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Dynamic analysis of the impact of free-air CO_2 enrichment (FACE) on biomass and N uptake in two contrasting genotypes of rice

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Abstract. Elevated CO_2 concentrations ([CO_2]) in the atmosphere often increase photosynthetic rates and crop yields. However, the degree of the CO_2 enhancement varies substantially among cultivars and with growth stage. Here, we examined the responses of two rice cultivars, Wuyunjing23 (WYJ) and IIyou084 (IIY), to two [CO_2] (~400 vs ~600) and two nitrogen (N) provision conditions at five growth stages. In general, both seed yield and aboveground biomass were more responsive to elevated [CO_2] in IIY than WYJ. However, the responses significantly changed at different N levels and growth stages. At the low N input, yield response to elevated [CO_2] was negligible in both cultivars while, at the normal input, yield in IIY was 18.8% higher under elevated [CO_2] than ambient [CO_2]. Also, responses to elevated [CO_2] significantly differed among various growth stages. Elevated [CO_2] tended to increase aboveground plant biomass in both cultivars at the panicle initiation (PI) and the heading stages, but this effect was significant only in IIY by the mid-ripening and the grain maturity stages. In contrast, CO_2 enhancement of root biomass only occurred in IIY. Elevated [CO_2] increased both total N uptake and seed N in IIY but only increased seed N in WYJ, indicating that it enhanced N translocation to seeds in both cultivars but promoted plant N acquisition only in IIY. Root C accumulation and N uptake also exhibited stronger responses in IIY than in WYJ, particularly at the heading stage, which may play a pivotal role in seed filling and seed yield. Our results showed that the more effective use of CO_2 in IIY compared with WYJ results in a strong response in root growth, nitrogen uptake, and in yield. These findings suggest that selection of [CO_2]-responsive rice cultivars may help optimise the rice yield under future [CO_2] scenarios.

Additional keywords: elevated [CO_2], growth stages, rice cultivars, root, nitrogen, yield.

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Introduction

The burgeoning human population, declining quality of existing arable land, and decreasing availability of new arable land, exert increasing pressure on global agricultural productivity. Rice (*Oryza sativa* L.) is the world's leading food crop, providing staple food for nearly half the human population. By 2050, food production is projected to increase by ~70 percent globally, and by nearly 100 percent in developing countries (FAO 2011). A recent study revealed that more than 78, 37 and 81% of rice growing areas in China, India, and Indonesia (the top three producers globally), respectively, experienced yield stagnation between 1961 (the onset of the Green Revolution) and 2008 (Ray *et al*. 2012), illustrating both the challenge and potential of increasing crop yields further in the coming decades. Meanwhile, global crop production will also be, and already is being, profoundly affected by global climate change (Long *et al*. 2004, 2006). For plant biologists and breeders, one of the paramount objectives, therefore, is to identify and select the varieties that are responsive to higher [CO_2] in a changing climate. (Ainsworth 2008; Ainsworth *et al*. 2008).

The atmospheric CO_2 concentration has been rising at an accelerating rate since the beginning of the industrial revolution in the 1760s. It has increased from ~280 $\mu mol\,mol^{-1}$ before the 1760s to ~404 $\mu mol\,mol^{-1}$ in 2017 (Dlugokencky and Tans 2018), and is expected to continue to increase and reach values of 530–970 $\mu mol\,mol^{-1}$ by the end of the 21st century (Bloom *et al*. 2010). CO_2 is an essential substrate for photosynthesis in plants. For C_3 species such as rice, because of the more readily saturated state for the CO_2 substrate at higher CO_2 levels of Rubisco in mesophyll cells, carbon fixation can be improved; additionally, elevated [CO_2] inhibits the oxygenation reaction at Rubisco, and, thus, photorespiration and the associated cycles of energy consumption (Long *et al*. 2004; Chen *et al*. 2005). Therefore, increased CO_2 availability could directly and indirectly enhance rice vegetative growth and final crop yield (Makino and Mae 1999; Zhu *et al*. 2016). However, the degree of this enhancement varies not only among species but also among cultivars. In the rice cultivar Akitakomachi, a yield increase of 14% was reported under free-air carbon dioxide enrichment (FACE) (Kim *et al*. 2001), FACE increased brown rice yield of the Koshihikari cultivar by ~16% (Zhang *et al*. 2013), and yield of the three-line Indica hybrid Shanyou63 was increased by 34% (Liu *et al*. 2008). For most observed rice cultivars, FACE increased production by less than 20%. Yet yields in some cultivars increased by more than 30% under FACE conditions (Kim *et al*. 2001; Liu *et al*. 2008; Zhang *et al*. 2013; Zhu *et al*. 2014). Obviously, understanding the basis for this variation would represent an important advance to help guide future selection of cultivars and developments in the breeding of high-response crop lines under elevated [CO_2]. Little is known about the dynamic response of biomass response to elevated [CO_2], especially at different growth stages. It is unknown when during the development cycle of the plant, response differences begin and how they are maintained.

When soil nutrient supply is sufficient, root uptake depends on root size and activity (Yang *et al*. 2008). Because root sampling in the field is labour-intensive, and soil sampling can be destructive, there are few observations of root traits under elevated CO_2 concentrations in the field as compared with shoot traits. According to Kim *et al*. (2001), the rice cultivar Akitakomachi shows greater root and total dry matter under FACE conditions. Furthermore, there was a positive relationship between root dry matter and crop N uptake across CO_2 treatments and sampling periods (Kim *et al*. 2001). For the Japonica cultivar Wuxiangjing14, adventitious root number and adventitious root length were, on average, 25–31% and 25–37% greater, respectively, under elevated [CO_2] than those under ambient conditions (Yang *et al*. 2008). Recently, Zhu *et al*. (2013) reported increases in root growth in four cultivars in the range of 12–38% under FACE. It is not known whether root and shoot responses are synchronised or whether they occur independently. Further, also unknown is how soil N availability affects the responses in C allocation.

