACE plots (Kobayashi *et al.* 2001). Gas exchange was measured using two portable photosynthesis systems (LI-6400; LI-COR, Lincoln, NE, USA). The $p(CO_2)$ was controlled with the LI-COR CO_2 injection system, and an irradiance of 1700 μmol photons m^{-2} s^{-1} was supplied by an in-built LED lamp (red/blue). The temperature in the 6-cm^2 chambers was set at 25°C, and the leaf temperature, measured by a thermocouple, ranged from 24.5 to 25.5°C. The vapour pressure deficit (VPD) at the leaf surface was between 1.9 and 2.1 kPa. Measurements were made between 10:00 and 15:00 local time, and each leaf was allowed 10–15 min to reach a steady state before measurements were taken. Initial linear slopes of *A versus* P_i response curves were used to estimate V_{cmax} according to Farquhar (1980), incorporating the temperature correction factor. J_{max} was calculated according to von Caemmerer and Farquhar (1981).

Concentrations of soluble protein, Rubisco, carbohydrate, free amino acid, SPS and N

Immediately after the gas exchange measurements were completed, the leaf was detached and five 2 cm long segments were excised (excluding the tip and base) and their width measured for calculation of area, prior to storage in liquid N_2. These samples were used for biochemical measurements. Three of the six samples taken from each N treatment from the two replicates of FACE and ambient plots were used for estimation of concentration of: Rubisco, soluble protein and SPS; soluble carbohydrates and amino acids. All six samples from each treatment combination were used for determination of total N and C concentration.

For soluble protein, Rubisco and SPS protein determination, each segment was ground in liquid N_2, and soluble protein was extracted in buffer containing NaH_2PO_4 (100 mM, pH 7), phenylmethylsulfonyl fluoride (1 mM), polyvinylpyrrolidone (1% w/v) and β-mercaptoethanol (1% v/v). The extract was centrifuged, and the soluble protein concentration of an aliquot of the supernatant was measured according to Bradford (1976). For Rubisco, an aliquot containing 2 μg of soluble protein was loaded onto a polyacrylamide (12.5% w/v) gel containing SDS (0.1% w/v). After separating the proteins by electrophoresis, gels were stained with Coomassie brilliant blue, and the large subunit of Rubisco was quantified using image analyser (UMAX PowerLook 2000; UMAX Data System Inc., Dallas, TX, USA). For SPS, 25 μg soluble protein was loaded onto SDS–PAGE (polyacrylamide, 10% w/v; SDS, 0.1% w/v). After electrophoresis, proteins were transferred to a nitrocellulose membrane (Protran; Schleicher and Schuell, Keene, NH, USA), and probed with a rabbit polyclonal antibody raised against maize SPS and alkaline phosphate-conjugated goat anti-rabbit immunoglobulin G antibody (Bio-Rad, Hercules, CA, USA). The amount of SPS protein was quantified using an image analyser, and expressed on relative amount on a soluble protein basis.

For soluble carbohydrate and amino acid determination, leaf segments were ground in liquid N_2 and extracted with boiling 80% ethanol. The mixture was centrifuged, and the residue was re-extracted twice with boiling ethanol. The supernatants were combined, and an aliquot was used to measure free amino acid concentration (Moore and Stein 1948). The method was slightly modified for rice. Aliquots (50 μL) of the combined supernatants were mixed with 1.5 mL of citric acid buffer (0.3 M citric acid, 0.5 M NaOH), after which 1.2 mL of ninhydrin reagent (50 mM ninhydrin, 2 mM ascorbic acid in methoxyethanol) was added. The mixture was heated in a boiling water bath for 20 min, after which 3 mL of 60% (v/v) ethanol was added.

