Paddy Rice Responses to Free-Air CO₂ Enrichment

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Abstract

We conducted free-air CO₂ enrichment of rice growing in farmers' paddies in Shizukuishi, Iwate, Japan. The three year experiment (1998 to 2000) showed that the CO₂ enrichment of 220-295 ppmV above ambient CO₂ concentration [CO₂] increased the rice yield by 7, 14, and 15 % with nitrogen (N) fertilizer applied at low, standard, and high levels, respectively. With little changes in harvest index, the yield increase was nearly proportional to the increase in plant biomass. This may lead one to hypothesize a simple source-driven mechanism: the yield gain by the CO₂ enrichment is caused by the photosynthetic enhancement, which is limited by low N availability. Nonetheless, a detailed analysis of the yield components, plant biomass and N accumulation has led us to a sink-mediated mechanism for the interaction between N and grain yield in response to elevated [CO₂]. They are linked together by the close relationship between the N accumulation through to panicle initiation (PI) and the number of grains at harvest. The elevated CO₂ stimulated N uptake through to PI with standard and high N levels, but not with low N, and resulted in a [CO₂] x N interaction for the number of grains, which dominated the grain yield response to elevated [CO₂].

Key words: Carbon dioxide, FACE, Free-air CO₂ enrichment, Nitrogen, Rice

1. Introduction to FACE

Free-air CO₂ enrichment (FACE) enables us to observe the responses of intact terrestrial ecosystems to elevated atmospheric CO₂ concentrations ([CO₂]) without artificially altering the above- and below-ground environment of the vegetation. Since it is likely that vegetation responses to elevated [CO₂] are influenced by biotic as well as abiotic stressors, the observations under FACE probably better predict the real-world changes in the future than those in enclosures, which can decouple the vegetation from the stressors.

CO₂ in the atmosphere is well mixed because of its relatively long residence time, and its concentration varies little temporally and spatially. As such, FACE can be seen as a unique opportunity along with the natural CO₂ springs, where CO₂ is released from the ground to create a CO₂ gradient around the source. Besides this uniqueness, however, FACE is no more than an ordinary field experiment with CO₂ being manipulated like fertilizers or crop varieties are in agronomic field trials. FACE thus shares the advantages and disadvantages of field experiments in comparison with experiments conducted in environmentally-controlled chambers. Advantages include the realism mentioned above and the size of the plots which al-

lows destructive samplings of the plants without altering the environment for remaining ones. Disadvantages include the lack of control over most of the environmental variables, and as a consequence the possible influences of the uncontrollable variables on the vegetation responses to elevated [CO₂]. The observations in a FACE experiment could therefore be specific to the time and the location, like those in any field experiment, and to make generalizations would require replication across years and locations.

Here we report on the FACE experiment conducted with rice in Japan from 1998 to 2000. Since rice is the most important crop in Asia on the basis of area grown and tonnage harvested, there is a need to determine how the projected increase in [CO₂] will affect rice yield. The three years of the FACE experiment included one year with cooler-than-normal weather and two warmer years. The experiments were therefore done under a reasonable range of year-to-year fluctuation in weather at this site. In a subsequent year (2003) of FACE at the same site, we experienced unusually cool temperatures that induced floral sterility. The results of the FACE experiment in this cool spell are not included in this paper, but a preliminary report can be found in the same volume (Okada *et al.*, 2005). Our FACE experiments in Japan up to now thus cover

a fairly large range of climatic variability. In view of the large environmental and genetic variability in rice culture in Asia, however, there is an obvious need to conduct FACE experiments at different locations. It is noteworthy that there has been a FACE experiment conducted on a rice-wheat rotation system in Wuxi, Jiangsu, China from 2001 through 2003, and that the results have been published recently (Huang *et al.*, 2004).