Past research on cultivar variation in response to elevated [CO_2] mainly focussed on the aboveground part of the plant, including spikelet number, grain weight, sink : source ratios, photosynthesis acclimation, and Rubisco content and expression (Zhu *et al*. 2014). According to Zhu *et al*. (2014), the greater response seen in the S63 line may be associated with enhanced carbon sinks relative to sources, and the ability to maintain photosynthetic capacity during grain development. However, little is known about the CO_2 response of the root system in terms of both C allocation and N uptake in contrasting cultivars. The differential responses of total N uptake to elevated [CO_2] between rice cultivars have not been reported. Could yield improvement in high-response rice cultivars under FACE relate to improved coordination of the balance in carbon and nitrogen status?

In the present study, we chose two rice cultivars, WYJ and IIY, which have been reported to have a weak and strong response, respectively, in yield trials under the FACE platform, to explore (i) how shoot and root biomass respond dynamically to elevated [CO_2] and whether they respond synchronously; (ii) whether the different response in yield is associated with different N acquisition traits between cultivars; and (iii) what the probable explanations are that permit the high-response cultivar to effectively respond to FACE at different stages of development. The exploration of these mechanisms will be essential to future breeding efforts aimed at improving crop performance under elevated CO_2 concentrations.

Materials and methods

Experimental site

The rice FACE facility was located at Zhongcun village (119°42′0″E, 32°35′5″N), Yangzhou city, Jiangsu province, China. A rice–wheat rotation system prevailed in this region. The soil properties were as follows: bulk density, 1.16 g cm^{-3}; total porosity, 54%; pH 7.2; organic carbon, 18.4 g kg^{-1}; total N content, 1.45 g kg^{-1}; total P content (as P_2O_5), 0.63 g kg^{-1}; clay (<0.002 mm), 13.6%; silt (0.002–0.02 mm), 28.5%; and sand (0.02–2 mm), 57.8%. The soil was classified as Shajiang Aquic Cambiosol (Cooperative Research Group on Chinese Soil Taxonomy 2001) and had a sandy-loamy texture, as per the US soil classification system.

FACE system

Plants were exposed to elevated [CO_2] by being grown in three rings (12 m diameter) under FACE, and three control rings under natural, ambient [CO_2]. In order to minimise CO_2 contamination, the distance between any two rings remained at least 90 m. For each ring, pure CO_2 was sprayed into the centre from peripheral emission tubes surrounding the experimental area 50–60 cm above the canopy, and connected a 10 m tall container full of carbon dioxide ice. Carbon dioxide release was controlled by a computer program, set based upon wind speed and direction. The CO_2 concentration in the centre of the elevated FACE rings was ~200 µmol mol^{-1} above ambient. Elevated [CO_2] concentrations were within 80% of the set point, >90% of those in each given year.

Crop cultivation

Two rice cultivars, WYJ (Japonica) and IIY (hybrid Indica), were chosen in the present study. Seeds of two lines were sown on seedling beds on 21 May 2014 under ambient [CO_2] conditions. A month later, seedlings were transplanted to ambient and elevated [CO_2] rings. Spacing of the hills was 16.7 × 25 cm (24 hills m^{-2}). A 30-cm polyvinyl chloride (PVC) barrier was inserted more than 10 cm into the soil to isolate an area characterised by zero nitrogen and water exchange with other areas in the ring. Phosphorus and potassium were applied as basal fertiliser before transplanting, as P_2O_5 (9 g m^{-2}) and K_2O (9 g m^{-2}). Urea (N, 46%) was used as the N fertiliser. N (22.5 g m^{-2}) was applied as a basal dressing (40% of the total) 1 day before transplanting (20 June), as a top dressing at early tillering (30% of the total, 28 June) and at panicle initiation (30% of the total, 1 August), which followed the local normal N application (NN). There was no N added in field at zero N treatment (0N). All experimental plots were submerged in water from 13 June to 10 July, then drained dry several times until 4 August, and flooded with intermittent irrigation from 5 August to 10 days before harvest. The plants in all plots were surrounded with border plants treated the same way as the plants inside. Other field managements, such as pesticides and weed control, were consistent with local farming practice.

Sampling and analysis

The plants were sampled destructively at five periods over the plant growth process, including the tillering, panicle initiation, heading, mid-ripening, and maturity stages. In order to extract the root as completely as possible from the soil, root bags made of nylon material were buried in the soil before transplanting, with one bag for each hill. The bottom of the bags was below the soil surface by more than 20 cm. For every CO_2 and N treatment, no less than nine repeats were set and sampled. During each sampling, a bag full of soil and root was removed, leaving a hill-size hole. The root-soil block was washed under water in a box, and the resulting mud precipitation was placed back into the field hole to maintain stable soil performance. The vacant spaces left after sampling were replanted with the border hills to maintain canopy conditions, and such plants were not sampled again. Samples were separated into shoot and root parts, which were

