Effects of free-air CO₂ enrichment (FACE) and nitrogen (N) supply on N uptake and utilization of indica and japonica cultivars (*Oryza sativa* L.)

Qian Jiang¹², Jishuang Zhang¹², Xi Xu¹², Gang Liu¹ and Jianguo Zhu¹*

**Abstract**

**Background:** Increasing atmospheric [CO₂] can increase photosynthesis and promote plant growth, consequently influencing nitrogen (N) cycling. Yet, there is no systematic information on the N response among different organs of japonica and indica rice genotypes to elevated [CO₂] as affected by N application. To investigate the impacts of elevated [CO₂] and N fertilization on N uptake and utilization of different genotypic rice (*Oryza sativa* L.) during grain filling, a free-air CO₂ enrichment (FACE) experiment with indica cv. Liangyou 084 (IIY084) and japonica cv. Wuyunjing 23 (WYJ23) was conducted in Eastern China. Crops were exposed to ambient [CO₂] and elevated [CO₂] (200 μmol mol⁻¹ above ambient) at two levels of N: control (0N) and 22.5 g N m⁻² (normal N, NN), and they were sampled at 82 days after transplanting (DAT), 99 DAT, and maturity, respectively.

**Results:** Under FACE, significant declines of N concentration in all tissues and the whole plants were observed with the greater decrease in leaves and stems at three stages. Positive responses of N use efficiency (NUE) to elevated [CO₂] were recorded over the study period. The total N accumulation remained unchanged, while a large amount of N was partitioned to panicles at the expense of leaves and stems. As compared to WYJ23, greater N transportation from roots to aboveground, especially the panicles, was observed on IIY084 accompanied by higher panicle biomass (82 DAT and 99 DAT), N concentrations (maturity), and greater NUE for leaves through the study season. Across all [CO₂] and cultivars, N fertilization increased N partitioning to leaves and stems while decreasing that to panicles. Additionally, N supply decreased NUE while stimulating N concentrations and N amounts of rice plants. Among all treatments, IIY084 had the highest N accumulation and allocation in panicles under elevated [CO₂] in combination with N fertilizer at maturity.

**Conclusion:** Data from this study were helpful for understanding the temporal N uptake and utilization of different rice genotypes as affected by N availability and suggest that IIY084 promises a considerable prospect for its grain yield and quality under future elevated atmospheric [CO₂].

**Keywords:** Free-air CO₂ enrichment (FACE), N use efficiency, N allocation, N concentration, Rice genotypes
Introduction

Due to the activities of human beings, such as the increasing demands for cultivated lands and energies, global atmospheric [CO$_2$] is increasing from 280 μmol mol$^{-1}$ before industrial revolution to 402 μmol mol$^{-1}$ in recent years. By the end of this century, 700 μmol mol$^{-1}$ or more is predicted for atmospheric [CO$_2$] (Metz et al. 2007). In general, the projected increase in [CO$_2$] is able to directly promote plant growth through raising photosynthesis, especially in C3 plants (Kim et al. 2001, 2003; Tausz et al. 2013). Subsequently, nutrient cycling and utilization, residue decomposition, and even insect-plant interaction might be affected to different extents (Coviella and Trumble 1999; Leakey et al. 2009; Viswanath et al. 2010; Zhu et al. 2016).

Nitrogen (N), accounting for 1–3% of the whole plant, is one of the essential elements required for plant growth and yield as well as quality and may play a critical role in the responses of plants to elevated [CO$_2$] (Kim et al. 2003). Based on both area and tonnage harvested, rice (Oryza sativa L.) is one of the most important crops in the world and the first food in Asia, providing dietary needs to a large population. Considering its vital socio-economic impacts, the variation of N uptake and utilization of rice in the future has become a focus (Liang et al. 2013; Myers et al. 2014; Roberts et al. 2011; Wei et al. 2011). Overall, previous studies have reported that under elevated [CO$_2$], whole plant N uptake increased, but the concentration on a per unit weight of tissue basis declined with the ranges depending on factors such as experimental facility, N supply, cultivar, and growth stage (Terao et al. 2005; Yang et al. 2007a, 2007b; Zhu et al. 2018).

Studies with different rice cultivars have presented clear genotype-dependent effects of elevated [CO$_2$] on yield and grain quality in chambers, greenhouses, and free-air CO$_2$ enrichment (FACE) (Baker 2004; Lv et al. 2020; Zhang et al. 2015; Zhu et al. 2018; Ziska et al. 1996a). Using japonica cv. Wuxiangjin 14 as testing material, Yang et al. (2007a) reported that shoot N concentration under elevated [CO$_2$] declined, but N uptake and use efficiency (NUE) increased by different magnitudes. Also, shoot N uptake of indica cv. Liangyoupeijiu was revealed to be increased by elevated [CO$_2$] (Yang et al. 2009). However, cultivation techniques (such as N application strategy and irrigation management) and environmental conditions (such as rainfall and temperature) are quite different among the experimental sites and years, especially under field conditions (Yang et al. 2007b). Moreover, the effect of increasing [CO$_2$] inside chambers and enclosures can be markedly different from those field experiment which could provide undisturbed field conditions and more reliable measurements (McLeod and Long 1999). Thus, an experiment with different genotypes such as japonica and indica cultivars at FACE is needed. Previous studies have found that most of the crop responses to elevated [CO$_2$] were directly or indirectly associated with N fertilization (Kim et al. 2003; Kimball 2016; Yang et al. 2007a). For example, positive response of photosynthesis to increasing [CO$_2$] was highly dependent on the N levels, increasing proportionally as N availability increased (Ziska et al. 1996b). In addition, N uptake and utilization of rice plants could vary between growth stages (Yang et al. 2007a). As Kim et al. (2003) reported, FACE decreased N concentration in leaves across the season, while it increased total N uptake at panicle initiation but not at maturity. Seed filling stage is crucial to the formation of yield and quality in many crops, and obviously, N is the determinant (Gregersen et al. 2008). Yet, little detailed information was given on the temporal N uptake and utilization over the grain filling duration. Also, there is no systematic information on the N response among different organs of japonica and indica genotypes to FACE as affected by N application.