After cooling, the absorbance was measured at 570 nm. Standards containing a mixture of asparagine and glutamine were treated in the same way as the plant extracts, and the concentration of the latter was determined. The remaining alcohol extract was vacuum dried to remove ethanol, and the residue was resuspended in water. An aliquot was used to measure sucrose and soluble carbohydrate concentrations. Sucrose was assayed enzymatically using a Boehringer-Mannheim kit (catalogue No. 716260; Germany). Soluble carbohydrate concentration was measured according to Yemm and Willis (1954). For total N determination, leaf segments were dried at 80°C and ground before being analysed using a Carbon and Nitrogen Analyser (Sumigraph NC-900; Sumika Chemical Analysis Service Co. Ltd, Chiba, Japan). Soluble protein, soluble sugar, free amino acids, and sucrose concentrations are expressed g^{-1} N.

Statistical analysis

For dry mass measurements, 3–12 hills (three plants per hill) at each N supply were sampled from all four replicates, and the results were analysed with ANOVA as a randomized complete block design with split plots for the N-supply levels. For logistical reasons, the measurements of photosynthetic and biochemical parameters were carried out in only two replicates. Each individual leaf measurement was considered as a separate replicate in the ANOVA (SAS 1998).

Results

Plant development

The first growth phase of the rice crop, from transplanting to panicle initiation, corresponded to the period between 0 and 62 DAT. Mid-tillering occurred between 25 and 35 DAT, and the eighth leaf had fully expanded during this period. The second phase, from panicle initiation to maturity, was from 62 to 123 DAT. During this phase, the flag leaf was fully expanded at about 76 DAT. Development was slightly accelerated by CO_2 enrichment, and flowering was about 2 d earlier in FACE than in ambient plots (Kobayashi *et al.* 2001).

Plant growth

Shoot dry mass was increased by higher N supplies at panicle initiation and maturity (Table 1). Across all N-supply levels, the shoot dry mass was greater in FACE relative to ambient treatment (43 and 16%, respectively) for the two harvests (Table 1). At panicle initiation, the magnitude of the CO_2 effect was greater with high N supplies (41, 45 and 49% in low-, medium- and high-N treatments, respectively), but the interaction between the effects of $p(CO_2)$ and N supply was not significant (Table 1). At maturity, the response of shoot dry mass to high $p(CO_2)$ was smaller than at panicle initiation (11, 15 and 19% at low-, medium- and high-N treatments, respectively). At panicle initiation, elevated $p(CO_2)$ increased the number of tillers by an average of 16%, and enhanced leaf area and mass by an average of about 25% (Table 1). These effects of elevated $p(CO_2)$ on tillering and leaf growth had diminished at maturity (Table 1).

Table 1. Number of tillers, leaf area, leaf and shoot mass of plants at panicle initiation and maturity in ambient and FACE plots

Values are means of variables across the four replicates, and the statistical significance level (P) for the effects of $p(CO_2)$ treatment, N-supply level, and their interaction

Growth stage	N supply	$p(CO_2)$	Number of tillers ($hill^{-1}$)	Leaf area ($\times 10^2$ m^{-2} $hill^{-1}$)	Leaf mass (g $hill^{-1}$)	Shoot mass (g $hill^{-1}$)
Panicle initiation	Low	Ambient	27	15.8	7.6	17.2
		FACE	28	18.1	9.0	24.2
	Medium	Ambient	26	17.8	8.4	20.0
		FACE	32	23.7	10.9	29.0
	High	Ambient	28	19.4	9.8	21.3
		FACE	33	23.5	12.7	30.6
		Effects		P (CO_2)		
		$p(CO_2)$	0.008	0.017	0.001	0.000
		N	0.080	0.003	0.000	0.001
		$p(CO_2) \times N$	0.198	0.303	0.140	0.566
Maturity	Low	Ambient	22	11.0	5.4	69.9
		FACE	22	10.0	5.2	77.7
	Medium	Ambient	24	11.8	5.4	77.2
		FACE	27	11.1	5.8	89.6
	High	Ambient	27	16.2	7.0	78.8
		FACE	29	15.0	7.1	94.1
		Effects		P (CO_2)		
		$p(CO_2)$	0.166	0.177	0.200	0.017
		N	0.001	0.000	0.000	0.003
		$p(CO_2) \times N$	0.978	0.882	0.608	0.482