2. The Rice FACE experiment in Japan

We installed four FACE apparatuses based on pure- CO_2 injection (Okada *et al.*, 2001) in farmers' fields in Shizukuishi near Morioka, Iwate Prefecture in northern Japan (Fig. 1). This area is representative of the

Fig. 1. Overview of a FACE ring in rice field in Shizukuishi, Iwate, Japan.

agro-climatic region that grows a large proportion of the rice crop in Japan. The four FACE 'rings' were placed in separate paddies, and four other plots were located as ambient CO_2 controls in other paddies. The eight plots were blocked into four pairs of one FACE and one Control plot according to the agronomic and soil management history of the paddies.

A FACE ring consisted of eight plastic tubes with numerous tiny holes. Pure CO_2 was injected into the air through the tiny holes in the tubes on the upwind sides, and was mixed with the air while being transported downwind. The amount of CO_2 injected was computer-controlled to keep $[\text{CO}_2]$ at the ring center close to the target level, which was 200 ppmV above the continuously monitored ambient $[\text{CO}_2]$.

The CO_2 enrichment was carried out continuously from transplanting to harvest during the rice growing seasons for three years from 1998 to 2000. No crops were grown outside the rice season. The area within each FACE ring and Control plot was split into subplots with different amounts of nitrogen (N) fertilizer: low N (40 kg N ha^{-1} in all years), standard N (80 kg N ha^{-1} in 1998, and 90 kg N ha^{-1} in 1999 and 2000), and high N (120 kg N ha^{-1} in 1998, and 150 kg N ha^{-1} in 1999 and 2000) to study the rice and soil responses to elevated $[\text{CO}_2]$ under various levels of N availability. Phosphate and potassium were supplied in sufficient amounts. We used the Japanese variety 'Akitakomachi' in the experiment because of its popularity among the local farmers. The variety is well adapted to the local climate, and has good grain quality. Some subplots were planted with other varieties, and varietal difference in the plant responses to elevated $[\text{CO}_2]$ was studied. The varietal responses to elevated $[\text{CO}_2]$ are not reported in this article, however.

Table 1. Grain yield and harvest index of the variety 'Akitakomachi' in the Rice FACE experiment in Japan. 'Ambient' denotes the plots in ambient $[\text{CO}_2]$, and 'Elevated' denotes the plots in the elevated $[\text{CO}_2]$. See text for the nitrogen application rates.

Unhulled fertile grain yield (dry matter tonne ha^{-1})						
(Percentage change relative to the ambient $[\text{CO}_2]$ plots in parentheses)						
Year	Low-N		Standard-N		High-N	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
1998	5.14	5.73 (+11 %)	5.88	6.81 (+16 %)	6.47	7.84 (+21 %)
1999	6.86	7.14 (+4 %)	7.67	8.70 (+13 %)	8.36	9.31 (+11 %)
2000	6.37	6.78 (+7 %)	7.23	8.28 (+15 %)	7.85	8.87 (+13 %)
Harvest index (Percentage change relative to the ambient $[\text{CO}_2]$ plots in parentheses)						
Year	Low-N		Standard-N		High-N	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
1999	0.523	0.498 (-4.8 %)	0.530	0.523 (-1.3 %)	0.523	0.513 (-1.9 %)
2000	0.495	0.475 (-4.0 %)	0.510	0.503 (-1.4 %)	0.530	0.525 (-0.9 %)

3. Plant responses to elevated [CO₂] in Rice FACE

Grain yield of 'Akitakomachi' was significantly increased by CO₂ enrichment across the three years (Table 1; Kim *et al.*, 2003). With standard N fertilization, the grain yield in the FACE plots was 13 to 16 % higher than the Control plots despite the significant difference in the yields between the three years due primarily to the year-to-year difference in the weather. The grain yield response to CO₂ enrichment was obviously influenced by the N fertilization: the response with low N was only about a half that with standard and high N rates.

Harvest index was reduced by CO₂ enrichment, but the change was only marginal with standard and high N (Table 1; Kim *et al.*, 2003). These results may lead one to a simplistic view of the mechanism of the crop yield response to elevated [CO₂]: photosynthesis was enhanced by CO₂ enrichment, leading to a larger accumulation of plant biomass, and, with the small change in harvest index, the grain yield increased proportionately. With low N, limited N availability constrained not only the photosynthetic response to elevated [CO₂] but as a consequence, the yield gain. In this source-driven hypothesis, the photosynthetic response to elevated [CO₂] determined the yield response.