Indica cv. Liangyou 084 (IY084) and japonica cv. Wuyunjing 23 (WJ23) were extensively planted in East and Southeast China (Zhu et al. 2018), and previous studies have represented that their grain yield increased by different magnitudes at elevated [CO$_2$] (Chen et al. 2015; Li et al. 2017). Here, we hypothesized the response of N traits to elevated [CO$_2$] also differs between these two cultivars. In this work, N concentration, amount, allocation, and NUE in tissues were measured during grain filling using the Chinese Rice FACE platform. Our objectives are (1) to compare whether there are differences in these N parameters in response to CO$_2$ enrichment between IY084 and WJ23 under fully open-area field condition and (2) to determine whether the effects of FACE on N traits change with crop development. The results obtained here should provide critical implications to select desirable cultivars which present optimal responses of both grain yield and quality at the future [CO$_2$].

Materials and methods

Free-air CO$_2$ enrichment (FACE) site

The FACE experimental system was located at the Zhongcun village (119° 42′ 0″ E, 32° 35′ 5″ N), Yangzhou City, Jiangsu Province, where is a typical agricultural region for rice-wheat rotation system in China. The soil is classified as Shajiang Aquic Cambisol according to Chinese Soil Taxonomy with a sandy loam texture, and the soil properties were 1.16 g cm$^{-3}$ bulk density, 54% total porosity, 18.4 g kg$^{-1}$ organic C, 1.45 g kg$^{-1}$ total N, and 0.63 g kg$^{-1}$ total P (as P$_2$O$_5$) (Zhu et al. 2016). This area has a typical north subtropical monsoon climate, with mean annual temperature 14.9 °C, mean
annual rainfall 978.7 mm, a total annual sunshine time of 2132 h, and more than 220 frost-free days.

FACE system
A detailed description of the FACE system can be found in a previous report (Liu et al. 2002). Briefly, three rectangular paddy fields with similar soils and agronomic histories were used. In total, there were three FACE plots and three ambient controls, and the useful area of each plot was 120 m$^2$ in this FACE system. The centers of the plots were separated from 90 m to avoid movement of additional [CO$_2$] to the ambient plots. Each FACE plot was encircled with an octagonal ring with the diameter of 14 m. It consisted of eight emission tubes which could rise as the plant grew to maintain the [CO$_2$] at the top of the canopy. In the ambient plots, plants were grown under ambient [CO$_2$] without ring structures. The [CO$_2$] in FACE plots was enhanced with 200 μmol mol$^{-1}$ above that of ambient control plots. The targeted [CO$_2$] within the FACE plots was automatically controlled using a computer system.

During the experiment in 2014, average daytime [CO$_2$] at canopy height was 378 μmol mol$^{-1}$ for the ambient plots and 571 μmol mol$^{-1}$ for FACE plots, respectively. The average temperature was 22.1 ± 2.4 °C over the growing season.

Crop cultivation
Two rice cultivars (Oryza sativa L.), i.e., an indica cv. Liangyou 084 (hereafter called IIY084) and a japonica cv. Wuyunjing 23 (hereafter called WYJ23), were tested in this investigation. Seedlings of two cultivars were raised in seed trays under respective treatments on May 20, 2014. Then, two seedlings per hill were transplanted manually into their corresponding subplots at a spacing of 25 cm by 16.7 cm (i.e., 24 hills m$^{-2}$) on June 20, 2014. The tested treatments were conducted in each FACE and ambient plots as subplots of 6 m$^2$ each.

In this study, two N levels were conducted: control (0N) and 22.5 g total N m$^{-2}$ (normal N, NN) which is recommended to local farmers and was supplied as urea (N, 46%) and a compound fertilizer (N:P$_2$O$_5$:K$_2$O = 15:15:15). Both P and K fertilizers were adopted as compound fertilizer at equal rates of 9 g P$_2$O$_5$ m$^{-2}$ and 9 g K$_2$O m$^{-2}$, and they were applied as basal fertilizer. Separately, 40% of urea was used before transplanting as basal nutrition, and then 30% each was applied at tillering and heading stages (Supplementary Table S1). To minimize the mixing of paddy water, 0N and NN treatments were separated from the rest of the subplots with a 30-cm PVC barrier pushed 10 cm into the soil. The crops in both FACE and ambient plots were surrounded by border plants treated the same way as the experimental plants inside. During the study, standard cultivation practices such as irrigation, midseason drainage, weeding, and insecticide application common to the local region were followed, as described in detail by Shi et al. (2009). All the plants were harvested on October 17, 2014, except for WYJ23 at NN treatments which were reaped 9 days later.

Sampling and measurements
Across the season, plants were destructively sampled at different times. In order to coincide as close as possible to the flowering, dough, and grain maturity stages of two varieties, sampling dates were fixed at 82, 99 days after transplanting (DAT), and grain maturity. In each subplot, stem numbers of 20 hills were counted randomly so as to ensure representativeness of sampling. After that, two hills with average stem number were sampled at each stage. For each sample, a block of soil 25 cm wide, 16.7 cm long, and 15 cm deep around the individual hill was dug up. The plants were first rinsed carefully with tap water and then rinsed with deionized water. Then, they were separated into root, stem (including leaf sheath), leaf (including living and dead leaf), and panicle. After harvest, the grains were threshed carefully and then dehulled to produce brown rice using a chaff-removal machine (JLG-II, China). Tissues as well as brown rice (only at maturity) were oven-dried at 80 °C until constant weight to derive their dry weights, and then, they were ground to < 0.25 mm in a stainless mill (FW-100, China), respectively to further analyses.

