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Seasonal changes in the effects of free-air CO_2 enrichment (FACE) on nitrogen (N) uptake and utilization of rice at three levels of N fertilization

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Abstract

Over time, the relative effect of elevated [CO_2] on the photosynthesis and dry matter (DM) production of rice crops is likely to be changed with increasing duration of CO_2 exposure, but the resultant [CO_2] effects on rice N concentration, uptake, efficiency and allocation remain unclear, especially under different soil N availability. Therefore, we conducted a free-air CO_2 enrichment (FACE) experiment at Wuxi, Jiangsu, China, in 2001–2003. A japonica cultivar with large panicle was grown at ambient or elevated (ca. 200 μmol mol^{-1} above ambient) [CO_2] under three levels of N: low (LN, 15 g N m^2), medium (MN, 25 g N m^2) and high N (HN, 35 g N m^2 (2002, 2003)). The MN level was similar to that recommended to local farmers. Averaged across all N levels and years, shoot N concentration (dry base) was lower under FACE by 1.8%, 6.1%, 12.2%, 14.3%, 12.1%, and 6.9% at early-tillering, mid-tillering, panicle initiation (PI), booting, heading and grain maturity, respectively. Shoot N uptake under FACE was enhanced by 46%, 38%, 6% and 16% on average during the growth periods from transplanting to early-tillering (period 1), early-tillering to mid-tillering (period 2), mid-tillering to PI (period 3) and heading to grain maturity (period 5), respectively, but slightly decreased by 2% in the period from PI to heading (period 4). Seasonal changes in crop response to FACE in ratio of shoot N uptake during a given growth period to that over the whole season followed a similar pattern to that of shoot N uptake, with average responses of 33%, 26%, −3%, −11% and 10% in periods 1–5 of the growth period, respectively. As a result, FACE increased final aboveground N uptake by 9% at maturity. FACE greatly reduced the ratio of leaf to shoot N content over the season, while allocation of N to stems and spikes showed an opposite trend. FACE treatment resulted in the significant increase in N use efficiency for biomass (NUEp) over the season except at early-tillering and in N use efficiency for grain yield (NUEg) at grain maturity. These results indicate that, in order to maximize grain output in a future high [CO_2] environment, the recommended rates, proportion and timing across the season of N application should be altered, in order to take full advantage of strong N uptake capacity during the early growth period and facilitate N uptake after that.

Keywords: Free-air CO_2 enrichment (FACE); Nitrogen concentration; Nitrogen uptake; Nitrogen allocation; Nitrogen efficiency; Rice

1. Introduction

Global atmospheric CO_2 concentration is increasing, increasing the productivity of crops as higher [CO_2] enhances plant photosynthesis. Nitrogen (N) is the most important element that not only is the biggest amount of elements that plants absorb from the soil, but also contributes to the plant growth and development more than other nutritional elements (Ingestad, 1981; Crawford and Glass, 1998), and may play a role in the response of plant to [CO_2]. Studies with cereal crops suggest that N can have effect on the responses of crop growth to elevated [CO_2] (Kimball et al., 1995, 2002; Kim et al., 2001, 2003a,b); however, very few reports are made on the effects of elevated [CO_2] in combination with N availability on the seasonal N nutrient response of rice (*Oryza sativa* L.), the world's most important cereal crops (Ziska et al., 1996; Kim et al., 2001, 2003a). Ziska et al. (1996) grew plants until flowering inside open-top chambers placed within paddies and found the concentration of aboveground N decreased at elevated [CO_2] regardless of N treatments (0, 9, 20 g m^{-2}) and N uptake increased with elevated [CO_2] early in crop development but by the end of the experiment N uptake was similar for both CO_2 treatments. In the case of FACE experiment, we know only a single investigation on rice plants

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by Kim et al. (2001, 2003b), which showed that FACE decreased leaf N concentration of rice plants supplied MN (8 or 9 g N m^{-2}) over the season, while it increased N uptake of rice plants supplied MN and HN (15 g N m^{-2}) at panicle initiation (PI) but not at maturity; however, these authors give us little detailed information on seasonal changes in response of aboveground N concentration, N uptake and N use efficiency over the whole growth season (i.e. from transplanting to grain maturity). There is also no systemic information on the effect of CO_2-enrichment on N distribution patterns among different organs of rice as affected by N application.

Enclosures have been shown to affect microclimatological factors which may influence the response of plants to elevated [CO_2] (McLeod and Long, 1999). The free-air CO_2 enrichment (FACE) techniques were developed in the past decade (e.g. Kimball, 1992; Okada et al., 2001), providing undisturbed field conditions and more reliable measurements in comparison with enclosures (Kimball et al., 1997). The Chinese Rice/Wheat FACE platform, which is also the second Rice FACE system in the world, have been set up and operated in June 2001, with the objective of investigating the effects of FACE on rice growth, yield, quality and ecosystem processes under field conditions. As in the Japanese Rice FACE project (also first rice FACE facility in the world), rice crops were grown from transplanting to grain maturity under two levels of [CO_2] (ambient and ambient plus 200 μmol mol^{-1}) (Kim et al., 2001, 2003a,b). In contrast, however, according to the actual Chinese rice production, a cultivar with larger panicle (average 155 spikelets per panicle, while it was mean 81 spikelets per panicle in the Japanese Rice FACE trial) that has been used in large-scale production in China was tested and three relatively higher N levels (15, 25 and 35 g N m^{-2}, compared with 4, 8 or 9 and 12 or 15 g N m^{-2} in the Japanese Rice FACE trial) were supplied. In addition to this, the cultivation technique (such as N application strategy and irrigation management) and environmental conditions (such as air temperature and incident solar radiation) in our experiment were also quite different from those in Japanese Rice FACE experiment. The aims of the present study were to determine the effects of FACE on N concentration, N uptake, N efficiency and N allocation on the whole shoot basis under field condition, and to determine if the effects of FACE on these N parameters change with crop development. The results obtained here could provide important implications on N application strategies to sustain rice productivity in a future elevated [CO_2] world.

2. Materials and methods

2.1. Experiment site description and meteorology

The FACE experimental system was located at the Nianyu Farm of Wuxi, Jiangsu province, China (31°37′N, 120°28′E), where the soil is classified as stagnic anthrosols (local name, huangni soil). A rice–wheat rotation system prevails in this region. The details of soil properties, climate and regional agricultural practices can be found in previous publications (Yang et al., 2006a,b).

2.2. FACE system

The China Rice FACE system has eight rings located in different paddies having similar soils and agronomic histories. Three replicate plots were randomly allocated for the elevated CO_2 treatments (hereinafter called FACE plots) and five for the ambient treatments (hereinafter referred to as ambient plots). More replicates for the ambient condition were set to well cover the spatial variability in soil or water management. Each replicate plot was ca. 80 m^2. In the FACE plots, crops were grown within 12.5 m diameter 'rings' which sprayed pure CO_2 both day and night throughout the growing season except for a few days during transplantation towards the plot centre from eight peripheral emission tubes (5 m long) located about 50–60 cm above the canopy. In the ambient plots, plants were grown under ambient [CO_2] without ring structures. The target [CO_2] in the FACE plots throughout the rice growth season was controlled to 200 μmol mol^{-1} above that of ambient by computer system platform according to ambient CO_2 concentration variation, wind direction, wind speed, canopy height and day-night changing. Adjacent plots were buffered to avoid treatment cross-over. Details of the design, rationale, operation, and performance of the CO_2 exposure system used in this study can be found in Okada et al. (2001) and Liu et al. (2002).

