Acclimation of nitrogen uptake capacity of rice to elevated atmospheric CO2 concentration

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INTRODUCTION

The atmospheric carbon dioxide concentration [CO2] has been rising since the Industrial Revolution from 280 to 379 μmol mol−1, and is predicted to be double that in this century (IPCC, 2007). An increase of [CO2] can enhance leaf photosynthesis of C3 species, including rice and many other food crops, and can increase growth and grain yield (Kimball et al., 2002). Reported yield enhancement by elevated [CO2] varies widely due to different experimental methods, but canopy-level studies show it to be 10–20% at +200 μmol mol−1 [CO2] under non-stress conditions (Kimball et al., 2002). This enhancement can be reduced under stress conditions such as low temperature (Okada et al., 2005; Shimono et al., 2008) and high temperature (Kim et al., 1996; Matsui et al., 1997). To keep pace with the increasing world food demand under future climates (United Nations, 2005), yield increases with high [CO2] could be important.

Nitrogen (N) uptake by roots is an important factor in achieving higher yields under elevated [CO2]. It is commonly accepted that higher amounts of N input lead to greater yield enhancement by elevated [CO2] (Rogers et al., 1996; Kimball et al., 2002; Kim et al., 2003), but the increment of yield enhancement by [CO2] per unit of N input became smaller with higher amounts of N input (Smart et al., 1998; Kimball et al., 2002; Kim et al., 2003). This might be attributed to limited sink capacities for absorbing N and C under higher amounts of N input (Rogers et al., 1996). However, considering the fact that plant N concentration on a dry weight basis at any N regime was reduced by elevated [CO2], N uptake may be a bottleneck for yield enhancement. If plants could absorb N to keep up with their dry weight increase, greater yield enhancement under elevated [CO2] might occur.

N uptake of roots is determined by the two factors root mass and capacity to take up N per unit of root. It is generally reported that elevated [CO2] increased root mass due to enhanced photosynthesis and growth (Kimball et al., 2002; Kim et al., 2003); a larger root mass could increase N uptake due to increased root surface coming into contact with soil nutrients. On the other hand, there is limited and conflicting information concerning the effects of elevated [CO2] on the N uptake capacity of roots per unit of root tissue. Elevated [CO2] increased N uptake capacity in red maple (1-year-old seedling; BassiriRad et al., 1999), did not alter the capacity of sorghum (40-d-old seedling) and sugar maple (1-year-old seedling; BassiriRad et al., 1999), and decreased the capacity of cottonwood trees (40-d-old seedling; McDonald et al., 2002), rice (80- to 100-d-old seedling; Morita et al., 2005) and loblolly and ponderosa pine

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(160-d-old seedling; BassiriRad et al., 1997). In the case of soybean, Delhon et al. (1996) reported a positive effect of elevated [CO2] on N uptake capacity using 20-d-old seedlings under growth chamber conditions, but BassiriRad et al. (1999) reported no effect of elevated [CO2] on N uptake capacity using 40-d-old seedlings in an open-top chamber experiment. Most of these studies relied on evaluations at a single growth stage, and there are limited studies concerning seasonal changes of N uptake capacity under long-term [CO2] elevation in spite of ample evidence of acclimation responses of several physiological characteristics involved in photosynthesis (reviewed by Moore et al., 1999), N allocation (e.g. Makino et al., 1997; Kim et al., 2003) and growth (e.g. Kim et al.; 2003; Sakai et al., 2006). It is difficult to draw a clear picture of how N uptake capacity is affected by elevated [CO2].

N uptake capacity can be affected by various factors, most of which could also be affected by [CO2]. It is well known that elevated [CO2] increases photosynthesis and carbohydrate supply (Sasaki et al., 2005); the increased carbohydrate is expected to increase the N uptake capacity (Sasakawa and Yamamoto, 1978; Gastal and Saugier, 1989; Delhon et al., 1996; Lejay et al., 1999, 2003; Sehtiya and Goyal, 2000). Also, the enhanced photosynthesis and growth by elevated [CO2] promotes N utilization in plants, leading to a decrease in the concentration of N in plant tissues (Kimball et al., 2002). Since root N concentration is reported to be a signal for regulating N uptake capacity (Dluzniewska et al., 2006), the decreased N concentration in roots caused by elevated [CO2] might promote N uptake capacity. In contrast, elevated [CO2] generally reduces stomatal opening and transpiration (Kimball et al., 2002); the decreased transpiration stream caused by elevated [CO2] can decrease N uptake (McDonald et al., 2002). There are several reports of a close relationship between transpiration and N uptake (Delhon et al., 1995; Cerezo et al., 1999; Kiyomiya et al., 2001). Thus, elevated [CO2] could affect N uptake capacity both positively and negatively through variations in photosynthesis and transpiration. The present study examined the effects of elevated [CO2] on N uptake capacity in rice throughout the season in relation to the responses of photosynthesis, transpiration and growth. To help in interpreting the seasonal patterns, the effects of short-term elevation of [CO2] on N uptake at two growth stages were also examined for plants grown at ambient [CO2].