N concentration was determined by high temperature combustion (VarioMAX CNS, Elementar, Germany). The accuracy of the analyses was estimated by comparison with a certified reference material GBW07603 from Institute of Geophysical & Geochemical Exploration, Chinese Academy of Geosciences, and blanks were introduced regularly. N amount was calculated from measurements of plant biomass multiplied by N concentration. N partitioning of each tissue was calculated from the total N accumulation. N use efficiencies (NUE) of panicles, leaves, stems, roots, and whole plants were determined as amount of dry matter per unit absorbed N (g DM g$^{-1}$ N).

Statistical analysis
Datasets were based on the mean values of each treatment and were analyzed with the statistical package SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA) and EXCEL 2010 for Win. A three-way ANOVA was applied to test the effects of [CO$_2$], cultivar, and N fertilizer and their interactions on N concentration, uptake, use efficiency, and allocation of rice plants with [CO$_2$], cultivar and N as main factors, and ring identity as random factor. A significant level of $P < 0.1$ (LSD, $n = 3$) was used. Standard error was calculated where appropriate. When interactions were significant, a one-way ANOVA
was used to compare the differences among different treatments. Differences at $P < 0.05$ levels (LSD) were considered statistically significant.

Results

**Biomass of four tissues and whole plants**

Across two cultivars and N levels, FACE significantly increased panicle biomass by 24.8% at 99 DAT ($P < 0.05$) and raised stem biomass by 12.7% and 17.3% at 82 DAT ($P < 0.1$) and 99 DAT ($P < 0.05$), respectively. Over the sampling season, biomass of roots was consistently higher at the elevated [CO$_2$] across all cultivars and N levels with average increase of 27.7, 19.4, and 18.1% at 82 DAT ($P < 0.01$), 99 DAT ($P < 0.05$), and maturity ($P < 0.1$), respectively (Tables 1 and 2).

Significant cultivar effects on panicle biomass at 82 DAT ($P < 0.01$) and 99 DAT ($P < 0.05$) and on root biomass at 99 DAT ($P < 0.05$) and maturity ($P < 0.1$) were observed, separately. As expected, N application substantially increased the biomass of all four tissues or whole plants at most sampling stages. Take the maturity for example, the average biomass of panicles, leaves, stems, roots, and whole plants across all [CO$_2$] levels and cultivars increased by 14.4%, 104.8%, 26.1%, 10.7%, and 25.5%, separately (Tables 1 and 2).

During grain filling, significant cultivar × N interactions were observed for root biomass ($P < 0.01$) at 99 DAT and leaf and stem biomass ($P < 0.1$) at maturity, respectively. In fertilized treatments, WJY23 had much greater root biomass than IIY084 under both [CO$_2$] levels at 99 DAT. At maturity, IIY084 under FACE condition had the lowest leaf and stem biomass in all 0N treatments, while an opposite trend was found on stem biomass in NN treatments (Tables 1 and 2).

**N concentrations in four tissues and whole plants**

Through the sampling stages, the overall patterns of changes in N concentrations in each tissue were generally similar across all treatments (Fig. 1). Averaged

Table 1 Biomass of four tissues and whole plants of two cultivars (IIY084 and WJY23) subjected to ambient (A) and elevated [CO$_2$] (F) at none (0N) and normal N levels (NN) at three stages

| Growth stage | N level | Cultivar | CO$_2$ | Panicle (g hill$^{-1}$) | Leaf | Stem | Root | Whole plant |
|--------------|---------|----------|--------|--------------------------|------|------|------|-------------|
|              |         |          |        |                          |      |      |      |             |
| 82 DAT       | 0N      | IIY084   | A      | 10.7 ± 1.8 ab             | 7.1 ± 0.7 | 18.6 ± 1.5 c | 3.5 ± 0.5 b | 39.9 ± 4.3  |
|              |         |          | F      | 8.5 ± 0.7 bc              | 8.7 ± 1.0 | 25.3 ± 3.0 ab | 3.7 ± 0.1 b | 46.3 ± 4.7  |
|              |         |          | WJY23  | 5.1 ± 1.2 d               | 9.0 ± 0.6 | 20.7 ± 0.9 abc | 3.4 ± 0.5 b | 38.3 ± 2.6  |
|              |         |          | F      | 7.4 ± 0.1 bcd             | 7.7 ± 0.7 | 19.5 ± 1.8 bc | 4.0 ± 0.7 b | 38.6 ± 2.8  |
|              | NN      | IIY084   | A      | 12.7 ± 1.5 a              | 15.2 ± 0.9 | 25.1 ± 0.5 abc | 3.8 ± 0.2 b | 56.7 ± 2.7  |
|              |         |          | F      | 12.3 ± 0.3 a              | 15.5 ± 3.2 | 26.3 ± 0.4 a | 5.4 ± 0.6 a | 59.6 ± 3.0  |
|              |         |          | WJY23  | 6.3 ± 0.7 cd              | 13.5 ± 1.7 | 22.1 ± 3.0 abc | 4.1 ± 0.5 b | 46.0 ± 5.1  |
|              |         |          | F      | 8.8 ± 0.9 bc              | 14.9 ± 1.6 | 27.1 ± 2.5 a  | 5.8 ± 0.2 a | 56.6 ± 5.1  |
| 99 DAT       | 0N      | IIY084   | A      | 31.5 ± 3.5 ab             | 7.9 ± 1.2  | 16.2 ± 2.0   | 4.8 ± 0.4 bc | 60.1 ± 6.5 ab|
|              |         |          | F      | 28.9 ± 5.2 abc            | 6.7 ± 1.4  | 19.5 ± 1.8   | 6.0 ± 0.3 ab | 61.8 ± 7.3 ab|
|              |         |          | WJY23  | 19.6 ± 4.4 cd             | 7.6 ± 0.9  | 16.9 ± 2.1   | 4.6 ± 0.5 bc | 49.0 ± 8.1 b |
|              |         |          | F      | 23.7 ± 3.7 bcd            | 7.4 ± 0.6  | 16.6 ± 4.2   | 4.9 ± 1.0 bc | 51.8 ± 10.3 b|
|              | NN      | IIY084   | A      | 29.1 ± 0.1 abc            | 13.0 ± 1.4 | 18.7 ± 1.0   | 3.85 ± 0.1 c | 64.6 ± 2.5 ab|
|              |         |          | F      | 37.2 ± 2.8 a              | 13.9 ± 0.5 | 26.1 ± 2.7   | 5.04 ± 0.3 bc | 82.3 ± 5.7 a |
|              |         |          | WJY23  | 16.4 ± 2.6 d              | 14.2 ± 1.3 | 23.3 ± 2.0   | 6.07 ± 0.6 ab | 60.0 ± 5.0 ab|
|              |         |          | F      | 30.8 ± 3.6 abc            | 14.7 ± 1.8 | 25.9 ± 2.1   | 7.12 ± 0.5 a | 78.5 ± 8.0 a |
| Maturity     | 0N      | IIY084   | A      | 41.7 ± 4.2                | 6.1 ± 0.4 bc | 18.0 ± 1.4 bc | 3.1 ± 0.2 | 68.9 ± 6.2  |
|              |         |          | F      | 34.9 ± 4.1               | 4.4 ± 0.5 c | 15.8 ± 1.7 c | 3.2 ± 0.5 | 58.3 ± 6.0  |
|              |         |          | WJY23  | 32.7 ± 2.0                | 6.6 ± 0.8 b | 17.3 ± 2.1 bc | 3.9 ± 0.4 | 60.5 ± 5.1  |
|              |         |          | F      | 37.5 ± 6.7               | 5.9 ± 1.0 bc | 18.2 ± 2.6 bc | 4.7 ± 0.8 | 66.4 ± 11.0 |
|              | NN      | IIY084   | A      | 38.7 ± 1.3                | 11.7 ± 0.4 a | 21.7 ± 1.1 ab | 3.3 ± 0.5 | 75.3 ± 7.2  |
|              |         |          | F      | 48.4 ± 2.4               | 12.6 ± 0.5 a | 25.5 ± 0.6 a | 4.2 ± 0.3 | 90.7 ± 3.0  |
|              |         |          | WJY23  | 37.8 ± 3.1                | 11.3 ± 0.9 a | 20.0 ± 1.1 bc | 4.1 ± 0.4 | 73.2 ± 5.2  |
|              |         |          | F      | 43.0 ± 1.5               | 11.5 ± 0.3 a | 20.2 ± 0.9 bc | 4.9 ± 0.3 | 79.7 ± 2.2  |