2.3. Crop cultivation

A japonica cv. Wuxiangging 14 tested in the experiment was a major local cultivar with large panicle (average 155 spikelets per panicle) and a high-yielding potential. Standard cultivation practices as commonly performed in the area were followed in all experimental plots. Rice seeds were sown on 18 May. The seedlings for the ambient plots were grown under ambient [CO_2] conditions, while those for the FACE plots were grown under elevated [CO_2] conditions. On 13 June, the seedlings were manually transplanted at a density of three seedlings per hill into the FACE and ambient plots. Spacing of the hills was 16.7 by 30 cm (equivalent to 24 hills m^{-2}). Three levels of N were supplied as urea (N, 46%) and compound fertilizer ($N:P_2O_5:K_2O$ = 15%:15%:15%): low (LN, 15 g N m^{-2}), medium (MN, 25 g N m^{-2}) and high N (HN, 35 g N m^{-2} (2002, 2003)). For all N levels, phosphorous (P) and potassium (K) fertilizers were applied as compound fertilizer at equal rates of 3.5 g P_2O_5 m^{-2} and 3.5 g K_2O m^{-2} in 2001, 7 g P_2O_5 m^{-2} and 7 g K_2O m^{-2} in 2002 and 2003, respectively. The rates and dates of N, P, and K fertilizer applications are listed in Table 1. Mixing of paddy water between N treatments was minimized by separating the LN and HN subplots from the rest of the plot (which received MN) with a 30 cm PVC barrier pashed 10 cm into the soil. The paddy fields were submerged with water, about 5 cm in depth, from 13 June to 10 July, and drained dry several times from 11 July to 4 August, and flooded with intermittent irrigation from 5 August to 10 days before harvest. The plants in both FACE and ambient plots were surrounded with border plants treated the same way as the plants inside.

Each rice season was followed by a winter wheat season. In the wheat cropping season prior to the first investigated rice

Table 1
Timing and application rates of N, P and K fertilizers in the LN, MN and HN plots over three rice cropping seasons (2001–2003)

Year	Treatment	N (g N m^{-2})/P (g P_2O_5 m^{-2})/K (g K_2O m^{-2})		
		First fertilization[a]	Second fertilization[b]	Third fertilization[c]
2001	LN	60/45.5/45.5	30/24.5/24.5	60/0/0
	MN	100/45.5/45.5	50/24.5/24.5	100/0/0
2002 and 2003	LN	90/70/70	0/0/0	60/0/0
	MN	90/70/70	60/0/0	100/0/0
	HN	90/70/70	120/0/0	140/0/0

[a] The first fertilization was applied as basal dressing on 13 June (i.e. 1 day prior to transplanting).
[b] The second fertilization was applied as tillering fertilizer on 18 June.
[c] The third fertileization was applied as ear fertilizer on 5 August, 28 July, 30 July in 2001, 2002 and 2003, respectively.

season (2001), N fertilizers were applied at an equal rate of about 22 g N m^{-2}. In the wheat season prior to the second rice season (2002), N fertilizers were applied at 15 and 25 g N m^{-2} in the plots receiving LN and MN, respectively. In the wheat season prior to the third rice season (2003), N fertilizers were applied at 9 and 18 g N m^{-2} in the plots receiving LN and MN treatments, respectively. P and K fertilizers were applied in all plots at equal rates of 7 g P_2O_5 m^{-2} and 7 g K_2O m^{-1} in all wheat season. Harvested wheat straw from the previous wheat season was incorporated before rice transplanting back into the soil in which the wheat was growing. Further details of the management with crop residue (wheat straw) are descryibed elsewhere (Zheng et al., 2006).

2.4. Plant sampling and measurements

Areas of the crop were destructively sampled at different times over the season. Sampling dates were fixed so as to coincide as much as possible with the early-tillering, mid-tillering, panicle initiation (PI), booting, heading (50% of plant headed), and grain maturity stages of the plants. In 2001, Plants were sampled at 28 and 51 days after transplanting (DAT), heading stage and grain maturity. In 2002, plants were sampled at 16, 27, 47 and 58 DAT, heading stage and grain maturity. In 2003, sampled at 12, 28 and 45 DAT, heading stage and grain maturity. In order to ensure representativeness of the sampling, the number of stems in 100 hills was counted at different places in each subplot, and then five plants with the mean stem number were selected. To maintain canopy conditions, the vacant spaces left after sampling were replanted with hills taken from the borders and these replanted hills were not subject to sampling any more. The samples were separated into living and dead leaf tissue, stem (including leaf sheath), and panicle (when applicable). For two of the five hills green leaf areas were measured. All the plant parts were oven-dried at 80 °C for 72 h or until dry weights were constant. After milling (0.5 mm mesh), the N concentrations of all plant parts were determined separately using a micro-Kjeldahl procedure. Grain yield was determined of all the plants from a 2 m^2 patch (excluding plants in the borders) in each subplot after the grains were adjusted to a moisture content of 0.14 g H_2O g^{-1} fresh weight.

2.5. Statistical analysis

Data were analyzed with the statistical package SPSS10.0 and EXCEL'2000 for Win. The experimental was designed as a blocked split–split–plot. Experimental year was treated as a fixed effect and was the whole-plot treatment. The [CO_2] was the split–plot treatment, and N was the split-split plot treatment. Average values of the sampled hills from different locations in each N subplot were used in the statistical analyses. Statistical analysis of variance (ANOVA) was used to determine differences between treatment means.

3. Results

3.1. N concentration

The overall patterns of changes in shoot N concentration through the growing season were similar across the 3 years of experimentation (Fig. 1). At the first sampling time (early-tillering), average shoot N concentration was 32.1 mg N g^{-1}. Then following the maximum N concentration, there was a steady decrease in N concentration through the rest of the growing season. At the end of the season, the mean N concentration had decreased to 10.0 mg N g^{-1}.

Overall, FACE decreased shoot N concentration over the season regardless of N addition rate ($P < 0.01$), except at early-tillering stage (Figs. 1 and 2a), the depression in shoot N concentration resulting from FACE showed a progressive increase with time during the season until middle growth stage, then there were declines in the negative responses starting at booting (for HN- or LN-crops) or heading (for MN-crops): when averaged across all N levels and years, shoot N concentration was lower under FACE by 1.8%, 6.1%, 12.2%, 14.3%, 12.1% and 6.9% at early-tillering, mid-tillering, PI, booting, heading and grain maturity, respectively (Fig. 2a). Similar patterns of N concentration response to FACE were observed for crops supplied LN, MN and HN, with LN-crops affected somewhat more than MN-, HN-crops at the most growth stages (Fig. 2a); however, no significant interaction was detected between [CO_2] and N supply due to considerable variation. Across [CO_2] levels, shoot N concentration increased apparently with increasing N supply: averaged across two [CO_2] levels and 3 years, the integrated shoot N concentration (calculated from Fig. 1) over the whole growing season was 20.1, 21.0 and 21.8 mg g^{-1} for LN, MN and HN-crops, respectively (Fig. 1); The year effects were significant ($P < 0.01$) for shoot N concentration at all growth stages (Table 2); however, interactions between all treatment variables were not detected at most stages of crop development.