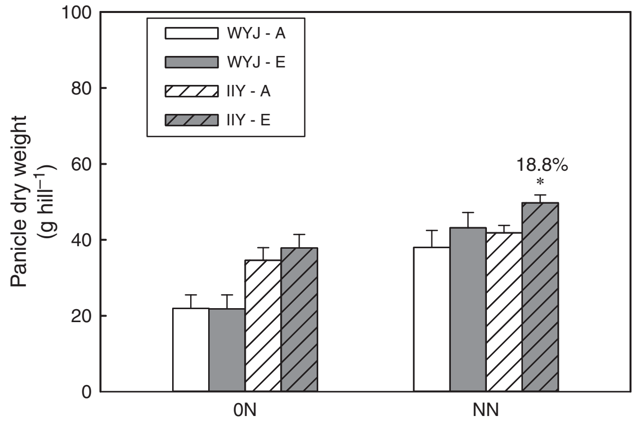

Fig. 1. Panicle DW per hill for the rice genotypes WYJ and IIY at ambient (A) and elevated (E) $[CO_2]$, with (NN) or without (0N) added nitrogen. Asterisks indicate significant differences between two levels of $[CO_2]$. The percentages are calculated as (E–A)/A. See 'Materials and methods' for additional details. Significant differences are indicated: *, $P < 0.05$; **, $P < 0.01$; bars indicate ±s.d.

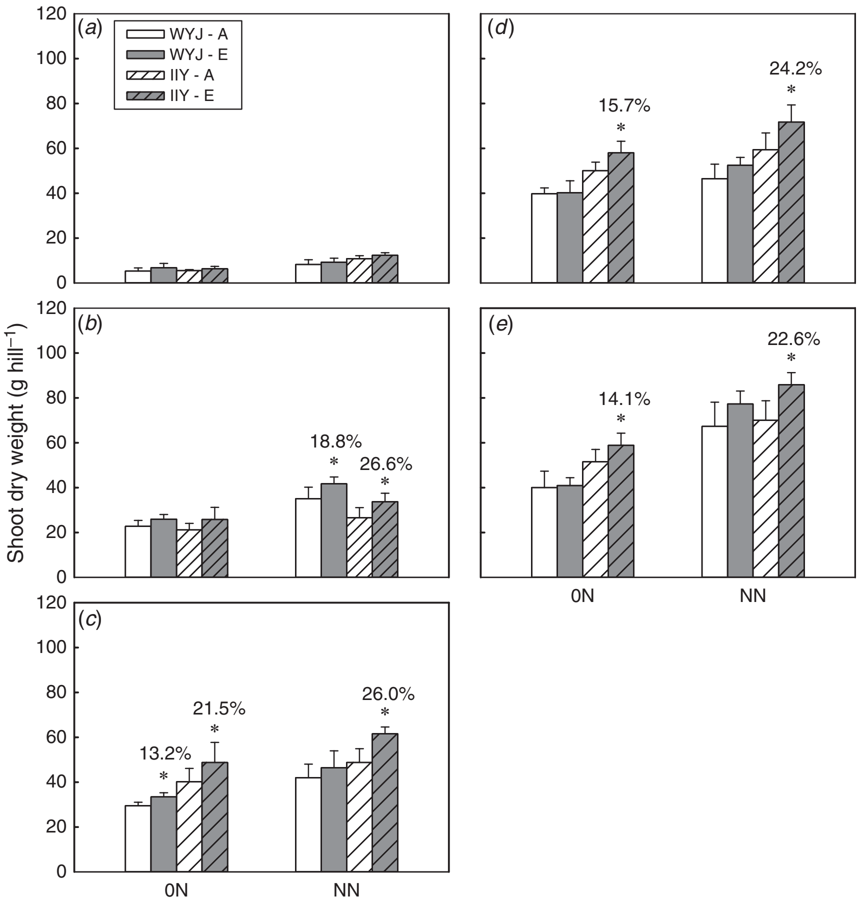

Fig. 2. Shoot DW per hill at different growth stages of the rice genotypes WYJ and IIY under ambient (A) and elevated (E) $[CO_2]$, with (NN) or without (0N) added nitrogen. Growth stages: (*a*) tillering; (*b*) panicle initiation, (*c*) heading, (*d*) mid-ripening; and (*e*) grain maturity. The numbers are calculated as (E–A)/A. See 'Materials and methods' for additional details. Significant differences between two CO_2 treatments are indicated: *$P < 0.05$; **$P < 0.01$; bars indicate ±s.d.

immediately placed in the oven steamed at 105°C for 30 min and then dried to constant weight at 80°C. For the final sampling at the maturity stage, panicles were excised from all shoots and analysed separately.

C and N concentrations in the rice plant

Oven-dried parts of plants were ground into powder using a grinding machine and then passed through a 0.2 mm sieve. C and N concentrations were analysed using a C-N analyser (Vario MAX CN). The uptake of C and N was calculated as follows: amount of total C or n = DW of relevant pat part × C or N concentration.

Statistical analyses

The experiment was set as a blocked split-split plot. The [CO_2] was treated as a fixed effect and was the whole-plot treatment. N was set as a split-plot treatment, and cultivars were set as the split-split plot treatment. Data were analysed using the statistical package IBM SPSS Statistic ver. 18.0 (IBM Corp.). Data were statistically analysed using ANOVA, to determine principal effects.