Table 2. N concentrations in eighth and flag leaves of rice grown in ambient or FACE plots

Leaves were sampled during the first phase of growth (transplanting to panicle initiation) at 32–36 DAT, and during the second phase (after panicle initiation) at 76–80 DAT. Values are means of 6–8 plants from two plots from each N treatment, expressed on an area basis with standard error

N treatment	$p(CO_2)$	N concentration (g m^{-2}) Eighth leaf	Flag leaf
Low	Ambient	2.7 ± 0.12	1.5 ± 0.06
	FACE	2.4 ± 0.13	1.2 ± 0.08
Medium	Ambient	2.7 ± 0.11	1.9 ± 0.18
	FACE	2.4 ± 0.19	1.6 ± 0.14
High	Ambient	2.7 ± 0.26	1.7 ± 0.14
	FACE	2.5 ± 0.31	1.6 ± 0.08

Leaf N concentration

At 32 DAT, N concentrations in the eighth leaf were unaffected by N supply, but tended to be lower at high $p(CO_2)$, although the effect was not significant (Table 2). At 76 DAT, N concentrations in the flag leaf were greater in the high-N and medium-N than in the low-N treatments, and were decreased by elevated $p(CO_2)$. The N to C ratios of the eighth leaf were unaffected by N treatment, and were 0.12 ± 0.002 and 0.12 ± 0.003 for the ambient and FACE treatments, respectively. The ratio decreased to an average of 0.073 ± 0.003 and 0.066 ± 0.003 for the same two treatments at 76 DAT.

Gas exchange parameters and Rubisco concentration

The response of A to P_i for the eighth and flag leaves is shown in Fig. 1. A was higher for the eighth leaf than the flag leaf at each P_i, especially for the FACE plants (Figs 1 and 2). There was strong evidence of acclimation to high $p(CO_2)$ in the flag leaves because at every P_i, A was lower for the FACE-grown plants compared with those grown at ambient $p(CO_2)$ (Fig. 1). This was not observed for the eighth leaf. Because there was no significant difference in N concentration due to the N treatments in the eighth leaf, and there was a small range of N concentrations in the flag leaf at each $p(CO_2)$, it was not possible to obtain a clear picture of the response of the photosynthetic parameters to varying N supply (Table 2). However, the trend was for a linear response of A and other parameters to increasing leaf N concentration. To simplify interpretation of the results, data for photosynthetic and biochemical measurements were expressed as g g^{-1} N, and pooled within CO_2 treatments (Tables 3, 4; Fig. 3), and A/N was calculated from the data of A in which the plants were grown and measured [i.e. at 39 or 59 $p(CO_2)$]. During the first phase of growth (vegetative phase), A/N of leaves of plants from FACE plots was higher than that of leaves from ambient plots (Fig. 3). In contrast, A/N of flag leaves was lower in plants from FACE than those from ambient plots. The reason for this difference was that V_{cmax}/N was significantly lower in flag leaves of plants from the FACE plot compared with those grown at ambient $p(CO_2)$ (Table 3). J_{max}/N was also lower in flag leaves from FACE relative to ambient CO_2 plots, but the opposite trend