Different letters in each column represent significant differences in each tissue among eight treatments at each sampling date according to LSD at 5% level.
across all treatments, N concentrations in panicles, leaves, stems, roots, and whole plants were 1.44%, 2.33%, 0.90%, 1.17%, and 1.37%, respectively at 82 DAT. Then, they varied to 1.22%, 1.71%, 0.69%, 1.25%, and 1.15%, respectively at 99 DAT. At the end of the season, N concentrations in all four tissues and whole plants have fallen to 1.21%, 0.91%, 0.60%, 1.00%, and 1.00%, respectively (Fig. 1).

Overall, elevated [CO₂] notably decreased (P < 0.05) plant N concentrations regardless of cultivars and N fertilization across the study period except for panicles and roots at 82 DAT (Fig. 1, Table 2). Compared to ambient, N concentrations in panicles, leaves, stems, roots, and whole plants exposure to increased [CO₂] significantly declined by 8.40%, 15.7%, 24.9%, 8.20%, and 14.8%, respectively at 99 DAT and by 10.2%, 22.4%, 30.7%, 18.0%, and 15.4%, respectively at maturity. In addition, N concentrations in leaves at 82 DAT (P < 0.01) and maturity (P < 0.1), as well as that in panicles at maturity (P < 0.01), were significantly affected by cultivar effects. At maturity, WYJ23 had 7.60% lower panicle N concentrations but 16.4% greater leaf N concentrations across all [CO₂] and N levels.

Averaged across all [CO₂] levels and cultivars, N fertilization significantly stimulated N concentrations in panicles, leaves, stems, roots, and whole plants by 14.5%, 38.6%, 83.5%, 31.4%, and 54.3%, respectively at 82 DAT and by 32.5%, 81.2%, 136.8%, 24.2%, and 77.3%, respectively at 99 DAT and by 30.0%, 91.2%, 105.7%, 65.6% and 44.8%, respectively at maturity. In addition, significant interactions of cultivar × N (P < 0.01) at both 99 DAT and maturity, and that of CO₂ × cultivar × N (P < 0.05) at maturity were observed on the N concentrations in panicles. Among all treatments, fertilized IIY084 under ambient had the greatest values at these two stages (Fig. 1, Table 2).

**Table 2** F-value and the results of three-way ANOVA (type III) testing the elevated [CO₂], cultivar, N fertilizer, and their interactions on biomass, N concentration, and NUE of rice crops at three growth stages