3.2. N uptake

The overall patterns of changes in aboveground N accumulation during the experimental period were similar

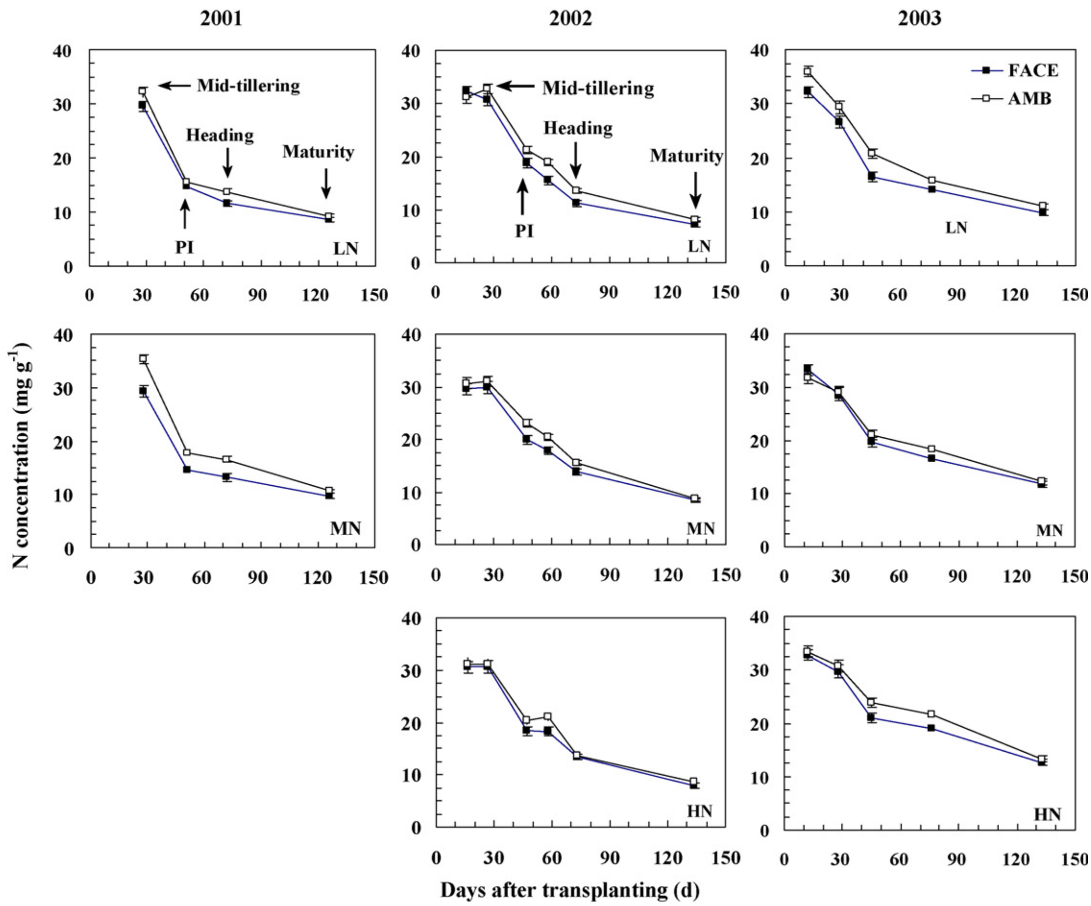

Fig. 1. Seasonal changes in nitrogen (N) concentration of aboveground rice plants subjected to ambient CO_2 (AMB; open symbols) and free-air CO_2 enrichment (FACE, 550 mol mol^{-1}) under low (LN, 15 g m^{-2}), medium (MN, 25 g m^{-2}) and high (HN, 35 g m^{-2}) levels of N application over three cropping seasons (2001–2003). Bars represent ± stardard error (n = 3 or 5) when it exceeds the size of the symbol.

for different treatments, with a rapid increase from early-tillering (mean value: 0.7 g N m^{-2}) to heading stage and then a slight increase until grain maturity (mean value: 18.4 g N m^{-2}) (Fig. 3). FACE significantly increased shoot N accumulation at all sampling dates (Table 2). The positive effect of elevated [CO_2] on N accumulation tended to decrease with crop development before heading, then at grain maturity, showed slightly increased responses: averaged across all N levels and years, shoot N accumulation was greater under FACE by 35%, 37%, 16%, 9%, 7% and 9% at early-tillering, mid-tillering, PI, booting, heading and grain maturity, respectively (Fig. 2b). Overall, the average response showed a tendency to be greater under a high N application than under a low N application, but [CO_2] × N interaction was not statistically significant due to the high variability between sampling replicate plants and seasons. Across [CO_2] levels, shoot N accumulation increased significantly with increasing N supply, except at early-tillering stage (Table 2); There was obvious variation between different

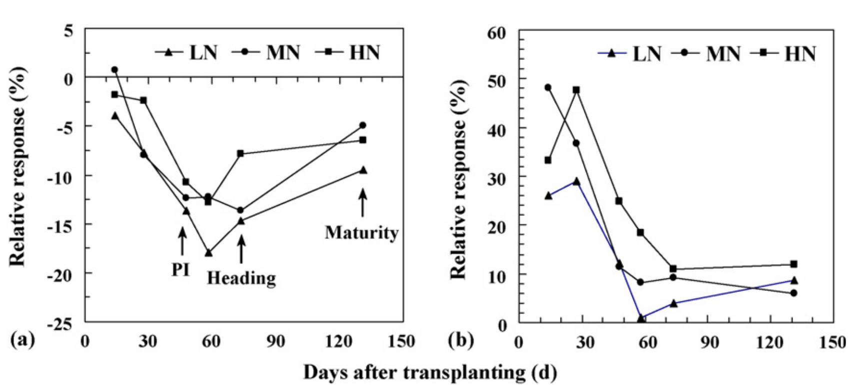

Fig. 2. Seasonal patterns of the relative response of shoot N concentration (a) and accumulation (b) to free-air CO_2 enrichment (FACE) under low (LN, 15 g m^{-2}), medium (MN, 25 g m^{-2}) and high (HN, 35 g m^{-2}) levels of N application (averaged across 2001–2003). The relative response is defined as (FACE-ABM)/ABM × 100; data are average values with ± one standard error shown.

Table 2
Significance of elevated CO_2 (C), nitrogen (N), year (Y), and their interaction on various nitrogen parameters of rice crops from the 2001–2003 experiments

Parameters	Stages or periods	C	N	C × N	Y	C × Y	N × Y	C × N × Y
Shoot N concentration (mg g^{-1})	A[a]	n.s.[b]	+	n.s.	**	n.s.	n.s.	*
	B	**	n.s.	n.s.	**	n.s.	n.s.	n.s.
	C	**	*	n.s.	**	n.s.	**	n.s.
	D	**	**	n.s.	–	–	–	–
	E	**	**	n.s.	**	n.s.	**	n.s.
	F	**	**	n.s.	**	n.s.	**	n.s.
Shoot N accumulation (g m^{-2})	A	**	n.s.	n.s.	+	**	n.s.	n.s.
	B	**	n.s.	n.s.	**	n.s.	n.s.	n.s.
	C	**	**	n.s.	*	n.s.	**	*
	D	*	+	n.s.	–	–	–	–
	E	**	**	n.s.	**	n.s.	**	n.s.
	F	**	**	n.s.	**	n.s.	+	+
Shoot N uptake (g m^{-2})	I[c]	**	n.s.	n.s.	+	**	n.s.	n.s.
	II	**	n.s.	n.s.	**	n.s.	n.s.	n.s.
	III	+	**	n.s.	*	+	**	*
	IV	n.s.	**	n.s.	**	+	n.s.	n.s.
	V	n.s.	*	n.s.	*	n.s.	n.s.	n.s.
Shoot N uptake ratio (%)	I	**	n.s.	n.s.	**	**	n.s.	n.s.
	II	**	+	n.s.	**	+	n.s.	n.s.
	III	n.s.	n.s.	n.s.	**	n.s.	*	*
	IV	+	**	n.s.	**	n.s.	n.s.	n.s.
	V	n.s.	*	n.s.	+	n.s.	n.s.	n.s.
Leaf N uptake ratio (%)	A	*	**	n.s.	**	+	+	n.s.
	B	+	n.s.	n.s.	**	n.s.	n.s.	n.s.
	C	**	n.s.	n.s.	n.s.	n.s.	n.s.	*
	D	*	n.s.	n.s.	–	–	–	–
	E	**	n.s.	n.s.	**	n.s.	n.s.	n.s.
	F	n.s.	n.s.	n.s.	**	**	n.s.	+
Stem N uptake ratio (%)	A	*	**	n.s.	**	+	+	n.s.
	B	+	n.s.	n.s.	**	n.s.	n.s.	n.s.
	C	**	n.s.	n.s.	n.s.	n.s.	n.s.	*
	D	*	n.s.	n.s.	–	–	–	–
	E	**	n.s.	n.s.	**	+	n.s.	n.s.
	F	n.s.	**	n.s.	**	n.s.	+	n.s.
Spike N uptake ratio (%)	E	n.s.	**	n.s.	**	n.s.	*	**
	F	+	+	n.s.	**	n.s.	+	*
NUEp (g g^{-1})	A	n.s.	n.s.	n.s.	**	n.s.	n.s.	+
	B	**	n.s.	n.s.	**	n.s.	n.s.	n.s.
	C	**	*	n.s.	**	n.s.	*	n.s.
	D	**	**	n.s.	–	–	–	–
	E	**	**	n.s.	**	n.s.	**	n.s.
	F	**	**	n.s.	**	n.s.	n.s.	n.s.