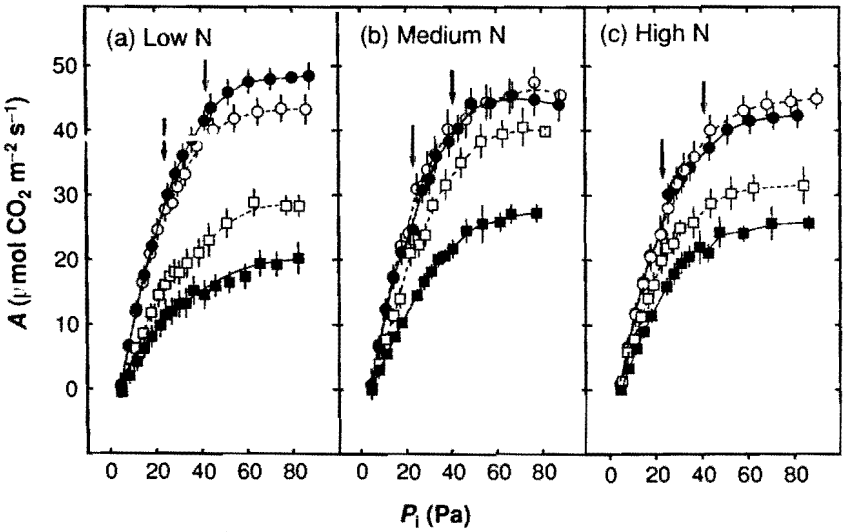

Fig. 1. Response of A to increasing P_i for eighth (○ and ●) and flag leaves (□ and ■) of rice grown in ambient (○ and □) or FACE (● and ■) plots at either low (*a*), medium (*b*) or high (*c*) N supplies. Arrows indicate growth $P_i(CO_2)$ concentration. Gas exchange measurements were made during the first phase of growth (transplanting to panicle initiation) at 32–36 DAT, and during the second phase (after panicle initiation) at 76–80 DAT. Values are means of 6–8 plants from two plots from each N treatment. Vertical bars represent one standard error.

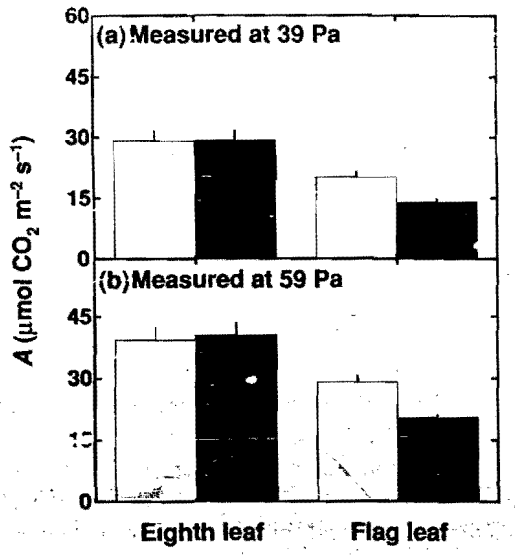

Fig. 2. *A* of eighth and flag leaves of rice grown in ambient (open bars) or FACE (solid bars) plots. Measurements were made at 32–36 and 76–80 DAT. Values are means of 6–8 plants (pooled between N treatments) for each of two CO_2 treatments. Vertical bars represent one standard error.

was observed for the eighth leaf (Table 3). However J_{max}/V_{cmax} was higher at elevated $p(CO_2)$ in both leaf types, but only significantly in the eighth leaf (Table 3).

Concentrations of soluble proteins, free amino acids, sucrose and SPS

As with gas exchange measurements, the concentrations of carbohydrates and N compounds are expressed g^{-1} N because of the small differences between leaf N concentrations within the N treatments. Soluble protein concentrations expressed on this basis tended to be higher in flag leaves than in eighth leaves, and were reduced by elevated $p(CO_2)$ (Table 4). Free amino acid concentrations were similar at both stages of development, but were reduced by high $p(CO_2)$ in the eighth leaves only (Table 4). Concentrations of soluble sugars and sucrose (expressed on an N basis) were lower in the eighth leaf than the flag leaf, and were increased by elevated $p(CO_2)$, except in the case of soluble sugars in the eighth leaf. The ratios of soluble protein/sucrose and amino acid/sucrose were higher in eighth leaf than the flag leaf, and were decreased by elevated $p(CO_2)$ (Table 4).