MATERIALS AND METHODS

Long-term [CO2] experiment

The rice cultivar ‘Akitakomachi’ (Oryza sativa L.) was grown hydroponically (not aerated) under two [CO2] conditions using two controlled environmental chambers (EGC Corp., Chagrin Falls, OH). The chambers were maintained at either 350 or 700 μmol mol⁻¹ [CO2], and with a 12 h photoperiod [photonically active radiation (PAR) of 650 μmol m⁻² s⁻¹ measured at pot height], 26/20 °C day/night temperature and 60 % relative humidity. Germinated seeds were sown on vermiculite held in plastic sieves (circular in shape with diameter 12 cm and 10 cm depth). After emergence (a week after sowing), the sieves were set into 4 L pots containing a complete nutrient solution (one plant per pot). The bottoms of the sieves were positioned above the nutrient solution, and all water and nutrient uptake occurred in roots which passed through the sieve into the nutrient solution. The nutrient solution contained 0.375 mM NH₄Cl, 0.250 mM NH₄H₂PO₄, 1.00 mM Ca(NO₃)₂·4H₂O, 0.875 mM MgSO₄·7H₂O, 0.625 mM KCl, 1.00 mM KNO₃, 0.250 mM K₂SO₄, 0.02 mM (NH₄)₂MoO₄·2H₂O, 5.15 mM H₂BO₃, 0.04 mM CuSO₄·5H₂O, 1.13 mM MnSO₄·2H₂O, 0.09 mM ZnSO₄·7H₂O and 26.9 mM Fe chelate (Sprint 330, Ciba-Geigy Corp., Greensboro, NC) with the pH adjusted to 5.0. The nutrient solution was made up from tap water. It provided 3-6 mM N (3-0 mM nitrate and 0-63 mM ammonium). The solution was refreshed and the pots were rotated inside the chamber once a week for the first month, twice a week for the next month and three times a week after that to supply enough nutrients and minimize error caused by locations. Each [CO2] chamber had 11 plants (one plant per pot). Note that in paddy fields, ammonium is abundant rather than nitrate, and the ammonium concentration is at most about 0.3 mM (Yamakawa et al., 2004).

Measurements of N uptake capacity, CO₂ assimilation rate and dry weight were conducted at three growth stages [panicle initiation (PI), full heading (HD) and mid-ripening (RP)] for 3–4 plants per each [CO2]. At each stage, firstly N uptake capacity and plant transpiration were measured [PI = 53 days after sowing (DAS), HD = 96 DAS, RP = 119 DAS], then the photosynthesis rate was measured using a portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE; PI = 55 DAS, HD = 98 DAS, RP = 125 DAS) and, finally, plants were harvested (PI = 59 DAS, HD = 101 DAS, RP = 133 DAS). The harvested plants were separated into green leaves, stem and culms, panicles (if present), roots and dead leaves, and dried for 72 h at 70 °C. The plant tissue samples were measured for N concentration using a CHN analyser (Perkin Elmer 2400, Series II CHNS/O Analyzer, Waltham, MA). Heading date, defined as the date when 50 % of productive tillers headed, was measured at 2 d intervals.

N uptake capacity was measured for 24 h using 2-0 L plastic beakers filled with 1-6 L of the nutrient solution described above. The volume and the concentration of nitrate and ammonium in the nutrient solution were measured at the beginning and at the end of the 24 h period using ion-specific electrodes (Cole-Palmer Inst. Co., Vernon Hill, IL). Electrodes were calibrated using five known concentrations of nitrate and ammonium solutions before the measurements. The reproducibility was ± 0-03 mm in the range from 1-1 to 6-3 mm for nitrate and ± 0-13 mm in the range from 0-3 to 2-5 mm. N uptake capacity (NUC) was calculated from the following equation separately for nitrate and ammonium.