[a] A–E denote five successive growth stages: early-tillering, mid-tillering, panicle initiation (PI), booting, heading, and maturity growth stage, respectively.
[b] Probability levels are indicated by n.s., +, * and ** for 'not significant', 0.1, 0.05, and 0.01, respectively.
[c] I–V indicate five successive growth periods: from transplanting to early-tillering, early-tillering to mid-tillering, mid-tillering to PI, PI to heading, and heading to grain maturity, respectively.

years with regards to shoot N accumulation throughout the cropping season. For the most part, however, interactions between all treatments variables were not detected.

The whole growth season of rice plant is consisted of different growth periods. Averaged over all N levels and seasons, shoot N uptake in the FACE plots was increased significantly by 46%, 38%, 6% and 16%, in periods 1–3 and 5 of the growth period, respectively, while it was reduced by 2% in period 4, the responses showing a progressive decrease with time during the season up to heading and then a slightly increase at grain maturity (Fig. 4a). The similar applied to shoot N uptake ratio (the ratio of shoot N uptake during a given growth period to final shoot N acquisition at maturity): averaged across all N levels and years, the response of shoot N uptake ratio to FACE was 33%, 26%, −3%, −11% and 10% in periods 1–5 of the growth period, respectively (Fig. 4b). Except early in the season (from transplanting to PI), N fertilization had significant influence on shoot N uptake and its ratio (Table 2). There was obvious variation between different years with regards to shoot N uptake and its ratios over the season. However, for the most part, interactions between all treatments variables were not detected.

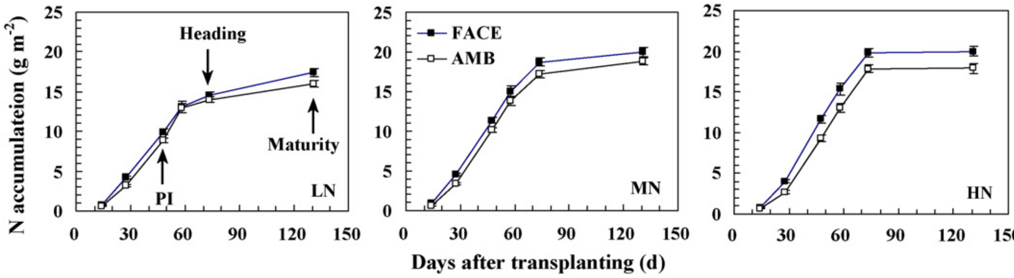

Fig. 3. Seasonal changes in N accumulation of aboveground rice plant subjected to ambient CO_2 (AMB; open symbols) and free-air CO_2 enrichment (FACE, 550 mol mol^{-1}) under low (LN, 15 g m^{-2}), medium (MN, 25 g m^{-2}) and high (HN, 35 g m^{-2}) levels of N application over three cropping seasons (2001–2003). Bars represent ± stardard error (n = 3 or 5) when it exceeds the size of the symbol.

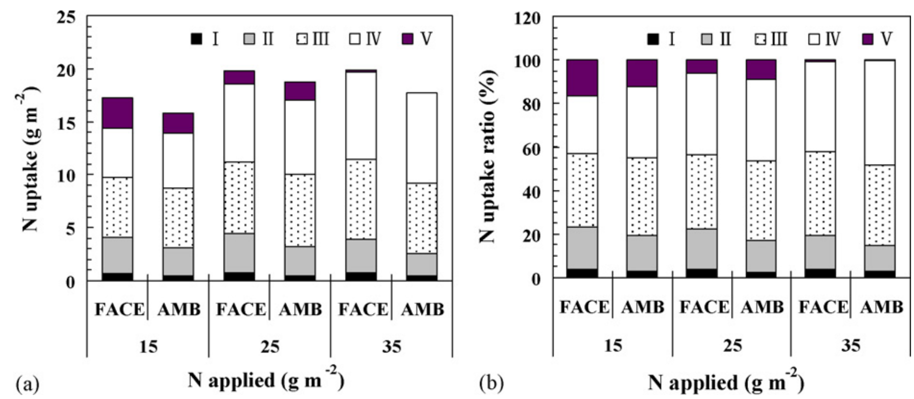

Fig. 4. Shoot N uptake (a) and its ratio (b) during five successive growth periods of rice crops subjected to ambient CO_2 (AMB; open symbols) and free-air CO_2 enrichment (FACE, 550 mol mol^{-1}) under low (LN, 15 g m^{-2}), medium (MN, 25 g m^{-2}) and high (HN, 35 g m^{-2}) levels of N application over three cropping seasons (2001–2003). Shoot N uptake ratio = shoot N uptake during a given growth period/the final N accumulation at maturity × 100. I–V indicate different growth periods: from transplanting to early-tillering, early-tillering to mid-tillering, booting to panicle initiation (PI), PI to heading, heading to grain maturity, respectively. For each N level, data are average values across 3 years (2001–2003).

3.3. N use efficiency

N use efficiency for biomass (NUEp, ratio of biomass to cumulative N absorption) represents the amount of dry matter yield produced per unit absorbed N. The overall patterns of changes in NUEp through the growing season were similar for different treatments (Fig. 5). There was no difference between early-tillering (36 g g^{-1}) and mid-tillering (33 g g^{-1}) stages, thereafter NUEp increased progressively through the rest of the growing season. At harvest, the mean NUEp had increased to 101 g g^{-1}. FACE significantly increased NUEp at all sampling stages except at early-tillering stage (Table 2): averaged across 3 years and all levels of N supplied, the NUEp was greater under FACE by 7%, 14%, 17%, 15% and 8% at early-tillering, mid-tillering, PI, booting, heading and grain maturity, respectively, with NUEp response at middle growth stage being larger than those at early or late growth stage (Fig. 5). Overall, the stimulatory effect of [CO_2] on NUEp was decreased slightly by higher N supply, though no significant interaction was detected between CO_2 and N (Table 2). Across [CO_2] levels, the NUEp decreased significantly with increasing N supply, except during early stage of development; Interac-

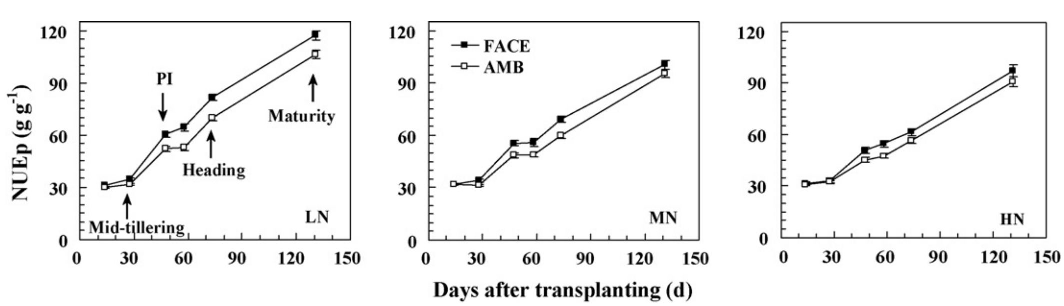

Fig. 5. N use efficiency for biomass (NUEp) at six successive growth stages of rice plants subjected to ambient CO_2 (AMB; open symbols) and free-air CO_2 enrichment (FACE, 550 mol mol^{-1}) under low (LN, 15 g m^{-2}), medium (MN, 25 g m^{-2}) and high (HN, 35 g m^{-2}) levels of N application over three cropping seasons (2001–2003). For each N level, data are average values across 3 years (2001–2003). Bars represent ± standard error (n = 3 or 5) when it exceeds the size of the symbol.