| Growth stage | Treatments | Biomass | N concentrations | NUE |
|--------------|------------|---------|------------------|-----|
|              |            | Panicle | Leaf | Stem | Root | Whole plant | Panicle | Leaf | Stem | Root | Whole plant |
| 82 DAT       | CO₂ 0.6    | 0.2     | 3.8** | 10.7** | 2.1 | 1.4 | 8.6** | 4.8** | 0.0 | 3.8** | 0.8 | 9.1** | 2.1 | 0.1 | 8.2** |
|              | Cultivar 30.7** | 0.1 | 0.8 | 0.5 | 3.0** | 0.9 | 19.0** | 0.0 | 0.5 | 0.0 | 0.9 | 25.4** | 1.9 | 0.4 | 4.6** |
|              | N 8.0** | 38.1** | 7.7** | 12.0** | 22.9** | 12.1** | 195.2** | 88.4** | 20.9** | 138.0** | 11.9 | 218.6** | 64.4** | 13.1** | 173.2** |
|              | CO₂ × cultivar 6.1** | 0.2 | 0.4 | 0.1 | 0.3 | 0.3 | 0.0 | 0.5 | 6.5 | 5.5** | 0.4 | 0.3 | 0.0 | 5.2 | 0.1 |
|              | CO₂ × N 0.5 | 0.1 | 0.0 | 4.1** | 0.1 | 0.4 | 0.2 | 0.2 | 1.1 | 0.5 | 0.4 | 0.2 | 0.2 | 1.3 | 0.6 |
|              | Cultivar × N 1.1 | 0.5 | 0.2 | 0.1 | 0.0 | 0.0 | 0.2 | 4.1** | 0.3 | 0.1 | 0.0 | 3.5** | 4.3 | 0.7 | 2.7 |
|              | CO₂ × cultivar × N 0.3 | 0.9 | 4.8** | 0.1 | 2.8 | 2.7 | 4.0** | 1.8 | 0.2 | 2.7 | 4.1** | 0.8 | 0.1 | 1.4 |
| 99 DAT       | CO₂ 5.9** | 0.0 | 3.6** | 6.8** | 5.7** | 9.5** | 19.5** | 7.5** | 5.2** | 5.5** | 15.3** | 39.4** | 19.8** | 5.4** | 22.0** |
|              | Cultivar 13.3** | 0.5 | 0.1 | 4.5** | 2.8 | 0.0 | 1.5 | 0.0 | 1.5 | 0.72 | 1.8 | 50.8** | 0.2 | 1.5 | 0.0 |
|              | N 1.0 | 58.3** | 13.2** | 1.6 | 13.5** | 100.0** | 229.5** | 59.6** | 32.5** | 51.5** | 141.9** | 510.5** | 176.7** | 36.2** | 208.2** |
|              | CO₂ × cultivar 1.7 | 0.0 | 1.6 | 0.4 | 0.1 | 0.1 | 0.0 | 0.2 | 0.7 | 0.0 | 0.3 | 0.4 | 0.2 | 0.9 | 1.1 |
|              | CO₂ × N 4.5** | 0.7 | 1.1 | 0.3 | 3.4** | 0.1 | 1.3 | 1.4 | 0.7 | 0.4 | 2.1 | 5.0 | 2.5 | 0.1 | 0.6 |
|              | Cultivar × N 0.0 | 0.2 | 1.0 | 15.2** | 0.5 | 9.5** | 31.3** | 0.0 | 0.9 | 1.3 | 14.2** | 113.3** | 0.0 | 0.7 | 0.4 |
|              | CO₂ × cultivar × N 0.0 | 0.2 | 0.0 | 0.2 | 0.0 | 0.0 | 0.8 | 0.2 | 0.1 | 0.6 | 0.6 | 0.0 | 0.0 | 0.5 | 1.4 |
| Maturity     | CO₂ 1.6 | 0.4 | 0.4 | 4.2** | 1.1 | 18.3** | 10.9** | 11.9** | 9.6** | 16.3** | 23.4** | 13.9** | 21.7** | 7.9** | 27.5** |
|              | Cultivar 1.6 | 0.1 | 1.3 | 9.7** | 0.7 | 9.0** | 3.7** | 1.4 | 0.1 | 1.9 | 3.6** | 10.1** | 0.9 | 0.4 | 0.6 |
|              | N 4.4** | 169.9** | 16.4** | 1.6 | 16.0** | 109.2** | 67.3** | 42.9** | 59.7** | 77.6** | 133.6** | 1169.1** | 171.3** | 83.7** | 161.1** |
|              | CO₂ × cultivar 0.5 | 0.1 | 0.0 | 0.2 | 0.2 | 2.7 | 0.2 | 1.2 | 0.0 | 1.3 | 1.4 | 0.2 | 0.0 | 0.4 | 0.4 |
|              | CO₂ × N 2.8 | 3.7** | 1.4 | 0.5 | 2.7 | 0.1 | 2.5 | 7.9** | 65** | 2.5 | 3.6** | 0.1 | 3.2** | 2.7 | 0.0 |
|              | Cultivar × N 0.0 | 3.5** | 3.8** | 0.4 | 0.6 | 30.1** | 0.2 | 1.4 | 0.1 | 5.3** | 28.7** | 4.1** | 0.5 | 0.5 | 6.4** |
|              | CO₂ × cultivar × N 2.6 | 0.9 | 2.3 | 0.3 | 2.4 | 4.5** | 0.2 | 2.1 | 1.9 | 2.3 | 1.9 | 0.4 | 2.5 | 2.8 | 1.6 |

*P < 0.1; **P < 0.05; ***P < 0.01
N amounts in four tissues and whole plants

Averaged across all cultivars and N levels, elevated [CO₂] significantly increased (P < 0.05) N amounts in roots by 28.8% at 82 DAT but decreased it in leaves (P < 0.05) and stems (P < 0.01) by 25.0% and 26.8%, respectively at maturity. As compared to WYJ23, IIY084 had 50.0%, 39.3%, and 18.2% greater panicle N amounts at 82 DAT (P < 0.01), 99 DAT (P < 0.01), and maturity (P < 0.05), separately across all [CO₂] and N levels. Also, N amounts in roots at maturity were dramatically affected (P < 0.01) by cultivar factor with greater values being observed in WYJ23 (Fig. 2).

N use efficiency (NUE) for four tissues and whole plants

NUE represents the amount of dry matter yield produced per unit absorbed N. In the present investigation, NUE for tissues and whole plants generally enhanced with the crop development (Fig. 3). Averaged across cultivars and N levels, NUE for all tissues and whole plants notably raised by 9.7%, 18.0%, 30.4%, 8.4%, and 16.5%, respectively at 99 DAT and by 11.2%, 23.3%, 24.2%, 15.3%, and 15.7%, separately at maturity under FACE condition (Table 2).