\[
\text{NUC} = \frac{(V_{\text{initial}} \times c_{N_{\text{initial}}}) - (V_{\text{final}} \times c_{N_{\text{final}}})}{\text{Time}} \times \frac{1}{R_{DW}} \tag{1}
\]

where \(V_{\text{initial}}, V_{\text{final}}, c_{N_{\text{initial}}}, c_{N_{\text{final}}}, \) and \(R_{DW}\) are the volume (V) and the concentration of nitrate or ammonium form (cN) at the start and the end of measurement, and the root dry weight that was in the nutrient solution (\(R_{DW}\)), respectively. The reduction in the volume and the concentration without
plants was very small \([<1–2\% \text{ (v/v)}]\), so this was not taken into account in the calculation. The transpiration rate was calculated from the volume change.

**Short-term [CO2] experiment**

The rice cultivar ‘Akitakomachi’ was hydroponically grown using a controlled environmental chamber with the same growth conditions as in the long-term [CO2] experiment, except for [CO2]. The chamber was maintained at a [CO2] of 350 \(\mu\text{mol mol}^{-1}\).

Plants were exposed to a 24 h treatment of two levels of [CO2] (350 or 700 \(\mu\text{mol mol}^{-1}\)) at the early vegetative stage (46 and 48 DAS) and to three levels of [CO2] (200, 350 or 700 \(\mu\text{mol mol}^{-1}\)) at the mid-reproductive stage (72–74 DAS). The experiment at the early vegetative stage was conducted using two independent growth chambers; six plants were moved from a control chamber to a treatment chamber at 700 \(\mu\text{mol mol}^{-1}\) at 46 DAS, and another six plants were moved to the treatment chamber at 350 \(\mu\text{mol mol}^{-1}\) at 48 DAS. The experiment at the mid-reproductive stage was conducted by using three independent chambers at 200, 350 or 700 \(\mu\text{mol mol}^{-1}\). A total of 12 plants were distributed into four groups (denoted as A, B, C and D) with three plants in the four replicate groups. Each group was exposed to a different [CO2] in a different order (at 72 DAS, group A, D = 200 \(\mu\text{mol mol}^{-1}\), C = 350 \(\mu\text{mol mol}^{-1}\), B = 700 \(\mu\text{mol mol}^{-1}\); at 73 DAS, group C = 200 \(\mu\text{mol mol}^{-1}\), A, B = 350 \(\mu\text{mol mol}^{-1}\), D = 700 \(\mu\text{mol mol}^{-1}\); at 74 DAS, group B = 200 \(\mu\text{mol mol}^{-1}\), D = 350 \(\mu\text{mol mol}^{-1}\), C, A = 700 \(\mu\text{mol mol}^{-1}\)). Leaf area averaged 503 \(\pm 24\text{ cm}^2\) per plant at the early vegetative stage and 1698 \(\pm 102\text{ cm}^2\) per plant at the mid-reproductive stage.

**Statistical analysis**

For the long-term [CO2] study, statistical analysis was conducted by \(t\)-tests. For the short-term [CO2] study, statistical analysis was conducted for parameters at the early vegetative stage by \(t\)-tests using individual plants as replicates for the experiment \((n = 4 \text{ or } 6)\), and statistical analysis was conducted for parameters at the mid-reproductive stage by one-way analysis of variance, using the least significant difference test with \(P = 0.05\) for separation of means of four replicates (= groups) as implemented by the SPSS for Windows 7.5J statistical software (SPSS, Inc., Chicago, IL). Regression analysis was conducted by comparing the residual variation about a common line with the sum of residual variations about individual lines (Mead et al., 2003). When the regressions were significantly different by this test, fitting parallel lines was tested by a comparison between the residual variation about parallel lines and the sum of residuals from fitting individual lines of different slope.

**RESULTS**

**Long-term [CO2] experiment**

Elevated [CO2] increased total dry weight and root dry weight, but the magnitude of the enhancement decreased with successive growth stages from 35 to 6\% for total dry weight and from 39 to 9\% for root dry weight (Fig. 1). Green leaf area was not affected by elevated [CO2] before the heading stage, but it was reduced by 17\% at the grain-filling stage \((P < 0.05)\), indicating that elevated [CO2] promoted leaf senescence. Because elevated [CO2] reduced plant N concentration per dry weight at all stages (data not shown), elevated [CO2] did not increase total plant N content at any growth stage in spite of the fact that root system mass had been increased by elevated [CO2] (Fig. 1). The ratio of root dry weight to top dry weight was not affected by elevated [CO2].

