The effects of free-air CO\(_2\) enrichment (FACE) on carbon and nitrogen accumulation in grains of rice (*Oryza sativa* L.)

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Abstract

Rising atmospheric CO\(_2\) concentrations will probably increase rice (*Oryza sativa* L.) yield but decrease grain nitrogen (GN) concentration. Grains attached to different positions in the panicles differ greatly in weight and quality, but their responses to elevated CO\(_2\) ([CO\(_2\)]) are poorly understood, which limits our understanding of the mechanisms of yield enhancement and quality degradation. Thus a free-air CO\(_2\) enrichment experiment was conducted to examine the effects of [CO\(_2\)] on grain mass (GM), grain carbon (GC), and GN accumulation in the spikelets attached to the upper primary rachis branch (superior spikelets; SS) and those attached to the lower secondary rachis (inferior spikelets; IS). [CO\(_2\)] stimulated the rice yield by 13\% but decreased the N concentration in the panicle by 7\% when averaged over two levels of N fertilizations (\(P < 0.01\)). The responses of SS and IS to [CO\(_2\)] were different particularly under higher N supply. For SS, [CO\(_2\)] decreased GN by 24\% (\(P < 0.01\)) but did not affect GM. For IS, [CO\(_2\)] increased GM by 13\% (\(P < 0.05\)) but GN was not affected. The reduction of GN due to [CO\(_2\)] started to appear at the beginning of grain filling. These results suggest that future [CO\(_2\)] levels probably stimulate the grain growth of IS, most of which are not marketable due to limited size, at the expense of GN reduction in SS. Translocation of N from SS to IS may be a possible mechanism for reduction in GN of SS. This may degrade the grain quality of marketable rice under [CO\(_2\)].

Key words: Dilution, free-air CO\(_2\) enrichment, grain filling, grain mass, inferior spikelets, nitrogen, *Oryza sativa* L., protein, superior spikelets, translocation.

Introduction

A substantial increase in global food production will be required, as the world’s population is expected to be >9 billion by 2050 (FAO, 2009). Rice (*Oryza sativa* L.) is a staple food for more than half of the world’s current population. Although rice production dramatically increased through genetic improvement along with application of a greater amount of chemical fertilizer during the green revolution in the last half century (Zhang, 2007), by the beginning of the present century, the rice yield increase had started to slow (Horie *et al.*, 2005; Long, 2012).

Anticipated environmental changes, such as high concentrations of ground-level ozone, elevated temperatures, and drought negatively affect rice production (Long, 2012), but the expected increase in the atmospheric carbon dioxide concentration ([CO\(_2\)]) is predicted to have a positive effect on the grain yield of rice (Ainsworth, 2008). [CO\(_2\)] has been steadily rising, from ~315 \(\mu\)mol mol\(^{-1}\) in 1959 to a current atmospheric average of ~390 \(\mu\)mol mol\(^{-1}\), and the rise is projected to continue and to reach as high as 550 \(\mu\)mol mol\(^{-1}\) by 2050 (Alley *et al.*, 2007). Although higher [CO\(_2\)] increases the grain yield
of rice (Kobayashi et al., 2006; Long et al., 2006; Hasegawa et al., 2007), it reduces grain quality, especially in terms of protein content (Seneewera et al., 1996; Lieffering et al., 2004; Terao et al., 2005; Yang et al., 2007; Taub et al., 2008; Seneewera, 2011). To maximize the yield enhancement and maintain grain quality in the future, a better understanding is needed of rice grain growth, yield stimulation, and grain quality degradation, especially the reduction in grain protein, under future elevated atmospheric [CO$_2$].

Grain yield stimulation by elevated [CO$_2$] is primarily and consistently achieved by increases in the number of grains (Baker, 2004; Ainsworth, 2008), whereas the response of individual grain weight to elevated [CO$_2$] has been inconsistent. It is apparently not affected (Baker and Allen, 1993; Ziska et al., 1997), increased (Yang et al., 2006), or has a minor effect on the overall yield increase (Kim et al., 2003). It should be noted that the reported individual grain weight is the average across all grains harvested at maturity, but that grain weight, quality, and development vary widely depending on the grain position within the panicle (Iwasaki et al., 1992; Jongkaewwattana et al., 1993; Wang et al., 2007).