In the case of cultivar effects, significant differences (P < 0.01) in NUE for leaves between IIY084 and WYJ23 were detected during the study period. Averaged across all [CO₂] and N levels, IIY084 had 10.4%, 13.8%, and 16.8% greater NUE for leaves at 82 DAT, 99 DAT, and maturity, respectively (Fig. 3, Table 2). Here, we can also find significantly negative N effects (P < 0.01) on the NUE across the grain filling. Compared to non-fertilized plants, N supply dramatically declined NUE for panicles, leaves, stems, roots, and even whole plants by 13.0%, 29.3%, 47.2%, 22.7%, and 35.5%, respectively at 82 DAT; by 24.8%, 45.5%, 56.6%, 18.9%, and 37.8%, respectively at
99 DAT; and by 22.6%, 47.5%, 46.8%, 37.5%, and 30.0%, separately at maturity regardless of cultivars and \([\text{CO}_2]\) levels.

Moreover, significant cultivar × N interactions were found on NUEs for leaves and stems \((P < 0.05)\) at 82 DAT, for panicles \((P < 0.01)\), and leaves \((P < 0.01, 99 \text{ DAT}; P < 0.1, 99 \text{ DAT}; P < 0.01, \text{ maturity})\), respectively (Table 2). Regardless of \([\text{CO}_2]\) levels, non-fertilized IIY084 had much greater NUE for panicles than other treatments at latter two stages. A similar trend of NUE for leaves was observed at 82 DAT and maturity (Fig. 3).

**N allocation among the four tissues**

In general, N partition to panicles sharply increased irrespective of \([\text{CO}_2]\), N levels, and cultivars during the grain filling (Fig. 4). At maturity, 57.5–77.1% of the N was allocated to the panicles. In contrast, those allocations to leaves and stems continually decreased and were 5.2–18.6% and 12.6–23%, respectively at harvest. During the study, N distributed to roots was lowest among four tissues, and it generally, especially for WYJ23, enhanced at 99 DAT and then declined at maturity.

The effects of elevated \([\text{CO}_2]\), N application, and cultivar on the N distribution differed among the four tissues. Significant \([\text{CO}_2]\) effects were observed on panicles \((P < 0.05, 99 \text{ DAT}; P < 0.01, \text{ maturity})\), leaves \((P < 0.01, 82 \text{ DAT}; P < 0.1, 99 \text{ DAT}; P < 0.01, \text{ maturity})\), and stems \((P < 0.05, 99 \text{ DAT}; P < 0.01, \text{ maturity})\). At maturity, the relative change ratios of N allocation to panicles, leaves, stems, and roots exposure to elevated \([\text{CO}_2]\) were 7.0%, −18.5%, −16.4%, and 9.6%, respectively across two cultivars and N levels when compared to ambient (Fig. 4).

Averaged across all \([\text{CO}_2]\) and N levels, WYJ23 had 37.0%, 12.1%, and 5.7% lower panicle N percentage at 82 DAT \((P < 0.01)\), 99 DAT \((P < 0.05)\), and maturity \((P < 0.05)\), respectively as compared to IIY084. However, significantly greater \((P < 0.01)\) N allocations to leaves at 82 DAT (22.3%) and maturity (32.9%) and to roots at 99 DAT (35.6%) and maturity (41.2%) were found in WYJ23. Regardless of \([\text{CO}_2]\) and cultivars, N fertilization significantly decreased the N fractions in panicles \((P < 0.01)\), while increasing those in leaves during study period. Also, positive N effects \((P < 0.05)\) were observed on the stem N percentages at the latter two stages.

Moreover, there were significant \([\text{CO}_2] \times \text{cultivar}\) interactions on the N fractions in panicles at 82 DAT \((P < 0.01)\) and 99 DAT \((P < 0.1)\). Also, significant \([\text{CO}_2] \times \text{cultivar}\) interactions on the N fractions in leaves at 82 DAT \((P < 0.01)\) and 99 DAT \((P < 0.1)\).
cultivar × N interactions were detected on the N fractions in panicles (P < 0.01) and leaves (P < 0.05) at 82 DAT. At this sampling stage, we can find that under ambient, non-fertilized IIY084 had the greatest N fractions in panicles, and fertilized WYJ23 had the greatest values in leaves (Fig. 4).

**Discussion**

**N concentration**

In the present investigation, N concentrations in each tissue across all treatments generally declined with the crop development. The biggest decrease appeared to be in the leaves, where the N concentrations at the first stage were highest among four tissues, but was almost one quarter at maturity. This, together with the increased leaf senescence observed in this study, indicated a fast N remobilization from green leaves to grains during the grain filling (Kim et al. 2003), which also can be testified by the result of N distribution (Fig. 4). Moreover, panicles had the greatest N concentrations among all tissues to ensure the grain quality at the harvest time.

Except for panicles and roots at 82 DAT, elevated [CO₂] significantly suppressed N concentrations in the four tissues as well as the whole plants. The reduction in N concentrations in rice plants under rising [CO₂] was widely reported (Myers et al. 2014; Pang et al. 2006; Zhu et al. 2018). Recently, a comprehensive meta-analysis involving 386 published reports and including 4481 observations confirmed that elevated [CO₂] significantly reduced the plant N concentration by 9.73% (P < 0.001) (Du et al. 2019). Furthermore, the mechanisms underlying the phenomenon have been well illustrated. There have been various explanations such as the limited soil available N supply, restricted N absorption, and transportation by roots, increased plant N loss, and decreased root N uptake efficiency, and unbalanced translocation of C and N in plants at increasing [CO₂] (Feng et al. 2015; Guo et al. 2015a; Leakey et al. 2009; Pang et al. 2006). Here, given the unchanged total N accumulations and N allocation to roots, and the increasing trend of soil available N (Wu et al. 2020), it is more likely attributed to the C gain outstripped the N uptake of rice plants exposed to rising [CO₂] (Guo et al. 2015a). In addition, the responses of different tissues to increasing [CO₂] were of different magnitudes as exhibited by Fig. 4, N concentrations decreasing more in leaves and stems than in other tissues. This is in accordance with the view
that different plant tissues have different metabolic pathways that get changed at elevated [CO$_2$] (Du et al. 2019).