Leaf photosynthetic response to intercellular [CO2] \((C_i; \text{ A} \leftarrow \text{C} \text{ response})\) was identical between the growth [CO2] treatments at both panicle initiation and heading (Fig. 2). However, at grain filling, the initial slope of plants grown under elevated [CO2] was lower than that of plants grown under ambient [CO2], although there was no difference in leaf photosynthesis at \(>1200 \mu\text{mol mol}^{-1} C_i\).

Uptake capacities of nitrate and ammonium at panicle initiation based on root dry mass were not affected by elevated [CO2], but at the two later stages they were decreased by elevated [CO2] by about 40\% (Fig. 3). When the capacities were expressed based on root N, a similar pattern existed, but with somewhat smaller negative effects of elevated [CO2] (17 and 36\% at the two later stages, respectively) because root N concentration was reduced by elevated [CO2] (data not shown). Similar responses were observed for plant transpiration rate (Fig. 3). Positive relationships between total N uptake capacity and transpiration rate were observed, and the relationships were independent at each growth stage \((F_{4,14} = 153, P < 0.001)\; \text{Fig. 4}. \) The slopes in the regressions at the heading and grain-filling stages were identical, but not at the panicle initiation stage. Compared at the same transpiration rate, the N uptake capacity was greater for earlier growth stages than later growth stages.

**Short-term [CO2] experiment**

Uptake capacities of N, for both ammonium and nitrate, increased with elevated [CO2] at the early vegetative stage \((P < 0.05)\), but the capacities were decreased with elevated [CO2] at the mid-reproductive growth stage \((P < 0.05)\; \text{Fig. 5}. \) However, at both the early vegetative and mid-reproductive stages, the transpiration rate was decreased by elevated [CO2].

There were close positive relationships between N uptake capacity and transpiration rates at the mid-reproductive growth stage over all levels of [CO2] (Fig. 6). However, at the early vegetative stage, the relationships were different between [CO2] \((F_{2, 8} = 4.29, P < 0.05)\ using all data; \(F_{2, 6} = 69.6, P < 0.001\ using data excluding one data point for each [CO2]). \) When including all data, significant relationships between N uptake capacity and transpiration rate could not be detected at either [CO2], but data excluding one data point for each [CO2] (circled in Fig. 6) showed significant and positive relationships between N uptake capacity and transpiration rate at each [CO2].
DISCUSSION

The present study showed that responses of uptake capacities of both ammonium and nitrate to elevated [CO₂], estimated based on uptakes per unit root mass, changed with plant growth stage and/or duration of exposure to elevated [CO₂]. This was evident in the long-term study by the lower capacities.
of N uptake at elevated [CO₂] in the later growth stages, and in
the short-term study by the contrasting responses of N uptake
to elevated [CO₂] at the two growth stages. The short-term
effect of elevated [CO₂] in the vegetative stage was an increase
in N uptake capacities (Fig. 5), and this contrasts with the lack
of increase in N uptake capacities at elevated [CO₂] even at the
earliest stage measured in the long-term study (Fig. 3), and the
lack of increase in total N content per plant throughout
the season despite greater root dry weight (Fig. 1). The con-
trast between the short-term and long-term responses
suggested that acclimation of N uptake capacity to elevated
[CO₂] occurred in the early growth stages. However, the
lower capacities at elevated [CO₂] at the later growth stages
in the long-term experiment (Fig. 3) were consistent with the
decrease in N uptake at elevated [CO₂] in the short-term experi-
ment at the later growth stage (Fig. 5). Because the short-term
[CO₂] response for plants exposed to long-term [CO₂] was not
measured, it cannot be concluded from the present results that
acclimation of N uptake to elevated [CO₂] continued in the
later growth stages. However, the lower capacities at elevated
[CO₂] at the later growth stages in the long-term experiment
(Fig. 3) were consistent with the decrease in N uptake at elevated [CO₂] in the short-term experiment at the later growth stage (Fig. 5). Because the short-term [CO₂] response for plants exposed to long-term [CO₂] was not measured, it cannot be concluded from the present results that acclimation of N uptake to elevated [CO₂] continued in the later growth stages. However, the lower capacities at elevated [CO₂] at the later growth stages in the long-term experiment (Fig. 3) could have resulted from the acclimation response in addition to the direct effects of elevated [CO₂] because it is generally observed that the acclimation of several physiological characteristics did not recover with prolonged duration of exposure to elevated [CO₂] once plants had acclimated
to elevated [CO₂] (Makino et al., 1997; Moore et al., 1999;
Kim et al., 2003; Sakai et al., 2006). The present study
clearly demonstrated that growth stage and duration of
exposure to elevated [CO₂] were important in determining the
patterns of N uptake in response to elevated [CO₂].
Decreased transpiration might be one factor responsible for
regulating N uptake capacity. In the long-term [CO₂] study at
each growth stages (Fig. 4) and in the short-term [CO₂] study