In rice, one spikelet generally bears one grain. The earlier-flowering spikelets located on the upper primary rachis branches are referred to as superior spikelets, and the later-flowering spikelets located on secondary rachis branches of the lower primary rachis branches are referred to as inferior spikelets. The inferior spikelets usually fill more slowly and are smaller and lighter than the superior spikelets (Murty and Murty, 1982; Matsumoto and Yoshida, 1994; Yang and Zhang, 2006, 2010; Zhang et al., 2012).

While elevated [CO$_2$] increases the carbohydrate supply to the panicle (Sasaki et al., 2005; Madan et al., 2012), the responses of superior and inferior grains to the elevated [CO$_2$] may be different. There are several possible ways in which elevated [CO$_2$] might affect the grain filling of superior and inferior spikelets. First, both the superior and inferior spikelets are better filled, producing heavier overall grains; secondly, the superior spikelets might be better filled and the inferior ones are unaffected, producing heavier superior grains; and, thirdly, the inferior spikelets are better filled and the superior ones are not affected, resulting in heavier inferior grains. It is yet to be determined which change actually occurs.

The distinction between the superior and inferior spikelets is important for understanding the mechanisms of reduction in nitrogen (N) concentrations in the grain due to elevated [CO$_2$], which are not fully understood (Taub et al., 2008). The N and protein concentrations may be diluted by increased carbohydrates under elevated [CO$_2$] (Gifford et al., 2000). Superior spikelets usually accumulate N earlier and faster than inferior spikelets (Iwasaki et al., 1992, 1993). Therefore, under elevated [CO$_2$], a limited quantity of available N may be more likely to be allocated to the superior spikelets, causing the protein content of the inferior spikelets to be reduced. If too little N is supplied by fertilization, there may be less available N in the whole plant, which would allow reductions in N and protein to be more easily detected.

Grain growth is mainly accomplished by the accumulation of grain carbon (GC), grain N (GN), and grain mass (GM) during grain filling. Because elevated [CO$_2$] alters both the rate and duration of grain filling (Li et al., 2000; Hu et al., 2007), the coordinated accumulation of GC, GN, and GM may be disturbed. Because the superior spikelets are favoured in the assimilate partitioning during grain filling, they fill early in the grain-filling period, and a reduction in protein may not be detected. The inferior spikelets, however, begin to fill rapidly ~20 days after heading (DAH) (Iwasaki et al., 1992), after the period of rapid accumulation in the superior spikelets has ended and much of the available N has already been taken up. As a result, they may be more likely to show reduced protein.

Over the past three decades, various laboratory and chamber experiments have been conducted to examine plant responses to elevated [CO$_2$], but the need to test findings and hypotheses under field conditions has become increasingly apparent (Leakey et al., 2009). Free-air CO$_2$ enrichment (FACE) experiments provide a unique platform for investigating how future ecosystems are likely to respond to higher [CO$_2$] within various ecosystem-scale interactions. Therefore, a FACE experiment was conducted with two N fertilization levels to answer the following questions. (i) Do grains located at different panicle positions respond differently to an elevated [CO$_2$]? (ii) Under an elevated [CO$_2$], if the average grain N concentration is decreased, is the reduction restricted to superior or inferior grains or is it observed in all grains? (iii) Will the N concentration in rice grains be reduced by an elevated [CO$_2$] more at a lower nitrogen fertilization level? (iv) When does the elevated [CO$_2$] start to decrease the GN concentration during grain filling, and is the response of the superior and inferior spikelets the same with respect to that timing?

The answers to these questions will improve our understanding of grain filling and growth responses to an increased source supply, and of the mechanisms of rice yield enhancement, under elevated [CO$_2$]. In addition, the results may suggest how grain yield might be increased and grain quality might be maintained in the future.

### Materials and methods

**Site description, [CO$_2$] control, and weather conditions**

The experiment was conducted at the Tsukuba FACE site in Tsukubamirai city, Ibaraki Prefecture, Japan (35°58′N, 139°60′E; 10 m above sea level) in 2010 and 2011. The climate is humid subtropical with an average temperature of 13.8 °C and annual precipitation of 1280 mm. The soils are Fluvisols, typical of alluvial areas. Additional soil properties are given in Hasegawa et al. (2013).