At 82 DAT and 99 DAT, IIY084 had greater panicle biomass but undifferentiated N concentrations compared to WYJ23 across all [CO$_2$] and N levels. At maturity, however, IIY084 had unchanged panicle biomass and greater N concentrations. This result might be explained by the accelerated N accumulation in IIY084 over the study period reflecting a better nutrition quality of grain. As expected, N supply significantly increased N concentrations in four tissues and whole plants throughout three stages. Similar results have been found with rice plants (Kim et al. 2003; Yang et al. 2007a). Moreover, we can find that fertilized IIY084 had the greatest N concentrations in panicles, revealing that the beneficial effect of N supply on grain N quality was larger for IIY084 than for WYJ23.

**N use efficiency**

The NUE has been used as a long-term indicator on availability of N utilization for C acquisition in plants. In the current experiment, NUE for stem was greater than other rice tissues across the three stages regardless of all treatments variables, reflecting with the same available N; greatest biomass would be observed on stems among all tissues. It had been reported that NUE for rice shoots was enhanced by FACE from middle-tillering to grain maturity stages (Yang et al. 2007a). In this study, greater NUE for whole plants was recorded under elevated [CO$_2$] during grain filling, which represented that the same amount of N accumulated in plants at elevated [CO$_2$] and ambient, greater dry matter will be produced under FACE. Similarly, the positive responses of NUE to elevated [CO$_2$] were reported in other plant species (Wei et al. 2018). These results could be attributed to the increased biomass and/or decreased N content of plants exposure to elevated [CO$_2$] (Kim et al. 2001; Reddy et al. 2010). At the same FACE platform, significantly increased NUE for leaves, stems, panicles, and the total aboveground was found at harvest (Guo et al. 2015b). However, the NUE for each tissue during the grain filling is not well reported, which could reveal the responses of the grain formation to elevated [CO$_2$] comprehensively. In the present investigation, increased NUE for leaves was detected at three stages, while that for panicles, stems, and roots were observed at latter two stages. Early at 40 DAT, NUE for leaf blades of rice was also enhanced by increased [CO$_2$] (Seneweera 2011).

---

**Fig. 4** The N allocation in four tissues of two cultivars (IIY084 and WY123) subjected to ambient (A) and elevated [CO$_2$] (F) at none (0N) and normal N levels (NN) at three stages and the results of three-way ANOVA (type III) (different letters represent significant differences in each tissue among eight treatments at each sampling date according to LSD at 5% level)
Thus, we can find that as directly exposed to elevated [CO$_2$], leaves are more sensitive, and then, its physiology of N use is easier to be changed than other tissues.

Besides, the NUE could be influenced by cultivar types, cultivation techniques, and nutrients circumstance (Cheng et al. 2011; Gifford et al. 2000; Wei et al. 2018). Here, we can find that IIY084 had greater NUE for leaves than WYJ23 averaged across all [CO$_2$] and N levels, showing it could produce higher leaf dry matter with the similar N uptake. This may be closely correlated with the different photosynthesis rate of the two cultivars. As revealed by an experiment at the same FACE platform, photosynthetic rate of IIY084 was significantly greater than that of WYJ23 at both elevated [CO$_2$] and ambient (Chen 2015). It is well known that a large portion of leaf N is invested in photosynthetic machinery since it is one of the most critical component of chemical compounds which are related to photosynthesis (Kattge et al. 2009; Wang et al. 2018). Thus, as supported by the result of N concentrations, lower N was needed in the photosynthesis of IIY084 leaves which consequently led to increased NUE as compared to WYJ23. Likewise, Seneweera (2011) considered that the increased NUE in both rice leaf blades and sheaths resulted from a smaller partitioning of N to photosynthetic processes. However, the interactive effects of [CO$_2$] and cultivar were undetected during the study period except for the root NUE at 82 DAT, indicating that in most cases, the differences resulting from the diverse intrinsic properties of the two tested genotypes can not be significantly affected by elevated [CO$_2$].

In addition to increasing [CO$_2$] and cultivars, NUE for all rice tissues and whole plants was predominantly affected by N fertilizer with greater values in non-fertilized plants across three stages. Regardless of [CO$_2$] levels, Yang et al. (2007a) found the NUE decreased significantly with increasing N supply, except during early stages of rice development. Using tomato as a testing material, Wei et al. (2018) also observed greater NUE in 1.5 g N pot$^{-1}$ than in 3.0 g N pot$^{-1}$ treatments. Distinctly, the greater NUE in lower N condition resulted from the exacerbated shortage of N relative to C in the plants, whereas the decreased NUE in N fertilized rice plants would be attributed to the more improvement in uptake of N than C.

N uptake and allocation
With the crop development, a rapid increase in N accumulation in panicles, while a steady decrease in leaves and stems were observed regardless of all treatment variables. In accordance with this, N partitioning to panicles sharply increased while those to leaves and stems continually declined. These were consistent with the observation that grain N can be mainly obtained by N transfer from the vegetative components of the rice during grain filling (Yang et al. 2007a). As reported by Gregersen et al. (2008), for the small-grained cereals such as barley, wheat, and rice, up to 90% of the N were mobilized from vegetative parts.

With respect to the FACE effects, N amount of total plants remained unchanged. Similar results have been reported elsewhere when rice plants were grown under various conditions (Kim et al. 2001, 2003; Seneweera 2011). On the contrary, other experiments demonstrated that the N uptake was significantly enhanced by elevated [CO$_2$] (Wang et al. 2020; Yang et al. 2007a). Besides the differences induced by cultivars and environmental conditions, the levels of N fertilization would be responsible for this contrast. The N supply both in this investigation and the studies cited here (Kim et al. 2001, 2003) was 0–22.5 g N m$^{-2}$, while at the experiment of Yang et al. (2007a), it was 15–35 g N m$^{-2}$. Therefore, it is considered that the appropriate effect of increasing [CO$_2$] on N uptake closely linked to the soil N availability (Yang et al. 2007a). Although the total N amount was unaffected, those in leaves and stems declined by 25.0% and 26.8% over ambient plants, respectively at maturity. Furthermore, N allocations to leaves and stems were suppressed while that to panicles was enhanced at elevated [CO$_2$]. Both Seneweera (2011) and Yang et al. (2007a) observed the significantly decreased N fractions in leaves at elevated [CO$_2$] and considered that there was an internal mechanism controlling N partitioning at the whole plant level. In the current experiment, it is possible that as the storage pools, N would be reallocated away from leaves and stems to the developing grain when the requirement arose, which was triggered by the rising [CO$_2$] (Kim et al. 2003). For example, as exposed to elevated [CO$_2$], more C can be fixed by the crops including panicles, and thus, more N was needed to be transported from vegetative parts to panicles in order to keep the balance between C and N as much as possible (Du et al. 2019; Guo et al. 2015b).