Results

The IIY cultivar had a greater yield response relative to the WYJ at elevated [CO_2]

The DW of panicles in IIY at ambient [CO_2] was 41.88 g per hill, whereas it was 49.76 g per hill at elevated [CO_2]. Exposure to elevated [CO_2] significantly increased the panicle weight of cultivar IIY, by 18.8%, at the local N level of the experiment (Fig. 1), whereas no significant difference was detected between two levels of CO_2 in the absence of N fertiliser. By contrast, the cultivar WYJ did not present any significant change between FACE and ambient [CO_2] at either N level.

The difference began to emerge at the PI stage

With no additional nitrogen fertiliser in the soil, IIY displayed significant increases in shoot DW, by 21.5, 15.7, and 14.1%, at the heading, mid-ripening, and grain maturity stages, respectively,

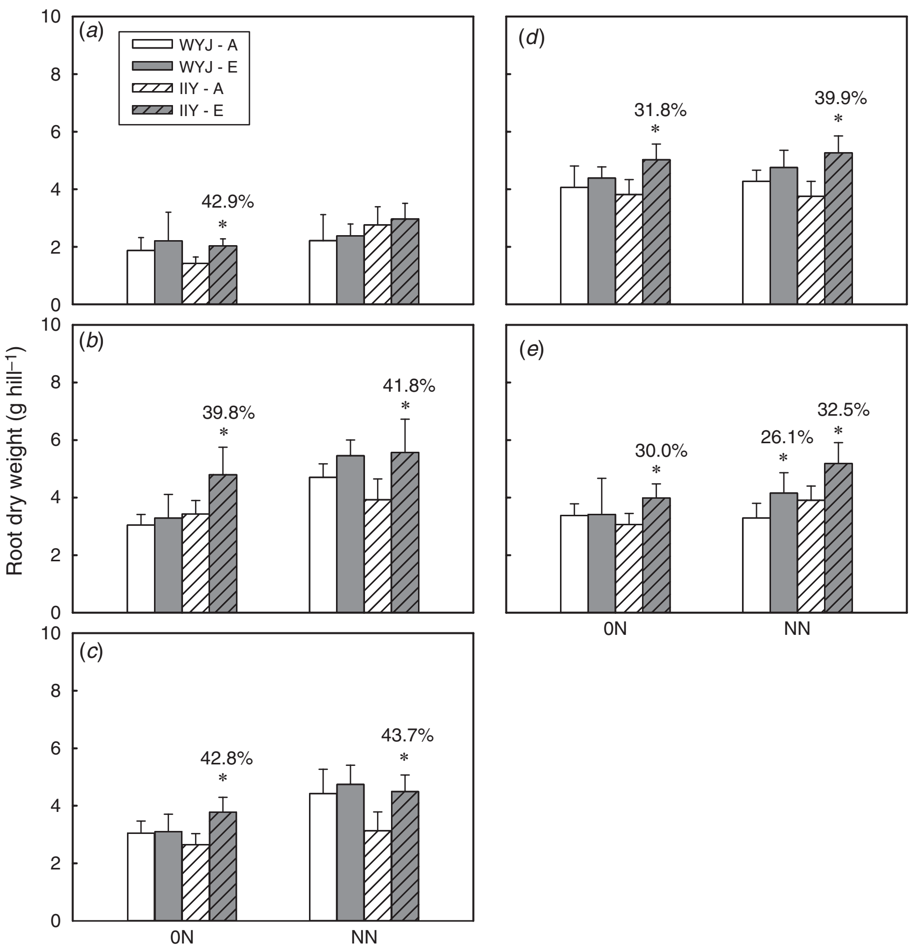

Fig. 3. Root DW per hill at different growth stages of the rice genotypes WYJ and IIY under ambient (A) and elevated (E) [CO_2], with (NN) or without (0N) added nitrogen. Growth stages: (*a*) tillering; (*b*) panicle initiation, (*c*) heading, (*d*) mid-ripening; and (*e*) grain maturity. The numbers are calculated as (E–A)/A. See 'Materials and methods' for additional details. Significant differences between two CO_2 treatments are indicated: *P < 0.05; **P < 0.01; bars indicate ±s.d.

under FACE (Fig. 2*c–e*). WYJ production in the FACE circle was significantly increased only during the heading phase (Fig. 2*c*). At normal N levels applied to soil, FACE increased the dry weight of IIY shoots by 26.6, 26.0, 24.2 and 22.6%, at the PI, heading, mid-ripening, and grain maturity, respectively (Fig. 2*b–e*), whereas WYJ only showed an increase of 18.8% during PI (Fig. 2*b*). Independent of N level, FACE increased shoot DW to a larger extent in IIY than in WYJ. Although WYJ showed more shoot biomass production than IIY at both CO_2 levels at the second stage observed, this phenomenon did not persist. Following this, the high-response cultivar IIY began to accumulate more biomass during the heading period (Fig. 2).

The response of IIY root growth was more significant, in keeping with the response in aboveground biomass

Along with the response difference in aboveground biomass between two contrasting lines, the growth response of the root system to FACE was also quite distinct. Root DW was monitored from the tillering period to maturity. For IIY, at all five sampling stages, root dry matter was greater under the FACE treatment (Fig. 3). Without additional N fertiliser, FACE significantly increased root dry matter, by 42.9, 39.8, 42.8, 31.8 and 30.0%, at the tilling, PI, heading, mid-ripening, and grain maturity stages, respectively, in comparison to their ambient controls (Fig. 3*a–e*). There was no significant difference observed in WYJ under the same conditions. Similarly, with normal N fertiliser levels in the soil, FACE significantly increased the root dry weight of IIY by 41.8, 43.7, 39.9 and 32.3% at the PI, heading, mid-ripening, and grain maturity stages (Fig. 3*b–e*). WYJ root dry matter was increased by 26.1% under FACE conditions only at the maturity stage (Fig. 3*e*). Although root growth in WYJ showed an upward trend at high [CO_2], the increase was far less than in IIY.