Nakamura et al. (2012) have described the method used to control [CO$_2$]. Briefly, four blocks were established in paddy fields, with each block consisting of two octagonal plots (240 m$^2$, 17 m across): an ambient CO$_2$ (ambient) plot and an elevated CO$_2$ (FACE) treatment plot. The FACE plots were equipped with emission tubes on the perimeter, where CO$_2$ was released from the windward side to keep the [CO$_2$] measured at the central point at 200 μmol mol$^{-1}$ above the ambient control level. The season-long daytime average [CO$_2$] in the ambient plots and in the FACE plots was 386 μmol mol$^{-1}$ and 584 μmol mol$^{-1}$ in 2010, and 386 μmol mol$^{-1}$ and 560 μmol mol$^{-1}$ in 2011, respectively. The seasonal mean air temperature was 25.0 °C in 2010 and 24.1 °C in 2011, higher than that of the average year.
Elevated \([\text{CO}_2]\) had no significant effect on the number of primary and secondary branches and the number of spikelets within a panicle (Table 2). N treatment had significant effects on the numbers of secondary branches, spikelets, and fertile spikelets, which were higher at SN than at 0N. The number of primary spikelets was sensitive to both \([\text{CO}_2]\) and N (0N versus SN) was tested by using the Mixed Model procedure of the SAS statistical analysis software package (SAS Add-In 4.3 for Microsoft Office, SAS Institute, Tokyo, Japan). For the GM data, the Richards’ function was fitted to the time course of GM accumulation in the superior and inferior spikelets and then the active grain-filling duration and average grain-filling rate were estimated according to Zhang et al. (2012).

Results

Grain yield and C and N concentrations of the bulk sample

Compared with the ambient treatment, FACE significantly increased the brown rice yield by 13% averaged across years and N treatments (\(P < 0.01\), Table 1), but the yield enhancement differed between the N treatments, as evidenced by the significant interaction between \([\text{CO}_2]\) and N (\(P < 0.05\)); 11% at 0N and 16% at SN. Harvest index (HI), defined here as the grain dry mass divided by above-ground dry mass, was slightly decreased by elevated \([\text{CO}_2]\) (\(P < 0.10\)), but this effect was only apparent in 0N where the HI decreased by 3.5%, and no reduction occurred in SN, resulting in a significant interaction between \([\text{CO}_2]\) and N (\(P < 0.05\)); elevated \([\text{CO}_2]\) did not affect panicle C concentration, but decreased N concentration significantly (\(P < 0.01\)). The reduction in the panicle N concentration was slightly larger in SN (8.5%) than in 0N (5.5%), with a marginally significant interaction (\(P < 0.10\)). Other yield components are shown in Supplementary Table S2 at JXB online.

Panicle structure

Elevated \([\text{CO}_2]\) had no significant effect on the number of primary and secondary branches and the number of spikelets. N treatment had significant effects on the numbers of secondary branches, spikelets, and fertile spikelets, which were higher at SN than at 0N. The number of primary spikelets was sensitive to both \([\text{CO}_2]\) and the N levels, namely at 0N the number of primary spikelets was increased by FACE, while at SN it was decreased by FACE.

Elevated \([\text{CO}_2]\) had no significant effects on the numbers of superior and inferior spikelets. N levels had a significant effect on the number of inferior spikelets; N fertilizer increased the number of inferior spikelets; N levels had a significant effect on the number of inferior spikelets. The effects of FACE on the number of fertile spikelets were dependent on the N level and the grain position: at 0N, the number of fertile superior spikelets was higher in FACE than in the ambient plots; at SN, the number of fertile superior spikelets in FACE was lower and the number of fertile inferior spikelets was higher than in ambient.