Table 3. Gas exchange parameters and concentrations of Rubisco (on an N basis) in the eighth and flag leaves of rice grown in ambient and FACE plots

Values are means of 18 plants (pooled between N treatments) for each of two ambient and FACE plots. Asterisks indicate significant differences between CO_2 treatments. *, $P \leq 0.05$; **, $P \leq 0.001$; ns, not significant

Measurement	$p(CO_2)$	Leaf number	
		Eighth leaf	Flag leaf
V_{cmax}/N (μmol s^{-1} g^{-1} N)	Ambient	56.69	60.95
	FACE	54.26 ns	48.50**
J_{max}/N (μmol s^{-1} g^{-1} N)	Ambient	74.0	104
	FACE	86.2*	90.1*
Rubisco/N (g g^{-1} N)	Ambient	0.94	1.10
	FACE	0.65**	0.89 ns
J_{max}/V_{cmax}	Ambient	1.46	1.63
	FACE	1.56*	1.82 ns
V_{cmax}/Rubisco (μmol s^{-1} g^{-1} Rubisco)	Ambient	55.2	37.9
	FACE	87.7**	47.3 ns

Discussion

There is strong support for the hypothesis that photosynthetic acclimation of field-grown rice occurs at elevated $p(CO_2)$ only after panicle initiation. During this phase of development, dry mass response to elevated $p(CO_2)$ was higher, and was accompanied by increased tiller number and leaf area. However, this was not sustained for longer, due to changes in source–sink balance at late ontogeny (Table 1). In this phase, the flag leaf is fully expanded, grain development commences, and the demand for remobilized N from the flag leaf is high (Mae 1997). In the flag leaf, photosynthetic acclimation occurred at all N supplies, but was more pronounced at medium N where reductions in N concentrations due to high $p(CO_2)$ were greatest (Figs 1, 2; Tables 2, 3). However, differences between N treatments in leaf N concentrations were small in the flag leaf and

Table 4. Concentrations of soluble proteins, carbohydrates, amino acids and SPS (on an N basis) in the eighth and flag leaves of rice grown in ambient or FACE plots

Values are means of 18 plants (pooled between N treatments) for each of two ambient and FACE plots. Soluble protein, soluble sugar, free amino acids, and sucrose are expressed g^{-1} N. SPS proteins are expressed (relative amount) g^{-1} soluble protein. Asterisks indicate significant differences between CO_2 treatments. *, $P \leq 0.05$; **, $P \leq 0.001$; ns, not significant

Measurement	Growth pCO_2	Leaf stage	
		Eighth leaf	Flag leaf
Soluble proteins (g g^{-1} N)	Ambient	2.7	3.23
	FACE	2.3 ns	2.45*
Free amino acids (g g^{-1} N)	Ambient	0.14	0.18
	FACE	0.12*	0.17 ns
Soluble sugar (g g^{-1} N)	Ambient	1.30	2.72
	FACE	1.48 ns	3.79*
Sucrose (g g^{-1} N)	Ambient	0.45	0.95
	FACE	0.52*	1.22**
SPS (relative amount)	Ambient	1.00	1.00
	FACE	1.19**	1.16**
Soluble protein/sucrose	Ambient	5.9	3.4
	FACE	4.4 ns	2.0**
Amino acid/sucrose	Ambient	0.31	0.18
	FACE	0.23*	0.13**

negligible in the eighth leaf (Table 2). For the flag leaf, A/N was lower in plants from the FACE treatment, indicating that reductions in leaf N concentrations did not fully account for acclimation (Fig. 3). In the eighth leaf, A/N was greater in plants grown at elevated $p(CO_2)$, possibly because of a larger demand for C to support rapid leaf growth at high $p(CO_2)$ (Table 1; Fig. 3). Lower Rubisco activity (V_{cmax}/N) accounted for acclimation of the flag leaf at elevated $p(CO_2)$ (Table 3). Electron transport capacity (J_{max}/N) was also influenced by growth at elevated $p(CO_2)$. However, the ratio of electron transport capacity to maximum Rubisco activity (J_{max}/V_{cmax}) was generally unaffected by $p(CO_2)$ (Table 3). In the New Zealand FACE experiment, in which C_3 pasture species were grown at 36 or 47.5 Pa CO_2, photosynthetic acclimation also resulted from a reduction in V_{cmax}, but J_{max}/V_{cmax} was unaffected (von Caemmerer *et al.* 2001). New Zealand results and data presented here show that acclimation occurs even when root growth is unrestricted under increased $p(CO_2)$, though the increase in $p(CO_2)$ is marginal.