FACE enhanced N content to a greater extent in IIY than in WYJ after the heading stage

In our experiment, no differences in shoot C concentrations were detected between ambient and elevated [CO_2] (data not shown), showing that shoot C concentration in the two cultivars did not respond to FACE. By contrast, FACE significantly increased root C concentration in IIY, but not WYJ, in our study. For IIY, an increase in root C concentration was found at the heading (3.6 and 5.6%) and grain maturity stages (7.3%, see Fig. S1, available as Supplementary Material to this paper), suggesting that the IIY root experienced more carbohydrate transport from the shoot than WYJ under the FACE conditions.

The accumulation and assimilation of nitrogen also required monitoring over the rice development cycle. Compared with IIY, WYJ accumulated more N in aboveground tissue during the early period of vegetative growth, and FACE significantly enhanced this process (Fig. 4). However, IIY absorbed more N after tillering. Fig. 4*b* shows that IIY accumulated more N than WYJ at NN. At the same time, FACE significantly enhanced shoot N uptake of IIY by 21% at NN. No difference was seen in WYJ under FACE conditions. With respect to N in panicles, Fig. 4*d* shows that panicles of IIY experienced higher N accumulation than WYJ at two levels of N. FACE significantly enhanced panicle N content in both cultivars by more than 10%.

For WYJ, FACE increased root N uptake by 24.5% in the 0N treatment and did not affect the uptake of N at normal N level

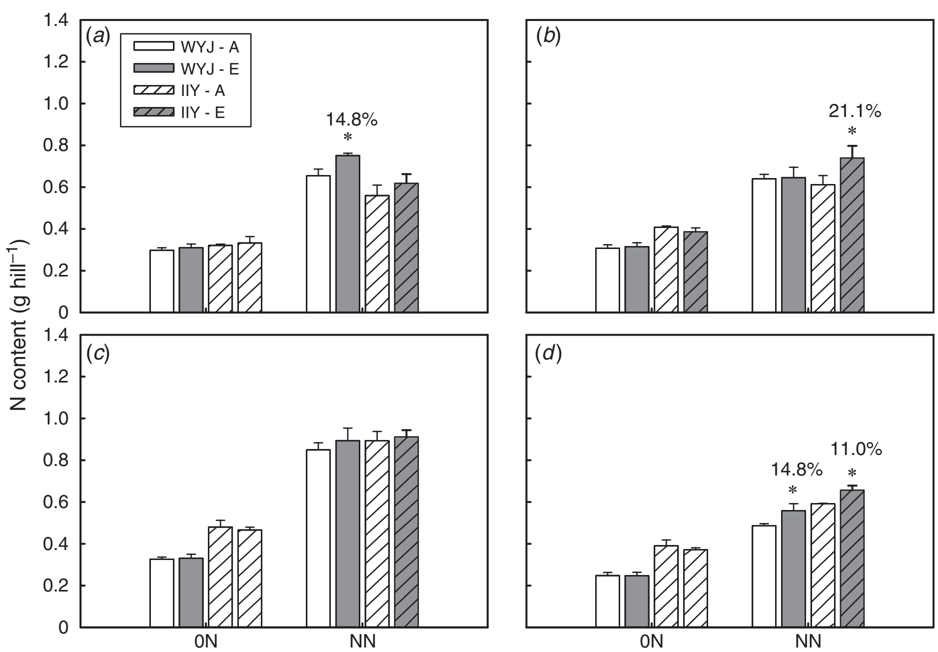

Fig. 4. Shoot N content of rice genotypes WYJ and IIY under ambient (A) and elevated (E) [CO_2], with (NN) or without (0N) added nitrogen. Shoot nitrogen content at: (*a*) panicle initiation, (*b*) heading; and (*c*) grain maturity. (*d*) Nitrogen content of panicles at grain maturity. The numbers are calculated as (E–A)/A. See 'Materials and methods' for additional details. Significant differences between two CO_2 treatments are indicated: $*P < 0.05$; $**P < 0.01$; bars indicate ±s.d.

during PI (Fig. 4*a*). The other cultivar, IIY, showed increases of 35.7 and 33.3% under FACE in the scenarios of 0N and NN. By the time of heading, FACE increased root N uptake in IIY by 30.1 and 36.5%, at the two levels of N application (Fig. 5*b*). However, WYJ did not present any difference either at ambient [CO_2] or at elevated [CO_2], independent of N level. During the maturity stage, FACE increased IIY root N uptake by 21.6% only in the scenario 0N (Fig. 5*c*).

FACE enhanced C : N ratio in two cultivars

Figs 6 and 7 present the C : N ratio of the two cultivars under four treatment combinations. FACE failed to significantly increase the C : N ratio of WYJ shoots at the tillering (Fig. 6*a*) and heading (Fig. 6*b*) stage, the C : N ratio of shoots without panicles (Fig. 6*c*) and the C : N ratio of panicles (Fig. 6*d*) at maturity. At the 0N level, FACE significantly increased the C:N ratio of IIY shoots, by 19.5 and 31.0% at the tillering and heading stage, respectively, and increased the C : N ratio of IIY panicles by 14.3%. At the NN level, FACE significantly increased the C : N ratio of IIY shoots by 21.1% at the tillering stage, and increased the C:N ratio of IIY shoots without panicles by 24.9% at grain maturity. For the belowground part at 0N, FACE significantly increased the C : N ratio of IIY roots by 13.9% at the heading stage (Fig. 7). At the NN level, the C : N ratio of roots was increased by 13.5 and 12.7% at the tillering and grain maturity stages in WYJ, and by 23.5% at grain maturity in IIY (Fig. 7).