Effects of elevated \([\text{CO}_2]\) and N levels on GM, GN, and GC contents in superior and inferior spikelets at maturity

Elevated \([\text{CO}_2]\) affected the GM of superior and inferior spikelets differently depending on the N fertilization level
Table 1. Effects of [CO$_2$] (A, ambient; E, elevated) and nitrogen fertilization levels (0 g m$^{-2}$ and 8 g m$^{-2}$) on the panicle number, grain yield, and harvest index of cultivar Koshihikari in 2010 and 2011 and the results of an analysis of variance (ANOVA)

| N applied (g m$^{-2}$) | Year | Brown rice yield$^a$ (g m$^{-2}$) | Harvest index$^b$ (%) | Panicle number (m$^{-2}$) | C concentration (mg g$^{-1}$) | N concentration (mg g$^{-1}$) |
|------------------------|------|-----------------------------------|------------------------|---------------------------|-------------------------------|-------------------------------|
|                        |      |                                   |                        |                           | A    | E    | E/A | A    | E    | E/A | A    | E    | E/A | A    | E    | E/A | A    | E    | E/A | A    | E    | E/A |
| 0                      | 2010 | 400                              | 455                    | 1.14                      | 44.1 | 43.5 | 0.99 | 429  | 426  | 0.99 | 9.00 | 8.57 | 0.95 | 257  | 272  | 1.06 |
|                        | 2011 | 430                              | 459                    | 1.07                      | 44.2 | 41.4 | 0.94 | 430  | 430  | 1.00 | 9.34 | 8.81 | 0.94 | 278  | 297  | 1.07 |
| 8                      | 2010 | 552                              | 642                    | 1.16                      | 46.1 | 46.7 | 1.01 | 427  | 426  | 1.00 | 10.10| 8.96 | 0.89 | 321  | 351  | 1.09 |
|                        | 2011 | 604                              | 698                    | 1.16                      | 45.7 | 45.5 | 0.99 | 433  | 433  | 1.00 | 10.61| 9.95 | 0.94 | 347  | 403  | 1.16 |

ANOVA$^c$

- Year: NS
- CO$_2$: NS
- Year×CO$_2$: NS
- N: NS
- Year×N: NS
- CO$_2$×N: NS
- Year×CO$_2$×N: NS

$^a$ Expressed on a 15% moisture content basis.

$^b$ Brown rice yield divided by the above-ground mass expressed on the 0% moisture (dry mass) basis.

$^c$ †P < 0.1; *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant.

Table 2. Effects of [CO$_2$] (A, ambient; E, elevated) and nitrogen fertilization levels (0 g m$^{-2}$ and 8 g m$^{-2}$) on the panicle structure of cultivar Koshihikari in 2011 and the results of an analysis of variance (ANOVA)

| N applied (g m$^{-2}$) | Branch number | Spikelet number | Fertile spikelet number |
|------------------------|---------------|-----------------|-------------------------|
|                        | Primary       | Secondary       | Total                   | Primary       | Secondary       | Total                   | Superior       | Inferior      | Total       | Superior       | Inferior      | Total       |
|                        | Primary       | Secondary       | Total                   | Primary       | Secondary       | Total                   | Superior       | Inferior      | Total       | Superior       | Inferior      | Total       |
|                        | A    | E    | E/A | A    | E    | E/A | A    | E    | E/A | A    | E    | E/A | A    | E    | E/A | A    | E    | E/A | A    | E    | E/A |
| 0                      | 9    | 10   | 16  | 16   | 52   | 55   | 42  | 44   | 93  | 99  | 49   | 52   | 38  | 40  | 87  | 92  |      |      |      |      |      |      |      |
| 8                      | 10   | 10   | 20  | 19   | 58   | 55   | 56  | 54   | 113 | 109 | 55   | 53   | 51  | 49  | 106 | 102 |      |      |      |      |      |      |      |

ANOVA$^a$

- CO$_2$: NS
- N: NS
- CO$_2$×N: NS

$^a$ *P < 0.05; **P < 0.01; NS, not significant.

Table 3. Effects of [CO$_2$] (A, ambient; E, elevated) and nitrogen fertilization levels (0 g m$^{-2}$ and 8 g m$^{-2}$) on the number of superior and inferior spikelets within a panicle of cultivar Koshihikari in 2011 and the results of an analysis of variance (ANOVA)

| N applied (g m$^{-2}$) | Spikelet number | % of sterile spikelets (%) | Fertile spikelet number | % in total fertile spikelets |
|------------------------|-----------------|----------------------------|-------------------------|-----------------------------|
|                        | Superior       | Inferior       | %                  | Superior       | Inferior       | %                  | Superior       | Inferior       | %                  |
|                        | E    | E    | E/A | A    | E    | E/A | A    | E    | E/A | A    | E    | E/A | A    | E    | E/A | A    | E    | E/A | A    | E    | E/A |
| 0                      | 7.0  | 7.5  | 5.8 | 5.8  | 5.8  | 1.3 | 1.8 | 6.8  | 7.3  | 4.8 | 4.8  | 7.8  | 7.7  | 5.5 | 5.2 |
| 8                      | 7.5  | 7.5  | 8.3 | 8.5  | 7.0  | 5.8 | 2.0 | 1.8 | 7.0  | 6.8  | 7.3 | 7.0  | 6.7 | 6.9 | 6.5 | 6.8 |