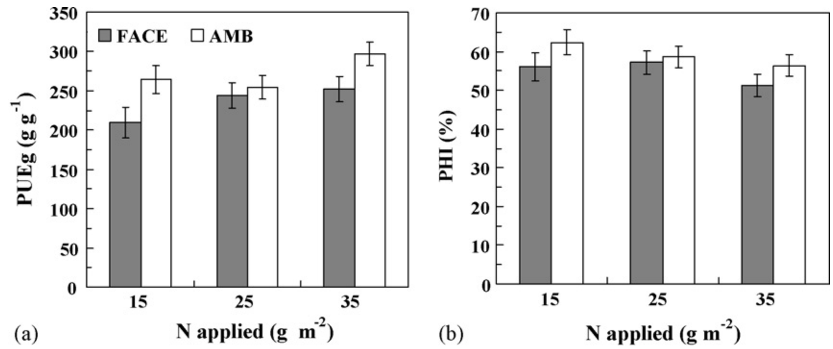

Fig. 6. N use efficiency for grain (NUEg) (a) and N harvest index (NHI) (b) of rice crops subjected to ambient CO_2 (AMB; open symbols) and free-air CO_2 enrichment (FACE, 550 mol mol^{-1}) under low (LN, 15 g m^{-2}), medium (MN, 25 g m^{-2}) and high (HN, 35 g m^{-2}) levels of N application over three cropping seasons (2001–2003). Data are average values across 3 years with ± one standard error (vertical bars). ANOVA results for NUEg: CO_2, $P = 0.044$; N, $P < 0.001$; year, $P < 0.001$; $CO_2 \times N$, $P = 0.617$; $CO_2 \times$ year, $P = 0.447$; N × year, $P = 0.326$; $CO_2 \times N \times$ year, $P = 0.383$; for NHI: CO_2, $P = 0.119$; N, $P = 0.036$; year, $P < 0.001$; $CO_2 \times N$, $P = 0.287$; $CO_2 \times$ year, $P = 0.678$; N × year, $P = 0.280$; $CO_2 \times N \times$ year, $P = 0.063$).

tions between all treatments variables were not detected at most stages of crop development although there was obvious variation between different years with regards to NUEp at all growth stages.

N use efficiency for grain (NUEg, ratio of grain yield to cumulative N absorption) represents the amount of harvested grain yield produced per unit N absorbed at maturity, while N harvest index (NHI = (N in grain)/(N in grain + straw)) represents the partitioning efficiency of N to the harvested part of the plant. FACE promoted NUEg and NHI, the promotion being somewhat larger under high or medium level of N application than under low N application (Fig. 6). Averaged across all N levels and years, NUEg and NHI increased by an average of 5.7% ($P < 0.05$) and 2.0%

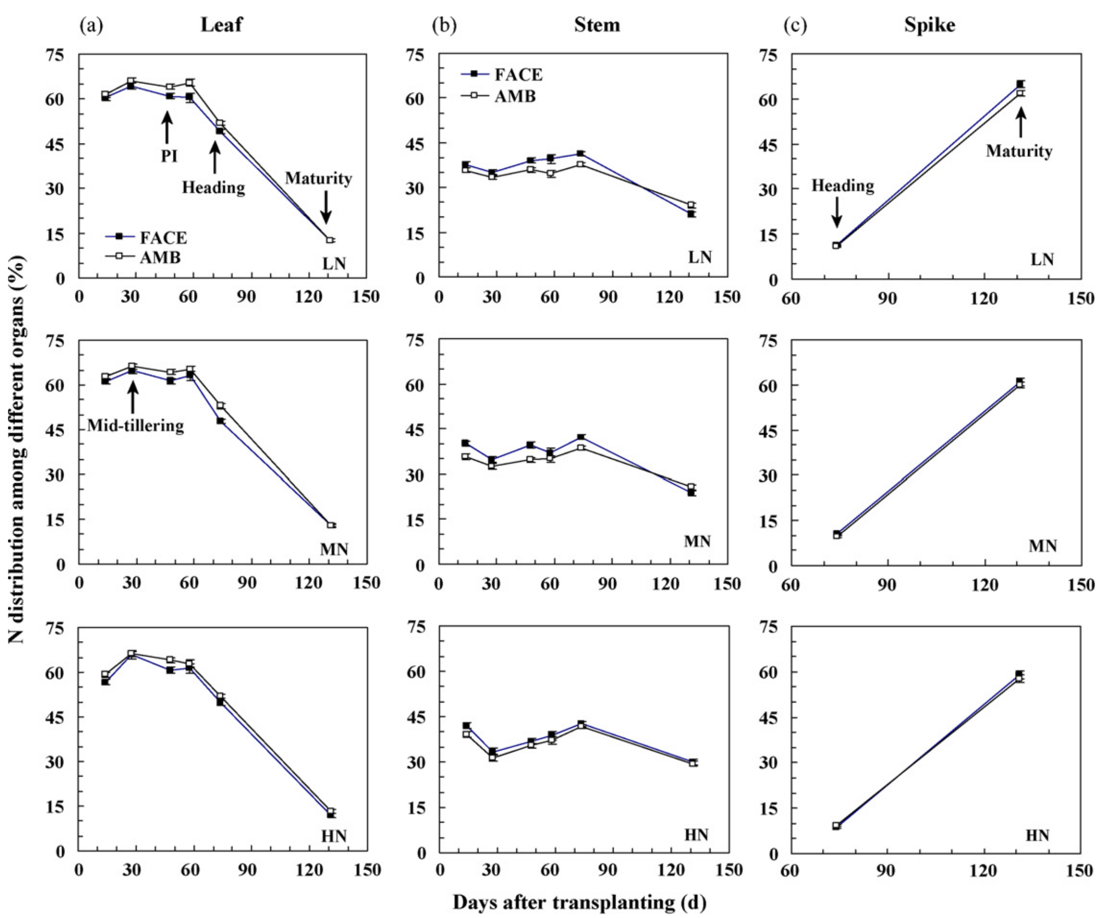

Fig. 7. Seasonal changes in N distribution among different organs of rice crops grown under ambient CO_2 (AMB; open symbols) or free-air CO_2 enrichment (FACE; closed symbols) in combination with three levels of N application (LN, 15 g m^{-2}; MN, 25 g m^{-2}; HN, 35 g m^{-2}) over three cropping seasons (2001–2003). Bars represent ± standard error ($n = 3$ or 5) when it exceeds the size of the symbol.

($P > 0.05$) with enriched [CO_2], respectively, relative to ambient [CO_2]. Across [CO_2] levels, the NUEg and NHI decreased significantly with increasing N supply (Table 2); Interactions between all treatments variables were not detected although there was obvious variation between different years with regards to NUEg and NHI.

3.4. N distribution

The proportion of leaves to the total aboveground N content was stable before booting stage (60–66%), while it declined apparently after that, with the lowest mean value of 13% at grain maturity, the trend was similar for different treatments (Fig. 7a). FACE significantly decreased N fractions in leaves at all growth stages. Averaged over all N levels and seasons, N fractions in leaves were reduced by 2.8%, 1.8%, 5.0%, 4.5%, 6.4% and 2.8% at early-tillering, mid-tillering, PI, booting, heading and grain maturity, respectively, with the negative response at middle growth stage being larger than that at early or late growth stage. The differences were significant between the two [CO_2] treatments throughout the cropping season except at grain maturity (Table 2). N fertilization had no influence on N fractions in leaves over the season except at early-tillering stage.