Elevated $p(CO_2)$ altered the balance between concentrations of C and N compounds in both the eighth and flag leaves, although these changes were not directly associated with acclimation (Table 4). In the flag leaf, soluble protein concentrations were reduced by elevated $p(CO_2)$, and the concentrations of soluble carbohydrates, sucrose and SPS protein were higher (Table 4). Consequently, the ratios of soluble protein and amino acid to sucrose concentrations were lower. Soluble sugar concentrations were only reduced in the flag leaf by high $p(CO_2)$ (Table 4). In the Swiss FACE experiment with *L. perenne*, lower ratios of amino acid to sucrose concentrations were correlated with reduced sink activity (Isopp *et al.* 2000). In our FACE experiment, this ratio was greater in the eighth than the flag leaf, and was reduced by elevated $p(CO_2)$ in both leaves (Table 4). The N to C ratio also declined from 0.11 in both CO_2 treatments at 32 DAT, to 0.073 and 0.066 at 76 DAT in ambient and FACE treatments, respectively. Prior to panicle initiation, the actively growing leaves are strong sinks for C from the source leaves (Mae 1997). It might be expected that elevated $p(CO_2)$ would increase or match the demand for C because of increases in dry mass and leaf area (Table 1; Fig. 3). Differences between species in their demand for N, as well as C, at elevated $p(CO_2)$ could explain the variation in the relationship between sink activity and the ratio of amino acid to sucrose concentration. Increased SPS protein in rice indicates the importance of SPS in regulating increased C flux under $p(CO_2)$. Our data were consistent with the results of the FACE experiment with *L. perenne* where SPS was expressed on the soluble protein basis (Isopp *et al.* 2000).

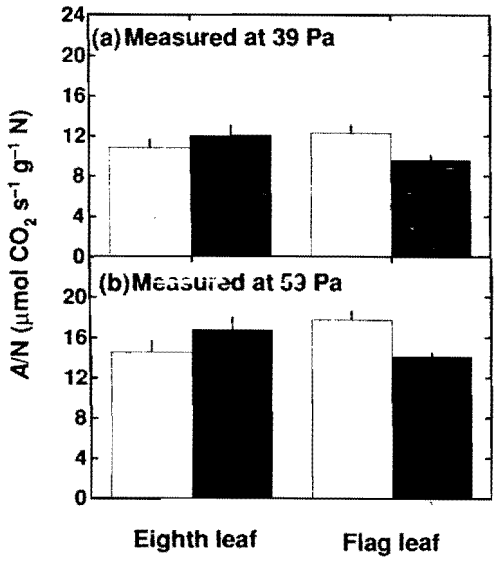

Fig. 3. A/N of eighth and flag leaves of rice grown in ambient (open bars) or FACE (solid bars) plots. Measurements were made at either 39 or 59 Pa CO_2 at 32–36 and 76–80 DAT. Values are means of 6–8 plants (pooled between N treatments) for each of two CO_2 treatments. Vertical bars represent one standard error.

There are several possible explanations for acclimation of rice at elevated $p(CO_2)$. The first is that acclimation may only occur below a threshold leaf N concentration. The range of leaf N concentrations differed at 32 and 76 DAT (2.4–2.7 g m^{-2} for the eighth leaf and 1.2–1.9 g m^{-2} for the flag leaf). We cannot rule out the possibility that acclimation only occurs in the lower range of leaf N concentrations. However, a previous study with rice showed that reductions in A due to high $p(CO_2)$ occurred over a range of about 1–2.5 g N m^{-2} for plants grown in controlled environments (Nakano *et al.* 1997). Consequently we think this explanation is unlikely.