ANOVA$^a$

- CO$_2$: NS
- N: NS
- CO$_2$×N: NS

$^a$ †P < 0.1; *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant.

(Table 4). At 0N, the GM of superior spikelets in FACE was higher than that in ambient, whereas the GM of inferior spikelets in FACE was lower than that in ambient. At SN, the GM of superior spikelets in FACE was lower than that in ambient, whereas the GM of inferior spikelets in FACE was higher than that in ambient. The GC content of superior spikelets
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Table 4. Effects of [CO₂] (A, ambient; E, elevated) and nitrogen fertilization levels (0g m⁻² and 8g m⁻²) on grain mass, grain carbon, and grain nitrogen in superior and inferior spikelets of Koshihikari in 2011 and the results of an analysis of variance (ANOVA)

| N applied (g m⁻²) | Grain mass (mg kernel⁻¹) | C concentration (mg g⁻¹) | C content (mg kernel⁻¹) | N concentration (mg g⁻¹) | N content (mg kernel⁻¹) |
|------------------|--------------------------|--------------------------|-------------------------|--------------------------|-------------------------|
|                  | Superior | Inferior | Superior | Inferior | Superior | Inferior | Superior | Inferior | Superior | Inferior | Superior | Inferior |
| 0                | A        | E        | A        | E        | A        | E        | A        | E        | A        | E        | A        | E        |
| 0                | 18.3     | 18.5     | 13.9     | 12.2     | 452      | 447      | 452      | 455      | 8.3      | 8.3      | 6.3      | 5.6      |
| 8                | 20.5     | 19.8     | 13.7     | 15.5     | 447      | 457      | 442      | 447      | 9.2      | 9.0      | 6.0      | 6.9      |

ANOVA

| N applied (g m⁻²) | Grain mass (mg kernel⁻¹) | C concentration (mg g⁻¹) | C content (mg kernel⁻¹) | N concentration (mg g⁻¹) | N content (mg kernel⁻¹) |
|------------------|--------------------------|--------------------------|-------------------------|--------------------------|-------------------------|
|                  | Superior | Inferior | Superior | Inferior | Superior | Inferior | Superior | Inferior | Superior | Inferior | Superior | Inferior |
| 0                | A        | E        | A        | E        | A        | E        | A        | E        | A        | E        | A        | E        |
| 0                | 18.3     | 18.5     | 13.9     | 12.2     | 452      | 447      | 452      | 455      | 8.3      | 8.3      | 6.3      | 5.6      |
| 8                | 20.5     | 19.8     | 13.7     | 15.5     | 447      | 457      | 442      | 447      | 9.2      | 9.0      | 6.0      | 6.9      |

was not affected by [CO₂], while GC of inferior spikelets was similar to the GM of inferior spikelets: at 0N, GC of inferior spikelets in FACE was lower than that in ambient, whereas the GC content of inferior spikelets in FACE was higher than that in ambient at SN. The GN content of superior spikelets was significantly decreased (by 24%) by FACE (P < 0.01), whereas GN in inferior spikelets was not affected by FACE. The N level had significant effects on the GM, GC, and GN content of superior and inferior spikelets: N fertilizer stimulated GM, GC, and GN in both the superior and inferior spikelets.

Time courses of GM, GN, and GC in superior and inferior spikelets

Grain-filling patterns differed between superior and inferior spikelets (Fig. 1). In the superior spikelets, GM accumulation started to increase immediately after panicle emergence (Fig. 1A), whereas in the inferior spikelets, rapid GM accumulation began at ~20 DAH (Fig. 1B). The final GM of inferior spikelets was lower than that of superior spikelets: at 0N, the GM of inferior spikelets was 52% lower in FACE and 32% lower in ambient compared with superior spikelets; and at SN, the GM of inferior spikelets was 28% lower in FACE and 50% lower in ambient compared with superior spikelets.