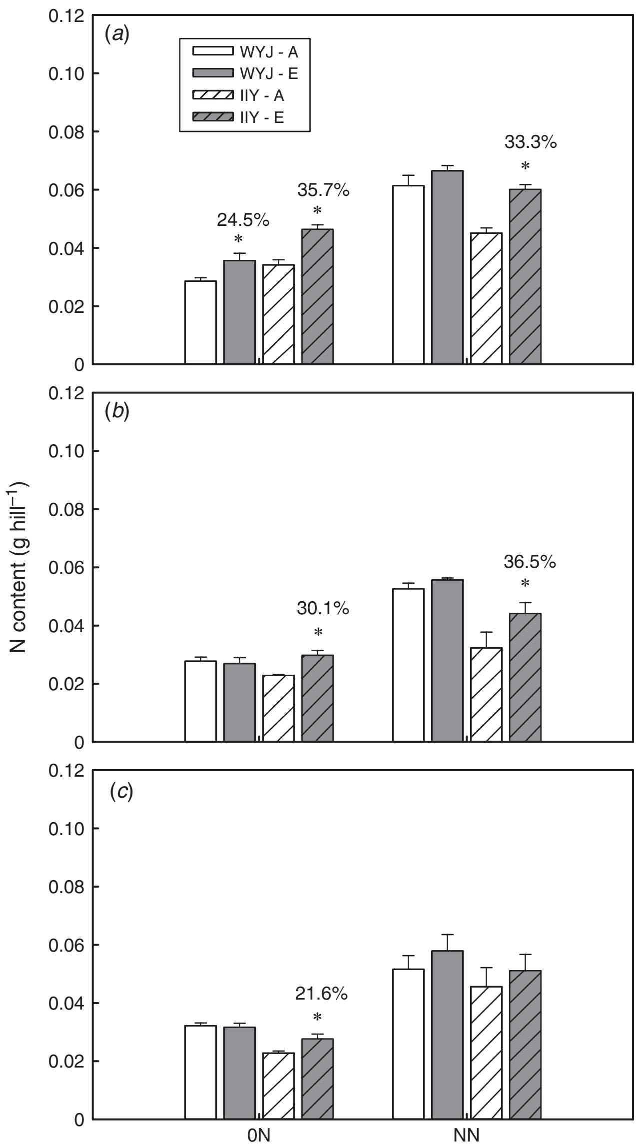

Fig. 5. Root N content of rice genotypes WYJ and IIY under ambient (A) and elevated (E) [CO_2], with (NN) or without (0N) added nitrogen. (*a*) Tillering, (*b*) heading; and (*c*) grain maturity. The numbers are calculated as (E–A)/A. See 'Materials and methods' for additional details. Significant differences between two CO_2 treatments are indicated: $^{*}P < 0.05$; $^{**}P < 0.01$; bars indicate ±s.d.

Discussion

In C_3 crops, a general observation is that the predicted enhancement in carbohydrate content will lead to greater aboveground DW and yield in FACE (Kim *et al*. 2001, 2003; Ainsworth and Long 2005). At the same time, elevated [CO_2] has been shown to affect belowground production and uptake of nitrogen (Bloom *et al*. 2002; Ainsworth and Long 2005; Yang *et al*. 2008). However, the differences in growth and production between different cultivars are rarely reported, especially in terms of root growth and nitrogen absorption. With longer exposures to elevated [CO_2], the initial stimulation of photosynthesis in leaves under FACE tends to diminish, which may be caused by diminished nutrient supply via the root system, resulting in a gap between ideal and realised yields and production (Long *et al*. 2004, 2006). Elevated [CO_2] increases rice seed yield more in some cultivars than others. (Yang *et al*. 2006; Liu *et al*. 2008; Zhu *et al*. 2016). In the present experiment, two cultivars with different yield response to elevated [CO_2] were analysed. Previous studies using various controlled systems have demonstrated that elevated [CO_2] increases panicle number and weight (Ainsworth 2008; Liu *et al*. 2008; Zhu *et al*. 2014). In our study, FACE increased the panicle weight of the highly responsive cultivar, IIY, by 18.8%, but not of the low-responder cultivar WYJ (Fig. 1).

Few studies have afforded comparisons between cultivars to elevated [CO_2] (Zhu *et al*. 2013, 2014), and the response of aboveground and belowground biomass to FACE throughout the whole growth cycle has not been examined. Our results show that although integrated over the whole growth cycle IIY presented a greater response to elevated [CO_2] in above- and belowground biomass than WYJ at the two N levels, the onset of the responses in the cultivars varied significantly with N level. Furthermore, the onset of the responses in the two cultivars differed in different parts of the plant. Under 0N, the difference in aboveground biomass emerged at the PI stage, whereas for root biomass, it emerged at the tillering stage, showing that the root responded faster. This allowed IIY under conditions of insufficient N supply, to respond quickly to acquire more nutrients via a larger root system. Under NN conditions, the differences in shoot and root emerged at the same time in the two lines, at PI. Comparing to the 0N condition, the application of N fertiliser accelerated rice