As mentioned before, N is important in photosynthesis (Kattge et al. 2009; Wang et al. 2018). In consistent with the findings of Seneweera (2011), accompanied by the greater photosynthetic NUE of IIY084, a large amount of N was allocated away from the photosynthetic machinery such as to the development of panicles as compared with WYJ23. Generally, roots are the first plant organ that receives nutrients such as N from the soil, traits like root biomass, length and rooting depth can significantly affect N uptake rates and NUE in crops (Kim et al. 2001). At 82 DAT, no significant cultivar effects on root biomass and N accumulation as well as N allocation were found. Through the rest of the growing stages, however, predominantly lower values were observed in IIY084. In spite of that, there was no
significant difference between IIY084 and WYJ23 with regard to NUE for roots, and an increasing tendency of N amount in whole IIY084 plants (maturity, \( P < 0.1 \)) was observed across the filling duration, which indicated IIY084 roots had greater ability to absorb N from soils as compared with WYJ23. Besides, we can find that with the rice development, IIY084 was more able to transfer N from roots to aboveground biomass, especially the panicles, leading to the greater panicle biomass regardless of [CO\(_2\)] and N levels.

Across the [CO\(_2\)] levels and cultivars, N accumulations in four tissues as well as the whole plants were dramatically enhanced by N supply, with a large proportion of N being partitioned to the leaves and stems at the expense of the panicles during study period. This agrees with other published findings (Yang et al. 2007a). Cheng et al. (2011) reported that the excessive N application decreased rice N harvest index, the ratio of N amount in grain to cumulative N absorption in whole plants, because excessively absorbed N was mainly deposited in leaves and straws but not allocated in grains. Those results advised proper application of N in rice production because excessive N would lead to wasting, decreasing N efficiency, and production benefit (Seneweera 2011; Yang et al. 2007b). As indicated by the significant cultivar \( \times \) N interactions at maturity, IIY084 in combination with N fertilizer had the highest N accumulations in panicles, stems, and whole plants regardless of [CO\(_2\)] levels. It is suggested that in addition to the reasonable N application, improvement of rice N efficiency is also closely related with the cultivar and the interaction of cultivar and N application.

Conclusions

Overall, the results here indicated that both elevated [CO\(_2\)] and N application would affect the N uptake and utilization of two tested rice genotypes to different extents. In most cases, IIY084 had greater panicle biomass and N concentrations since more N was transferred from roots to aboveground, especially the panicles. Furthermore, greater beneficial effects of N fertilization on the N uptake and utilization were observed on IIY084 regardless of [CO\(_2\)] levels. Accordingly, IIY084 shows a considerable prospect in terms of its grain yield and quality with additional [CO\(_2\)]. Nutritional deficits induced by rising [CO\(_2\)] are likely to occur for a large segment of the global population who are rice-dependent (Myers et al. 2014), and thus, it is necessary to select superior rice cultivar with greater nutrition utilization such as N, either through traditional breeding or genetic modification as well as the optimal fertilizing strategy under future CO\(_2\)-enriched environment. Additionally, other aspects of climate change need to be considered, especially temperature. An elevation in atmospheric CO\(_2\) could result in rising temperature, and thus, the integrated effects of CO\(_2\) and temperature on the nutrition uptake and utilization of different rice genotypes are necessary to be investigated in the future.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13717-020-0038-5.

Additional file 1: Supplementary Table S1. The schedule of fertilization in this experiment (g \( \text{m}^{-2} \)).

Abbreviations

CO\(_2\): Carbon dioxide; FACE: Free-air CO\(_2\) enrichment; N: Nitrogen; IIY084: Indica cv. Liangyou 084; WYJ23: Japonica cv. Wuyunjing 23; P: Phosphorus; K: Potassium; DAT: Days after transplanting; NUE: N use efficiency; ANOVA: Analysis of variance; ns: Not significant; C: Carbon

Acknowledgements

This work was financially supported by the National Basic Research Program of China (2014CB954500) and National Natural Science Foundation of China (31261140364, 31370457).

Authors’ contributions

QJ and JZ conceived the study and designed the experiments. QJ, JJ, and XX performed the experiments. QJ and GL analyzed the data with suggestions by JZ. QJ and JZ wrote the manuscript. All authors have read and approved the final manuscript.

Funding

National Basic Research Program of China (2014CB954500) and National Natural Science Foundation of China (31261140364, 31370457). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials

Not applicable

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author details

1State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, No. 71 East Beijing Road, Nanjing 210008, China. 2University of Chinese Academy of Sciences, Beijing 100049, China.

Received: 23 February 2020 Accepted: 26 May 2020

Published online: 29 June 2020

References

Baker JT (2004) Yield responses of southern US rice cultivars to CO\(_2\) and temperature. Agric For Meteorol 122:129–137
Chen C (2015) Spikelet formation and seed vigor of contrasting rice cultivars in response to elevated carbon dioxide. University of Chinese Academy of Sciences, Beijing (in Chinese)
Chen C, Jiang Q, Zhik LH, Zhu JG, Liu G, Zhang JS, Kang N, Seneweera S, Zhu OW (2015) Seed vigor of contrasting rice cultivars in response to elevated carbon dioxide. Field Crop Res 178:63–68
Cheng JF, Jiang HY, Liu YB, Dai TB, Cao WX (2011) Methods on identification and screening of rice genotypes with high nitrogen efficiency. Rice Sci 18:127–135