The effects of elevated [CO₂] on GM accumulation were more apparent in the inferior spikelets at SN (Fig. 1B). The duration of active grain filling estimated from the Richards’ function fitted to each plot (Zhang et al., 2012) was shorter at 0N, and the average grain-filling rate of superior spikelets was increased and that of inferior spikelets was decreased by the FACE treatment (P < 0.05; data not shown). In contrast, the duration of active grain filling was longer at SN, and the average grain-filling rate of superior spikelets was decreased and that of inferior spikelets was increased by the FACE treatment compared with ambient treatment.

Nitrogen levels had a significant effect on the accumulation of GM in both superior and inferior spikelets. In superior spikelets, a difference in GM accumulation between SN and 0N became apparent at ~20 DAH (Fig. 1A). In inferior spikelets, a difference became apparent at ~30 DAH, and the inferior spikelets apparently continued to accumulate GM even after 40 DAH (Fig. 1B).

FACE and N levels modified the relationship between GN and GM accumulation (Fig. 1, lower panels). FACE decreased the GN concentration, and SN increased the GN concentration in both superior and inferior spikelets. The effects were more apparent before 15 DAH, and from 30 to 40 DAH the trend differed between superior and inferior spikelets (Fig. 1C, D). The GN concentration in the superior spikelets was apparently (P < 0.10) decreased by FACE at 5 DAH, 30 DAH (P < 0.01), and 40 DAH (P < 0.05) (Fig. 1C). SN significantly increased the GN concentration in superior spikelets at 10 DAH (P < 0.05) and 20 DAH (P < 0.05). The GN concentration in inferior spikelets was significantly decreased by FACE at 30 DAH (P < 0.05) and increased by SN at 10 DAH (P < 0.10) (Fig. 1D).

With the increase of GM from inferior spikelets to superior spikelets, the GN concentration was decreased (Fig. 2); the slope of the relationship between the GN concentration and GM differed significantly between FACE and ambient (P < 0.05). For superior spikelets, although the GM was not affected by FACE, the GN concentration was decreased by FACE (P < 0.05).

Discussion

Grain yield stimulation by elevated [CO₂]

Elevated [CO₂] significantly increased the grain yield by 13% (P < 0.05, Table 2), as was commonly observed in the previous studies for the rice FACE experiments (Kim et al., 2003; Yang et al., 2006). The grain yield increase was primarily due to the increase in the number of panicles (Tables 1, 2), which was also previously shown (Kim et al., 2003; Yang et al., 2006). The number of spikelets located on primary and secondary branches of each panicle, on the other hand, was not affected (Tables 2, 3). These results show that elevated [CO₂] did not affect the ratio between inferior and superior spikelets, indicating that variations among superior and inferior spikelets with respect to grain weight and grain quality have important effects on the average grain response to elevated [CO₂].
Is a reduction of GN concentration by elevated [CO$_2$] a result of dilution?

It has been commonly reported that elevated [CO$_2$] decreases GN concentration in rice (Seneweera et al., 1996; Lieffering et al., 2004; Terao et al., 2005; Yang et al., 2007; Seneweera, 2011). A significant reduction in N concentration of the bulk panicle samples was also observed ($P < 0.01$, Table 2). Further analysis showed, however, that this decrease is apparent only in superior spikelets (Table 5). The mechanisms by which elevated [CO$_2$] decreases plant N and protein concentrations are not yet well understood, but one plausible mechanism is that the stimulated production of carbohydrates by elevated [CO$_2$] dilutes plant N (Gifford et al., 2000; Taub et al., 2008). A negative correlation was also found between the GM and GN concentration when superior and inferior spikelets were pooled (Fig. 2). When the effect of FACE in superior and inferior spikelets was examined separately, however, it was found that the FACE treatment decreased the GN concentration of the superior spikelets, but that their GM was not increased by elevated [CO$_2$]. Moreover, whereas the GM of inferior spikelets was significantly increased by elevated [CO$_2$], their GN concentration was not reduced. These results
suggest that the reduced GN concentration cannot be a simple result of dilution by increased GM. Rather, differential responses of C and N allocation to the grains in response to elevated [CO\textsubscript{2}] must be a part of the mechanism that accounts for the reduced GN.