Before heading, the proportion of stems to the total aboveground N content was stable (33–41%), while it declined apparently after that, with the lowest value of 26% at maturity, the trend was similar for different treatments (Fig. 7b). FACE enhanced N fractions in stems by 5.4%, 8.4%, 6.8%, 1.4% and 8.5% at early-tillering, mid-tillering, PI, booting and heading, respectively on average, but it decreased by 5.7% at grain maturity. The differences were significant between the two [CO_2] treatments throughout the cropping season except at grain maturity (Table 2). Fertilization had no influence on N fractions in stems over the season except at early-tillering and grain maturity.

With crop development, the proportion of spikes to the total aboveground N content increased substantially, with averages of 10.1% and 60.7% at heading and grain maturity, respectively (Fig. 7c). Such a rapid increase in the fractions of absorbed N found in spikes coincided with a sharp decline in fractions in leaves and stems, probably as N was translocated from vegetative tissues (especially leaves) to the developing grain (Fig. 7). The crop response to FACE in the ratio of spike to shoot N content varied with the crop development stages: remaining unchanged at heading stage, and showing a significant increase of 2.9% at maturity. Across [CO_2] levels, N fractions in spikes decreased significantly with increasing N supply (Table 2): averaged across all [CO_2] levels and years, the integrated ratio of spike to shoot N content (calculated from Fig. 7c) over the grain filling stage was 37.2%, 35.4% and 33.7% for LN, MN and HN-crops, respectively.

The year effects were significant for the proportion of N allocated to the leaves, stems and spikes at all growth stages (Table 2). However, for the most part, interactions between all treatments variables were not detected for these parameters.

4. Discussion and conclusion

The present study showed the final aboveground N uptake at maturity was increased significantly by 9% under elevated [CO_2] (Fig. 1), and this is in contrast to those with rice from a OTC study (Ziska et al., 1996) and a FACE study (Kim et al., 2001, 2003b; Yamakawa et al., 2004) which demonstrated that the total amount of N taken up was similar for both elevated and ambient [CO_2] crops by the end of the experiments (at anthesis or at maturity). In addition to the difference in test cultivar and environmental conditions, one possibility for this contrast may be due to the different levels of N supplied. The N application rate in this trial was 15–35 g N m^{-2}, while it was 0–20 g N m^{-2} in the two experiments cited above (Ziska et al., 1996; Kim et al., 2001, 2003a; Yamakawa et al., 2004). A result with cotton by Rogers et al. (1996) indicated that there was no CO_2 effects on N accumulation at low to moderate levels of N supply (0–17 mg kg^{-1}), while apparent [CO_2]-induced increases in N accumulation were observed at high level of N supply (133 mg kg^{-1}). Thus, there appears to be a fairly wide range in the effects of elevated [CO_2] on N yield depending on soil N availability.

The final shoot N acquisition of rice crops at maturity is related to dynamics of shoot N uptake during various growth periods. However, there is little information on effects of elevated [CO_2] on seasonal changes in shoot N uptake of rice crops. One rice FACE study related to seasonal changes in plant N uptake has been reported by Kim et al. (2001, 2003b), showing positive N uptake response to elevated [CO_2] in the period form transplanting to PI and negative response during the period from PI to maturity. In our experiment, the whole stage was further divided into five successive growth periods: from transplanting to early-tillering (period 1), early-tillering to mid-tillering (period 2), mid-tillering to PI (period 3), PI to heading (period 4), heading to grain maturity (period 5), respectively. The response in shoot N uptake declined gradually with crop development before heading, while it showed a slight increasing trend again after heading, with average responses of 46%, 38%, 6%, −2% and 16% during the periods 1–5, respectively. With respect to shoot N uptake ratio, the average responses to FACE were 33%, 26%, −3%, −11% and 10%, respectively, during the respective growth periods, similar to what occurred with shoot N uptake. Such seasonal responses in aboveground N uptake (ratio) are mainly regulated by soil N availaility, plant N uptake ability and also related to plant phenology, as discussed below.

Firstly, in this trial, the available N pool over the season appeared to be similar in both CO_2 treatments under each N levels. Because 60% of the total N fertilizer (equal to 9–21 g N m^{-2}) was applied before mid-tillering stage, soil N availability was greater during the early growth period (EGP) compared with during middle growth period (MGP) or late growth period (LGP), similar to the suggestion of Kim et al. (2001). As we know, for some crop species the ability to respond associated with CO_2 elevation depends on an adequate supply of nutrients. Hence, the FACE crops took up a greater amount of N during EGP, especially during productive tiller formation phase (i.e. before mid-tillering stage), which was similar to the previous studies (Ziska et al., 1996; Kim et al., 2001, 2003b; Yamakawa et al., 2004). Because of the markedly greater N uptake in the FACE plots during EGP, the N availability during MGP decreased; however, in this trial, 40% (e.g. 6–14 g N m^{-2}) of the total N was applied at about 25 days before heading, which could lead to increased soil N availability during LGP. Secondly, When soil N availability is not limiting (as in our trial), N uptake by crops depends on factors such as root size and physiological activity (Berntson et al., 1998). In the same experiments (in 2002 and 2003 season), our data showed that, on a per plant basis, FACE crops showed substantial increases in root biomass (ca. 42%), root volume (ca. 54%), adventitious root number (ca. 26%) and adventitious root length (ca. 35%) during EGP (unpublished). As root morphology is an important component of a plant's ability to take up nutrients, it is probable that overall N uptake potential during EGP was greater in FACE crops. Indeed, we found that there was a strong correlation between shoot N uptake and root biomass and no significant difference was observed in the regression of shoot N uptake against root biomass between the two CO_2 levels (Fig. 8), suggesting that the increased N uptake with FACE might be due to increased root growth. In contrary to the EGP, we found that there was no stimulation due to FACE detected for root growth during MGP, while the mean root activity per unit dry weight was reduced more during MGP as compared with that during EGS (unpublished), indicating overall depression in crop N uptake capability during MGP. Thirdly, it must be noted that an accelerated crop phenology with elevated [CO_2]—across the 3 years heading stage and grain maturity stage occurred 3–5 days (mean 3.4 days), and 4–9 days (mean 5.8 days) earlier, respectively, compared to those under ambient [CO_2] (Huang et al., 2005). Overall, because of plentiful N supply in the soil together with a greater relative N uptake capacity during early development, FACE crops showed the largest N uptake increase over the season as compared with ambient crops. Such enhanced N uptake corresponds to the findings for leaf area index (LAI), net assimilation rate (NAR), tiller occurrence rate

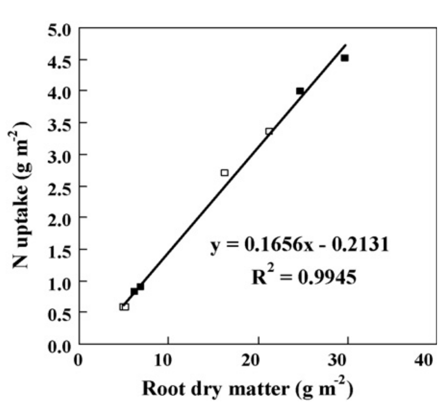

Fig. 8. The relationship between root dry matter (DM) and shoot nitrogen (N) uptake at early-tillering, mid-tillering stages of rice grown under ambient CO_2 (open squares) and free-air CO_2 enrichment (closed squares) conditions in 2002–2003. N application rates were 25 g N m^{-2}. Data points are the means of each sampling/year. First degree polynomial fitted; R^2 is shown.