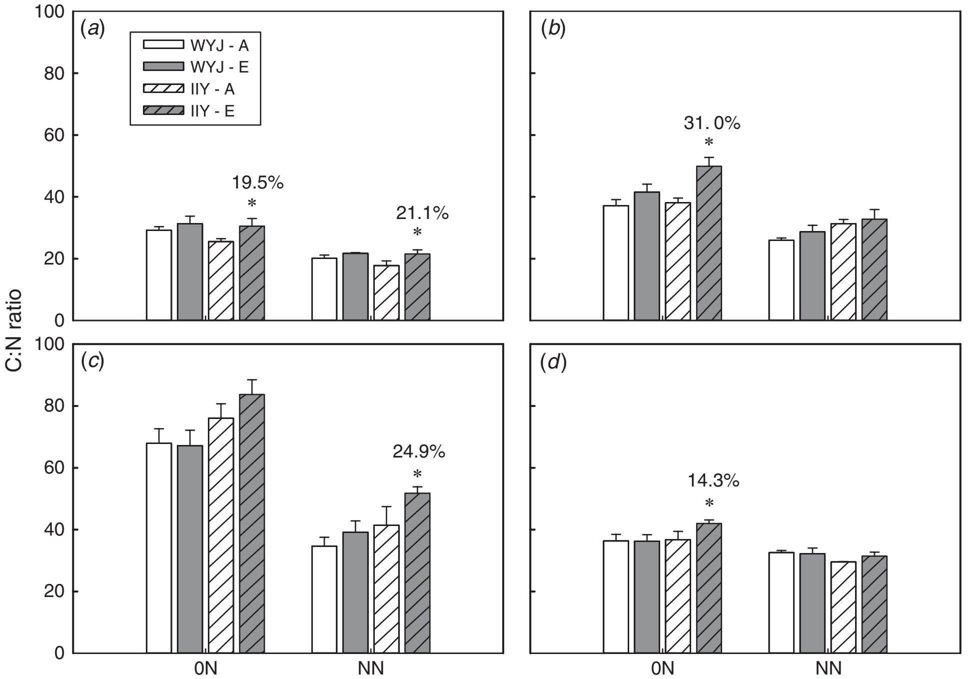

Fig. 6. Shoot C : N ratio of rice genotypes WYJ and IIY under ambient (A) and elevated (E) [CO_2], with (NN) or without (0N) added nitrogen. Shoot C : N ratio at (*a*) panicle initiation, and (*b*) heading stages, (*c*) shoot C : N ratio without panicles; and (*d*) C : N ratio of panicles at grain maturity. The numbers are calculated as (E–A)/A. See 'Materials and methods' for additional details. Significant differences between two CO_2 treatments are indicated: *$P < 0.05$; **$P < 0.01$; bars indicate ±s.d.

growth and synchronised the shoot and root responses. In our study, the use of a temporal sectional monitor helped identify the precise stage at which differences appeared. Knowing the stage at which differences emerge will be critical to the judicious application of fertiliser under elevated [CO_2] conditions.

Data from the field clearly suggest that elevated atmospheric [CO_2] stimulates root proliferation in cotton (Rogers *et al*. 1992). Elevated [CO_2] has also been observed to increase root weight in wheat during all observed developmental stages (Wechsung *et al*. 1995, 1999). In maize, an increase in nodal root volume was observed at elevated [CO_2] concentrations (Zong and Shangguan 2013). Similarly, previous reports in rice showed significant increases in both adventitious root volume (ARV) and adventitious root dry weight (ARD) under FACE treatment (Sun *et al*. 2013). In our experiments, IIY showed greater root weight under FACE conditions than WYJ at all growth stages (Fig. 3). In addition to this, it has been reported that high-response cultivars may have the ability to maintain photosynthetic capacity and carbohydrate content under FACE conditions, especially during grain development, and increased photosynthate can result in several secondary effects such as alterations in specific leaf area, biomass allocation, respiration, and/or carbon content (Poorter *et al*. 1992; Cotrufo and Gorissen 1997; Zhu *et al*. 2014). In our study, IIY roots displayed a larger increase in C content under FACE, whereas, at the same time, root C concentration in IIY was 5.6% higher at elevated [CO_2] than under control conditions at the heading stage with NN (Fig. S1*b*). By contrast, WYJ did not show any difference in root C concentration (Fig. S1). Hence, roots in IIY may experience more carbohydrate transport from the shoot than WYJ roots, under FACE conditions.

The ability to respond and maintain growth enhancement under FACE is expected to depend on an adequate supply of nutrients. In rice, increases in root biomass due to elevated [CO_2] allow the plant to exploit more N from the soil (Nam *et al*. 2013). Previous studies on rice have demonstrated that root dry matter is a good predictor of the plant's ability to take up nutrients (Kim *et al*. 2001); N content and dry matter were positively correlated for individual rice organs and the whole plant (Kim *et al*. 2011). Guo *et al*. (2015) reported that elevated [CO_2] can stimulate aboveground biomass and N accumulations in rice by 19.1 and 12.5% respectively. In the present study, Figs 4 and 5 present data that show that the weakly responding cultivar WYJ possesses strong N uptake capacity at elevated [CO_2] in the early stage of vegetative growth but that this cannot be sustained. During the heading stage, FACE increased IIY's N content in both root and shoot by more than 30 and 20%, which did not occur in WYJ (Figs 4, 5).