In previous experiments under current [CO\textsubscript{2}], removal of superior spikelets induced allocation of N to inferior spikelets at various stages during grain filling (Iwasaki et al., 1992, 1993; Kato, 2004). Seneweera (2011) showed that elevated [CO\textsubscript{2}] greatly affects rice growth and N partitioning among different organs, and the present study demonstrated that elevated [CO\textsubscript{2}] affects the partitioning of N between superior and inferior spikelets.

Reduction of GN under elevated [CO\textsubscript{2}] occurred at early grain filling

GN accumulated differently in superior and inferior spikelets during the grain-filling period in response to elevated [CO\textsubscript{2}], and the reduction in GN concentration occurred at the beginning of the grain-filling period (Fig. 1C, D). The superior spikelets started the rapid growth immediately after heading, whereas growth and N accumulation in inferior spikelets started only at ~20 DAH (Iwasaki et al., 1992), when superior spikelets had already completed their rapid growth. In this study, under ambient [CO\textsubscript{2}], N accumulation trends in superior and inferior spikelets were similar to those previously reported (Iwasaki et al., 1992). They were, however, different from the previous report under elevated [CO\textsubscript{2}] (Fig. 1C, D), which showed a reduced GN concentration in superior spikelets at both early (5 DAH) and late grain-filling stages (from 30 to 40 DAH). The mechanisms of these two reductions may be different. The late GN reduction in superior spikelets may be due to the preferential allocation of N to inferior spikelets whose growth has been stimulated by elevated [CO\textsubscript{2}], with a greater need for N. In contrast, the early drop in GN concentration may reflect dilution by stimulated carbohydrate accumulation in elevated [CO\textsubscript{2}] against lagging N accumulation. At this early stage, the growth of inferior spikelets is slow or has not yet begun, and they are not competing with the superior spikelets for N. No dilution effect was observed in the inferior spikelets at this early stage because of their slow growth. By the time the inferior spikelets began to grow rapidly, after 20 DAH, the rapid growth of superior spikelets had already slowed and much of the available N had been taken up. Therefore, the limited amount of available N was easily diluted by the stimulated carbohydrates. Thus, in inferior spikelets, the GN reduction by elevated [CO\textsubscript{2}] occurred at a late stage (30 DAH). The translocation of N to inferior spikelets from superior spikelets from 30 DAH to 40 DAH may have ameliorated the GN reduction in inferior spikelets at maturity under elevated [CO\textsubscript{2}], and, at the same time, caused a significant GN reduction in the superior spikelets. In addition to the N reduction, the GM of superior spikelets was also slightly decreased under elevated [CO\textsubscript{2}], whereas the GM of inferior spikelets was significantly increased at SN (Table 4). Inferior spikelets are usually limited by the C supply (Murty and Murty, 1982; Fu et al., 2011); thus, the increased C supply by elevated [CO\textsubscript{2}] stimulated the growth of inferior grains.

Limited N supply did not intensify the effects of elevated [CO\textsubscript{2}] on GN

The different responses of superior and inferior spikelets to elevated [CO\textsubscript{2}] were dependent on the N level (Table 4, Fig. 1A, B). The GM of superior spikelets was little affected by elevated [CO\textsubscript{2}]. On the other hand, the GM of inferior spikelets was lower at 0N and higher at SN under elevated than ambient [CO\textsubscript{2}]. Poor grain filling of inferior spikelets is reported to be attributed to a sink limitation due to poor activity of enzymes related to starch synthesis (Patel et al., 1996; Liang et al., 2001; Ishimaru et al., 2005), whereas the present results showed that when the source supply was increased by elevated [CO\textsubscript{2}], the grain filling of inferior spikelets was improved. Manderscheid et al. (2009) reported that grain growth of winter barley under elevated [CO\textsubscript{2}] is strongly sink limited, whereas the present results showed that the GM of superior spikelets, which have a larger sink capacity than inferior spikelets, responded negatively to elevated [CO\textsubscript{2}]. These results suggest that in rice, more than source supply and sink limitations, other mechanisms regulate the partitioning of assimilate and grain growth in response to environmental change.