and DM production, which also showed maximum CO_2 response to FACE during that period (Yang et al., 2006a). As soil N availability and plant N uptake ability decreased during MGP, together with accelerated crop phenology, relative stimulation of N uptake by FACE either disappeared or even slightly reversed (Fig. 5). Indirect evidence for reduced N uptake during the MGP in FACE crops was its faster rate of crop senescence: dead leaf DM (data not shown), tillering extinction speed and unproductive tillering ratio during that period (Yang et al., 2006b) were substantially greater in the FACE plots compared with those in the ambient plots. Contrary to seasonal trend in N uptake response to elevated [CO_2] before heading, FACE crops showed again enhanced N uptake during LGP. Such a phenomenon could primarily be attributed to N fertilization in this trial which induced higher soil N availability during LGP, though mean duration from heading to grain maturity shrank 2.4 days on average in FACE crops. Based on the seasonal aboveground responses in morphological traits reported previously (include DM production, LAI and NAR, see Yang et al., 2006a) and in N uptake in this trial, it can be concluded that the progressive acclimation of rice growth to higher [CO_2] does not occur inevitably, whether or not the plant acclimation to the higher [CO_2] was also affected by cultivation technique (such as N application strategies in the current experiments).

The differences in seasonal responses of shoot N concentration to elevated [CO_2] can be explained by changes in both shoot DM production and N uptake during various growth periods. Because rice plants showed the maximum CO_2 response of shoot N uptake to FACE at early-tillering stage over the whole season, shoot N uptake can match the increases in shoot DM production, so no significant decrease in shoot N concentration will be detected at that stage. With crop development, the magnitude of shoot N uptake increase due to FACE decreased more than that of aboveground DM, so shoot N concentration showed a progressive decrease with maximum CO_2-related decrease (−14%) being reached at booting stage. After booting stage, the again increased shoot N uptake in FACE crops due to higher soil N availability resulted in the relatively smaller decrease in shoot N concentration with only 7% decrease at grain maturity. Such seasonal pattern in shoot N concentration was similar to the results with Lolium perenne from a pot study within a FACE experiment reported by Daepp et al. (2001) who found that under low soil N, the shoot N concentration during a single reproduction stage was reduced, by about 20%, compared to reductions of about 9% during vegetative stages. In present study, the results demonstrated that, FACE significantly reduced spikelet number per panicle of rice by 7.6% due to the enhancement of spikelet degeneration per panicle (see Yang et al., 2006b, Table 1, Fig. 2b). Prior studies indicated that the degeneration of spikelets is closely related to the crop N nutrient during the panicle formation (Wada, 1969; You et al., 2000).We hypothesized that, in addition to panicle size of the test variety, greater degenerated spikelets per panicle due to FACE may be associated with significant reduction in shoot N concentration during the spikelet formation (Fig. 2a).

The NUEp was the reciprocal of N concentration, as a result, its seasonal response showed the reverse to that of shoot N concentration, with positive response at MGP being larger than those at EGP or LGP (Fig. 5); The enhanced N use efficiency under elevated [CO_2] was also reported in rice (Aben et al., 1999; Kim et al., 2001) and other species (Drake et al., 1997), assuming that the same amount of N taken up between elevated and ambient grown rice, greater dry matter or grain will be produced under elevated [CO_2] condition.

FACE not only change the amount of N uptake, but also alter N allocation patterns among different organs. Our results for the first time indicated the amount and proportion of N translocated from the roots to stems before heading and to panicles after heading were greater with elevated [CO_2], while N distribution ratio in the leaves showed the reverse, suggesting different strategies of crop N partitioning in a high [CO_2] environment (Fig. 7). Such changes in N allocation patterns are strongly linked with greatly increased dry weight of stems (a major sink of N before heading) and panicles (a major sink of N after heading) (see Yang et al., 2006a).

Many studies with rice (Ziska et al., 1997) and other species (Pettersson et al., 1993; Pettersson and MacDonald, 1994; Bowler and Press, 1996) under controlled environments have shown that the effect of [CO_2] is influenced by soil fertility or N application. For rice within a FACE experiment under low N application ranging from 4 to 15 g N m^{-2}, Kim et al. (2001, 2003a) reported that crop N uptake showed positive interaction between [CO_2] and applied N before PI and negative interaction in the period from PI to maturity. However, in the present experiments, no interactive effect of [CO_2] and N supply was observed over the whole season for all N parameters measured (Table 2), indicating that, under higher soil N availability, the interactive effect between elevated [CO_2] and N supply is not important as compared with the first order main effects of the CO_2 itself. Similarly, studies from the same FACE platform indicated, for the most part, no synergistic effect was detected between [CO_2] and N with respect to yield, biomass and other growth parameters (Yang et al., 2006a,b).

In this series of experiments, we found that overall DM accumulation at harvest was increased on average about 16% under FACE, which was mainly due to significant enhanced DM production during EGP, while the DM responses declined substantially during MGP (Yang et al., 2006a). Average grain yield at harvest was stimulated somewhat less, by an average of 13% by FACE, primarily due to substantial increase in panicle number (+19%) which was contributed to significant increase in tillering occurrence speed (+30%), meanwhile FACE crop showed the reduction in productive tiller ratio (−8%) and harvest index (−3%) as a result of increased tillering extinction speed and increased degenerated spikelet number per panicle (−52%) (Yang et al., 2006b). With respect to the yield response of FACE crops to N, previous results demonstrated that the grain yield response to FACE significantly increased with increasing N level from 15 to 25 g N m^{-2}, but further increases in N supply to 35 g N m^{-2} resulted in apparent reduction due to excessive N-induced infertility (e.g. filled spikelet percentage and grain weight), as well as decreased spikelet number per square meter, primarily because of severe self-shading experienced by HN-crops after PI (Yang et al., 2006b). We conclude that the seasonal changes in crop growth and yield formation response to FACE are directly or indirectly associated with the season patterns of N uptake. Incorporating the present results with growth and yield response to FACE reported previously, we think, in order to maximize rice productivity in a future high CO_2 environment, the N fertilizer strategies (the recommended N rates, proportion and timing across the season of N application) for rice production systems should be adjusted, at least for the similar conditions of this experiment. First, the recommended rates of N for rice may need to be higher than 25 g m^{-2} (current recommended rates of N in china), but less than 35 g m^{-2}. The heavy applications of N frequently lead to greater yield losses in rice due to lodging (Yoshida, 1981); however, in our experiment, FACE crops exhibited increased resistance to lodging: FACE crops supplied 25 g N m^{-2} were protected very well against lodging while the ambient crops experienced lodging damage. Such a phenomenon coincides with our previous findings which indicated that FACE-crops were commonly higher in rooting volume, stem weight and proportion, concentration and amount of soluble carbondydrates in stem throughout the season (Yang et al., 2006a,b). Secondly, in order to take full advantage of strong N uptake capacity during EGP and facilitate N uptake during MGP and LGP, proportions and timing of the N application across the season may need to be changed: (1) the proportion of N applied after PI (i.e., the ratio of ear-grain fertilizer to base-tillering fertilizer) may also need to be higher than current recommendations, from current 4:6 to 5:5 or 6:4 or higher, enabling the extra N to be taken after PI; (2) timing across the season of the N application also need to be changed: more fraction of the N applied before PI (i.e., base-tillering fertilizer) should be applied as basal dressing rather than tillering dressing in order to delay nitrogen release, which can be enhanced by using controlled-release N fertilizers (for example coated granular fertilizers) or method of deep-layer fertilization or both, and more fraction of N applied after PI (i.e., ear-grain fertilizer) should be applied at stage of leaf-remainder 1.0 (also referred to spikelet sustaining fertilizer in rice production) in order to reduce the number of degenerated spikelets; however, additional experiments with different cultivars would be needed to develop N fertilizer of high-yield cultivation under future elevated [CO_2] conditions.