The carbon to nitrogen (C : N) ratio is an important indicator of distributions that may occur in photosynthate production, and is a reflection of the coordination of carbon and nitrogen metabolism. On average, the C : N ratio in plant tissues has shown increases of ~15% at double the normal [CO_2] (Gifford *et al*. 2000), and for cotton, the C : N ratio was increased by 21–23% under doubled [CO_2] (Hendrey *et al*. 1993). According to Figs 6 and 7 in our study, the C:N ratios in IIY and WYJ were generally higher at the 0N level than at

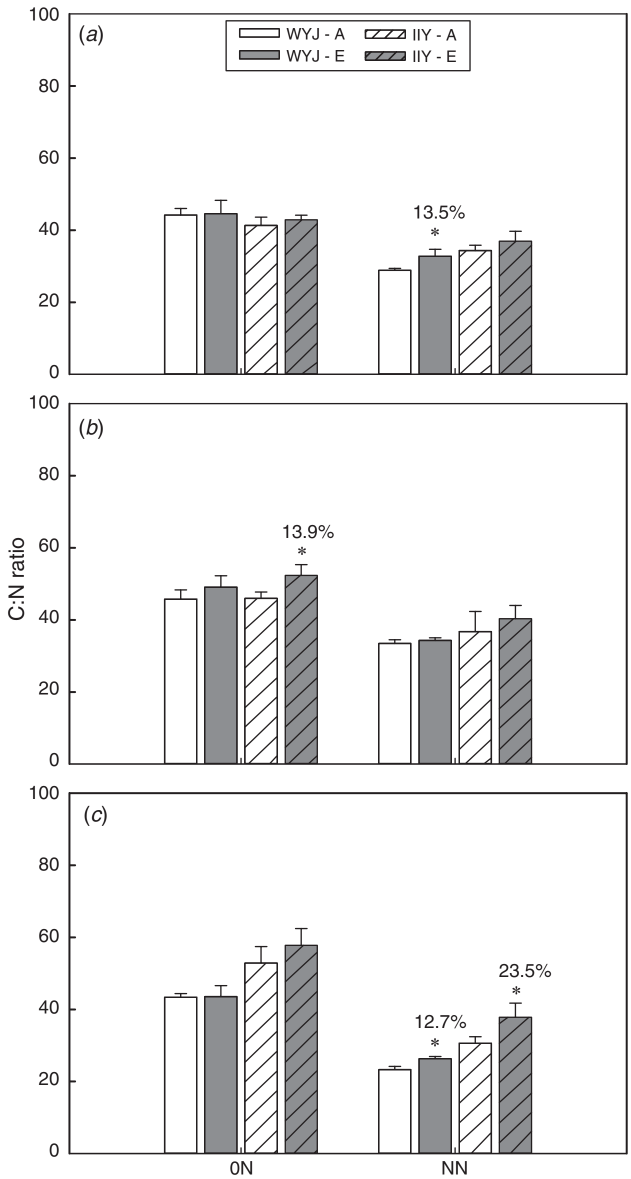

Fig. 7. Root C : N ratio of rice genotypes WYJ and IIY under ambient (A) and elevated (E) [CO_2], with (NN) or without (0N) added nitrogen. Root C : N ratio at (*a*) panicle initiation, (*b*) heading, and (*c*) grain maturity. The numbers are calculated as (E–A)/A. See 'Materials and methods' for additional details. Significant differences between two CO_2 treatments are indicated: *$P < 0.05$; **$P < 0.01$; bars indicate ±s.d.

the NN level, which may be explained by the lower N content at the 0N level. At the same time, IIY displayed a higher C : N ratio than WYJ in most cases, indicating that IIY did have a higher nitrogen use efficiency under elevated [CO_2] than WYJ. Per unit N, IIY accumulated more C than WYJ at elevated [CO_2]. Furthermore, IIY had higher root biomass and spikelet number (data not shown) than WYJ under FACE, indicating that IIY had greater sink strength than WYJ at elevated [CO_2]. A larger 'sink' capacity has been suggested as a critical factor for maximising plant production at elevated [CO_2] (Drake *et al*. 1997; Ziska *et al*. 2001; Ainsworth *et al*. 2004). This may partly explain the higher seed yield and larger biomass in IIY under FACE.

Our research compared the timing of the responses emerging under FACE between two contrasting cultivars of rice at two N treatments, and in the two major organs of the plant, and explored the probable causes for the differences in yield response between them. Based on this analysis, we propose that the high-response cultivar IIY efficiently uses CO_2 under FACE conditions, produces more carbohydrate and transports carbohydrate to other organs via the phloem. Roots receive and assimilate the added carbon to quickly attain a larger size and physiological activity, which can promote the uptake of N by the root, and maintain a balance of carbon and nitrogen in the root tissue. Clearly, a boost in root growth is the prerequisite to healthy shoot development. It is essential to an effective response to elevated [CO_2]. Hence, the yield potential of high-responder rice genotypes appears to partly depend on stimulations of root growth and nitrogen acquisition, which should be focussed on in cultivar selection. It has been argued in the ecological literature that N availability in particular will limit the response to elevated [CO_2]. In our study, compared with conditions with no N fertiliser application in experimental soil, the enhancement of biomass and yield in FACE were more significant with local N fertiliser application. Hence, more nitrogen must be applied so as to obtain the full potential of crop yield under the predicted future [CO_2] conditions, especially during the PI and heading stages. Therefore, the future demand for nitrogen fertiliser in rice production is expected to increase significantly and more attention needs to be directed towards assessing the environmental impacts due to fertiliser production and application.

Conflicts of interest

The authors declare no conflicts of interest.

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