A second explanation is that acclimation simply reflects accelerated leaf and/or plant development at high $p(CO_2)$, and that measurements made at the same chronological time do not coincide with phenological age (Pearson and Brooks 1995; Miller *et al.* 1997; Kauder *et al.* 2000; von Caemmerer *et al.* 2001). We took care to minimize the influence of faster development at high $p(CO_2)$ by measuring leaf A in the ambient treatment 2 d later than in the FACE treatment. This should have accounted for accelerated plant growth at high $p(CO_2)$ because flowering was about 2 d earlier (Kobayashi *et al.* 2001). However, we cannot be certain of this because A and Rubisco concentrations of rice grown at ambient $p(CO_2)$ reach a maximum 20 d after leaf emergence, while leaf expansion is complete 10 d after emergence (Mae and Ohira 1982; Makino *et al.* 1984). It is also possible that the developmental pattern of the flag leaf from emergence to full expansion and senescence is faster at elevated $p(CO_2)$, or that high $p(CO_2)$ accelerates senescence only. Experiments with wheat show that reductions in N and Rubisco concentrations due to high $p(CO_2)$ in the expanded fourth leaf (Rogers *et al.* 1996) are caused by reduced Rubisco synthesis in the elongation zone of the developing

leaf (S. Seneweera, unpublished data). This lends support to the proposal that accumulation of carbohydrates in the leaf reduces levels of the message (mRNA) for synthesis of the small subunit of Rubisco. In this experiment, soluble sugar concentrations were higher at elevated $p(CO_2)$ in the flag leaf but not the eighth leaf, but sucrose concentrations were greater at high $p(CO_2)$ at both developmental stages (Table 4).

The third and most likely explanation for acclimation is that supply from the source and demand by the sink for C and N vary during development of the rice plant. We favour this explanation because it is probable that regulation of Rubisco synthesis and activity during leaf development occurs as a result of changes in source–sink balance. At 32 DAT, at both ambient and elevated $p(CO_2)$, tillering and leaf area development were rapid, and there would have been a large demand for C and N for protein and carbohydrate synthesis, particularly at high $p(CO_2)$ (Mae 1997). However, N uptake was great enough to meet the demands for accelerated growth at high $p(CO_2)$, and leaf N concentrations were little affected (Table 2). Consequently, C supplied as photosynthate from the source leaves, and N coming from current uptake plus remobilized N from older leaves, was sufficient to meet the demand of the growing leaves in ambient and FACE treatments. Measurements of acquisition and allocation of dry mass and N in the FACE experiment support this conclusion (S. Miura, H. Kim, M. Lieffering, M. Okada and K. Kobayashi, unpublished data). After panicle initiation N uptake dropped dramatically, and panicles rather than leaves were major sinks for N and photosynthate. The flag leaf makes a large contribution of N and C to the developing grain (Mae 1997). Further, growth under FACE increased the number of panicles (9%), fertile spikelets (9%) and grain yield (14%) per square metre for the medium N supply (Kim *et al.* 2001), indicating that there was higher demand for C and N for grain development. However, the amount of N uptake after panicle initiation was smaller in FACE plots than in ambient plots (S. Miura, H. Kim, M. Lieffering, M. Okada and K. Kobayashi, unpublished data). This imbalance between the demand and supply of N was resolved by reallocating a large fraction of leaf N to panicles in the FACE experiment relative to the ambient $p(CO_2)$ experiment. This remobilization of leaf N is likely associated with the lower Rubisco concentrations under FACE due to reduced synthesis during leaf expansion and/or accelerated degradation during senescence. Based on results of an open-top chamber experiment with *Hordeum vulgare*, Fangmeier *et al.* (2000) suggested that elevated $p(CO_2)$ enhanced leaf senescence by increasing the N sink capacity of the grain. In the Swiss FACE experiment with ryegrass, Rogers *et al.* (1998) showed that photosynthetic acclimation to FACE occurred only at late ontogeny when the ratio of source to sink activity had declined.

We conclude that for field-grown rice, the greater demand for N for grain development under FACE compared with ambient $p(CO_2)$ reduces the Rubisco concentration of leaves including the flag leaf, and this explains photosynthetic acclimation. Acclimation does not occur at an early stage (eighth leaf) prior to panicle initiation, because the supply of N from current uptake matches the demand for vegetative growth.

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