Low N fertilization did not intensify N reduction in rice grains under elevated [CO\textsubscript{2}] (Table 4). The stimulation of biomass and yield by elevated [CO\textsubscript{2}] is usually small at low N levels (Amthor, 2001; Kim et al., 2003; Manderscheid et al., 2009). In this study, the grain yield enhancement and panicle N reduction by elevated [CO\textsubscript{2}] were 11% and 5.5% at low N, and 16% and 8.5% at ample N (Table 1). At low N, the carbohydrate stimulation by elevated [CO\textsubscript{2}] may not be enough to dilute the GN concentration significantly in well-filled superior spikelets or growth-depressed inferior spikelets. As inferior spikelets usually have a higher N content than superior spikelets (Matsue et al., 1994), inferior spikelets may become an N source for superior spikelets when N is deficient. The translocation of N from inferior to superior spikelets may ameliorate some of the N reduction in superior spikelets at 0N.

The different responses in GM and GN accumulation between superior and inferior spikelets under elevated [CO\textsubscript{2}] may be an indication of how rice adapts to environmental change. The present study suggests that under elevated [CO\textsubscript{2}], the better growth of superior spikelets occurs at the expense of the depressed growth of inferior grains when N is deficient. On the other hand, when N is ample, the better growth of inferior grains occurs without the greater growth of superior grains.

Relevance of the position-dependent response of grains to elevated [CO\textsubscript{2}] with rice quality

The differences in the translocation of N between superior and inferior grains may affect the grain quality response of rice to elevated [CO\textsubscript{2}]. The ratio of the number of superior to inferior
grains was not affected (Tables 1–3), but the number of panicles was increased by elevated [CO$_2$]. Therefore, the numbers of inferior and superior grains increased simultaneously. Compared with superior grains, the cooking quality and appearance of inferior grains are usually lower (Chaudhry and Nagato, 1970; Iwasaki et al., 1992; Jongkaewwattana et al., 1993). Moreover, although the GM in inferior grains was significantly increased by elevated [CO$_2$], some inferior grains were still too small and thus would be discarded when the grain was sieved to evaluate the thousand grain weight and grain quality (Lieffering et al., 2004), resulting in an overall protein loss. Some well-filled inferior grains might not be discarded during sieving. In fact, while inferior grains were 41% lighter than superior grains, inferior grain mass ranged from 11 mg to 17 mg, the heavier ones being close to the range of superior grain mass (17–21 mg). The reduced grain size would have a negative impact on milling quality (Jongkaewwattana and Geng, 2001). Head rice loss under elevated [CO$_2$] directly affects the market value of rice and the income of rice producers (Yang et al., 2007). Thus, the protein reduction in the superior grains under elevated [CO$_2$] might affect the overall grain quality of the yield.

**Implications for future experiments**

There is an urgent need to understand how future elevated CO$_2$ concentrations will affect yield and grain quality so that rice yields and grain quality can both be maximized. Elevated [CO$_2$] stimulates the grain yield of rice mainly by increasing the spikelet number, and the spikelet number increment is mainly achieved by an increase in the number of panicles. The grain yield of cultivars with fewer spikelets within a panicle may also become higher under elevated [CO$_2$]. The translocation of assimilate among spikelets should be examined in cultivars with fewer spikelets per panicle or with spikelets of uniform size within a panicle. Such cultivars may be better able to adapt to future elevated [CO$_2$]. Moreover, the differences between superior and inferior grains are also dependent on panicle type and cultivar (Liu et al., 2005; Wang et al., 2007). Thus, cultivars with different panicle types should be tested to better understand the mechanisms of yield enhancement and protein reduction in rice grains under future elevated [CO$_2$].

**Conclusion**

Future elevated [CO$_2$] levels are likely to stimulate the growth of inferior grains at the expense of reductions in the protein content of superior grains. More than a dilution effect caused by the accumulation of more carbohydrates under elevated [CO$_2$], the differential allocation of N and assimilates between superior and inferior grains is responsible for this reduction in grain protein content, which thus affects the grain quality of the rice.

**Supplementary data**

Supplementary data are available at *JXB* online.

**Table S1.** Weather conditions in the 2010 and 2011 growing seasons at the Tsukuba FACE site, Japan.

**Table S2.** Yield, yield components, and panicle C and N concentration of rice cultivar Koshihikari obtained in the 2010 and 2011 growing seasons at the Tsukuba FACE experiments.

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