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References

Aben, S.K., Seneweera, S.P., Ghannoum, O., Conroy, J.P., 1999. Nitrogen requirements for maximum growth and photosynthesis of rice, *Oryza sativa* L-cv. Jarrah grown at 36 and 70 Pa CO_2. Aust. J. Plant Physiol. 26, 759–766.

Berntson, G.M., Rajakaruna, N., Bazzaz, F.A., 1998. Growth and nitrogen uptake in an experimental community of annuals to elevated atmospheric CO_2. Global Change Biol. 4, 607–626.

Bowler, J.M., Press, M.C., 1996. Effects of elevated carbon dioxide, nitrogen form and concentration on growth and photosynthesis of a fast-and slow-growing grass. New Phytol. 132, 391–401.

Crawford, N.M., Glass, D.M.A., 1998. Molecular and physiological aspect of nitrate uptake in plants. Trends Plant Sci. 3, 389–395.

Daepp, M., Nosberger, J., Luscher, A., 2001. Nitrogen fertilization and developmental stage alter the response of Lolium perenne to elevated CO_2. New Phytol. 150, 347–358.

Drake, B.G., Gonzalez-Meler, M.A., Long, S.P., 1997. More efficient plants: a consequence of rising atmospheric CO_2? Ann. Rev. Plant Physiol. Plant Mol. Biol. 48, 609–639.

Huang, J.Y., Yang, L.X., Yang, H.J., Liu, H.J., Dong, G.C., Zhu, J.G., Wang, Y.L., 2005. Effects of free-air CO_2 enrichment (FACE) on growth duration of rice (*Oryza sativa* L.) and its cause. Acta Agron. Sinica 31, 882–887.

Ingestad, T., 1981. Plant growth in relation to nitrogen supply. In: Clark, F.E., Rosswall, T., Terrestrial Nitrogen Cycles. Ecol. Bull. Stockholm 33(303), 268–271.

Kim, H.Y., Lieffering, M., Kobayashi, K., Okada, M., Mitchell, M.W., Gumpertz, M., 2003a. Effects of free-air CO_2 enrichment and nitrogen supply on the yield of temperate paddy rice crops. Field Crops Res. 83, 261–270.

Kim, H.Y., Lieffering, M., Kobayashi, K., Okada, M., Miura, S., 2003b. Seasonal changes in the effects of elevated CO_2 on rice at three levels of nitrogen supply: a free air CO_2 enrichment (FACE) experiment. Global Change Biol. 9, 826–837.

Kim, H.Y., Lieffering, M., Miura, S., Kobayashi, K., Okada, M., 2001. Growth and nitrogen uptake of CO_2-enriched rice under field conditions. New Phytol. 150, 223–229.

Kimball, B.A., 1992. Cost comparisons among free-air CO_2 enrichment, open-top chamber, and sunlit controlled-environment chamber methods of CO_2 exposure. Crit. Rev. Plant Sci. 11, 265–270.

Kimball, B.A., Kobayashi, K., Bindi, M., 2002. Responses of agricultural crops to free-air CO_2 enrichment. Adv. Agron. 77, 293–368.

Kimball, B.A., Pinter Jr.P.J., Garcia, R.L., LaMorte, R.L., Wall, G.W., Hunsaker, D.J., Wechsung, G., Wechsung, F., Kartschall, T., 1995. Productivity and water use of wheat under free-air CO_2 enrichment. Global Change Biol. 1, 429–442.

Kimball, B.A., Pinter, P.J., Wall, G.W., Garcia, R.L., LaMorte, R.L., Jak, P.M.C., Frumau, K.F.A., Vugts, H.F., 1997. Comparisons of responses of vegetation to elevated carbon dioxide in free-air and open-top chamber facilities. In: Allen, L.H., Kirkham, M.B., Olszyk, D.M., Whitman, C.E. (Eds.), Advances in Carbon Dioxide Research. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, Wisconsin, pp. 113–130.

Liu, G., Han, Y., Zhu, J.G., Okada, M., Nakamura, H., Yoshimoto, M., 2002. Rice-wheat rotational FACE platform. I. System construct and control (in Chinese). Chin. J. Appl. Ecol. 13, 1253–1258 (in Chinese).

McLeod, A.R., Long, S.P., 1999. Free-air carbon dioxide enrichment (FACE) in global change research: a review. Adv. Ecol. Res. 28, 1–56.

Okada, M., Lieffering, M., Nakamura, H., Yoshimoto, M., Kim, H.Y., Kobayashi, K., 2001. Free-air CO_2 enrichment (FACE) using pure CO_2 injection: system description. New Phytol. 150, 251–260.

Pettersson, R., MacDonald, J.S., 1994. Effects of nitrogen supply on the acclimation of photosynthesis to elevated CO_2. Photosynth. Res. 39, 389–400.

Pettersson, R., MacDonald, A.J.S., Stadenberg, I., 1993. Response of small birch plants (*Betula pendula* Roth.) to elevated CO_2 and nitrogen supply. Plant Cell Environ. 16, 1115–1121.

Rogers, G.S., Milham, P.J., Thibaud, M.C., Conroy, J.P., 1996. Interactions between rising CO_2 concentration and nitrogen supply in cotton. 1. Growth and leaf nitrogen concentration. Aust. J. Plant Physiol. 23 (2), 119–125.

Wada, G., 1969. The effects of nitrogenous nutrition on the yield-determining process of rice plant. Bull. Natl. Inst. Agric. Sci. A16, 27–167 (in Japanese with English summary).

Yamakawa, Y., Saigusa, M., Okada, M., Kobayashi, K., 2004. Nutrient uptake by rice and soil solution composition under atmospheric CO_2 enrichment. Plant Soil 259, 367–372.

Yang, L.X., Huang, J.Y., Yang, H.J., Dong, G.C., Liu, G., Zhu, J.G., Wang, Y.L., 2006a. Seasonal changes in the effects of free-air CO_2 enrichment (FACE) on dry matter production and distribution of rice (*Oryza sativa* L.). Field Crop Res. 98, 12–19.

Yang, L.X., Huang, J.Y., Yang, H.J., Zhu, J.G., Liu, H.J., Dong, G.C., Liu, G., Han, Y., Wang, Y.L., 2006b. The impact of free-air CO_2 enrichment (FACE) and N supply on yield formation of rice crops with large panicle. Field Crop Res. 98, 141–150.

Yoshida, S., 1981. Fundamentals of Rice Crop Science. International Rice Research Institutes, Los Banos, Philippines.

You, Y.L., Yamamoto, Y., Yoshida, Y., Nitta, Y., Miyazaki, A., 2000. Response of differentiated and degenerated spikelets to top-dressing, shading and day/night temperature treatments in rice cultivars with large panicles. Soil Sci. Plant Nutr. 46, 631–641.

Zheng, Z.H., Zhou, Z.X., Wang, Y.S., Zhu, J.G., Wang, Y.L., Yue, J., Shi, Y., Kobayashi, K., Inubushi, K., Huang, Y., Han, S.H., Xu, Z.J., Xie, B.H., Butterbach-bah, K., Yang, L.X., 2006. Nitrogen-regulated effects of free-air CO2 enrichment (FACE) on methane emissions from paddy rice fields. Global Change Biol. 12 (9), 1717–1732.

Ziska, L.H., Namuco, O.S., Moya, T., Quilang, J., 1997. Growth and yield responses of field-grown tropical rice to increasing carbon dioxide and air temperature. Agron. J. 89, 45–53.

Ziska, L.H., Weerakoon, W., Namuco, O.S., Pamplona, R., 1996. The influence of nitrogen on the elevated CO_2 response on field-grown rice. Aust. J. Plant Physiol. 23, 45–52.