Fig. 3. Seasonal changes in nitrate and ammonium uptake capacities and
transpiration rate in rice plants exposed to long-term [CO₂] treatments. N
uptake capacities and transpiration rate are expressed per unit of dry mass of
root which reached through the sieve into the nutrient solution. Total N
uptake capacity was expressed as the sum of nitrate and ammonium uptake
capacities. Percentage changes due to elevated [CO₂] are given. Bars indicate
the s.e. for 3–4 plants. ** , * and ns indicate the 1 %, 5 % and the not signifi-
cantly different levels of significance, respectively.

Fig. 4. The relationship between total N uptake capacity and plant transpira-
tion rate of rice plants exposed to long-term [CO₂] treatments. Total N uptake
capacity was expressed as the sum of nitrate and ammonium uptake capacities.
Correlation coefficients and regression equations are given. DAS, days after
sowing. ***, * and ns indicate the 0.1 %, 5 % and the not significantly different
levels of significance, respectively.
at the mid-reproductive stage (Fig. 6), there was a close positive correlation between N uptake capacity and transpiration rate over \([\text{CO}_2]\) environments. McDonald et al. (2002) exposed a 40-d-old cottonwood tree potted in soil to different \([\text{CO}_2]\) and humidity for 10 d, and demonstrated that the reduction in N uptake rate at elevated \([\text{CO}_2]\) resulted from a decreased transpiration rate in the short-term study at the early vegetative stage. Their experiment could not separate a direct effect of elevated \([\text{CO}_2]\) on N uptake capacity and an indirect effect of elevated \([\text{CO}_2]\) on N movement in the soil. The present study was conducted under hydroponic conditions, which excludes effects of nutrient movement in

![Graphs showing N uptake capacity and transpiration rate](image)

**Fig. 5.** Nitrate and ammonium uptake capacities and transpiration rates of rice plants exposed to short-term \([\text{CO}_2]\) treatments at two growth stages. N uptake capacities and transpiration rate are expressed per unit of dry mass of root which reached through the sieve into the nutrient solution. Total N uptake capacity was expressed as the sum of nitrate and ammonium uptake capacities. Bars indicate the s.e. of six individual plants at the early vegetative growth stage and of four averaged groups of plants at the mid-reproductive stage. The same letter indicates that mean values were not significantly different at the 5 % probability level.

![Graphs showing relationships between N uptake capacity and transpiration rate](image)

**Fig. 6.** Relationships between total N uptake capacity and plant transpiration rate of rice plants exposed to short-term \([\text{CO}_2]\) treatments at different growth stages. Total N uptake capacity was expressed as the sum of nitrate and ammonium uptake capacities. Correlation coefficients and regression equations are given. ***, **, * and ns indicate the 0.1 %, 1 %, 5 % and the not significantly different levels of significance, respectively.
the soil, and showed that elevated \([\text{CO}_2]\) affected N uptake capacity.

However, in the short-term \([\text{CO}_2]\) experiment at the early growth stage (46–48 d old), the transpiration rate could not explain variations in N uptake capacity due to \([\text{CO}_2]\) (Fig. 6). At this stage, elevated \([\text{CO}_2]\) enhanced N uptake capacity even though elevated \([\text{CO}_2]\) significantly decreased and transpiration rate between different growth stages obtained the stage of growth and showed acclimation to elevated \([\text{CO}_2]\). Systems to take up nitrate and ammonium ions changed with hint for the present analysis. We thank Mrs Frances Caulfield for the maintenance of the growth chambers and plants, Dr Junko Sakurai, Dr Mari Murai, Dr Kensaku Suzuki and Dr Takami Hayashi, of the National Agricultural Research Center for Tohoku Region, Japan, for the information about environmental conditions for growing rice, and the anonymous reviewers for invaluable comments and suggestions. This study was supported by a research fellowship from the Japanese Society for the Promotion of Science for Young Scientists.

**LITERATURE CITED**