A detailed analysis of grain yield components, plant biomass and N accumulation has, however, revealed a more complex response of the plants to elevated [CO₂] than the simple source-driven hypothesis. Among the changes in the yield components, the increase in the number of spikelet per ground area dominated the grain yield response to elevated [CO₂] (Kim *et al.*, 2003). Notably, the maximum number of grains is determined before heading, i.e. before the accumulation of carbo-hydrates into the rice grains.

It is also noteworthy that neither the increase in total plant biomass from PI to maturity nor that from heading to maturity was increased by the CO₂ enrichment at all (Fig. 2). This is interesting because the dominant fraction of the grain biomass comes from current rather than stored photosynthesis during grain-filling, and the higher grain yield must have been supported by a higher rate of carbohydrate supply. It was found that, at least for standard and high N, under the elevated [CO₂] the larger biomass of the grains was balanced by the greater loss of shoot (leaf + stem) weight for the period from heading to maturity (Fig. 2).

Further, it is shown that across both CO₂ levels, the N accumulation through to the PI stage is the key determinant of the number of grains at harvest (Fig. 3; Kim *et al.*, 2001). A similar relationship has been reported by Horie *et al.* (1997) for another Japanese variety 'Koshihikari' planted at three locations with large differences in climatic and edaphic conditions (Fig. 3). The difference in the relationships between the studies may indicate a varietal difference in rice.

Combining these findings, we hypothesized a sink-mediated mechanism to explain the interaction between N-availability and grain yield increase due to CO₂ enrichment: up to PI, CO₂ enrichment increased N accumulation (Fig. 4) through greater root growth (Kim *et al.*, 2001), and thereby increased the number of grains in the crops receiving standard and high N levels. With low N, by comparison, N uptake through to PI was already limited by N availability and was unchanged by CO₂ enrichment. The potential grain number response was much less under low N compared to the higher N levels.

Fig. 2. Changes in total plant biomass weight from heading to maturity (TBM H-M) and that from panicle initiation to maturity (TBM PI-M), and the change in shoot biomass weight from heading to maturity (SBM H-M) under elevated [CO₂] (Elev) and ambient [CO₂] (Amb) in 2000.

Fig. 3. Relationship between the number of fertile grains at harvest and nitrogen accumulated through panicle initiation stage.

During the grain-filling stage, the larger number of grains under elevated $[\text{CO}_2]$ demanded more carbohydrates for grain growth than those under ambient $[\text{CO}_2]$ in the standard and high N levels. The higher carbohydrate demand of the grains under elevated $[\text{CO}_2]$ was met by increased re-translocation of stored carbohydrates from the stems and leaf sheaths, resulting in a larger loss of shoot weight during the grain-filling period (Fig. 2). This was not the case in the low N plots since the grain number response was much less.

The larger number of grains under elevated $[\text{CO}_2]$ also demanded more N than those under ambient $[\text{CO}_2]$. After panicle initiation, with soil N availability depending on the limited supply of N from mineralization, the extra demand for N had to be met by greater remobilization. Plants at standard and high N levels had accumulated more N through to PI under elevated $[\text{CO}_2]$ (Fig. 4), and their leaves served as the major source for grain N.

Fig. 4. Total plant nitrogen at panicle initiation (TN_PI) and at maturity (TN_M) under elevated $[\text{CO}_2]$ (Elev) and ambient $[\text{CO}_2]$ (Amb). Averages of the 1999 and 2000 results are given.

A consequence of greater export of N from the leaves to the grains is faster leaf senescence and hence quicker loss of photosynthesizing leaf area in the CO_2 -enriched plants. The greater export of N from the leaves to developing panicles might have also contributed to the decline in photosynthetic capacity of the flag leaves under CO_2 enrichment (Seneweera *et al.*, 2002).

These declines in leaf area and photosynthetic capacity in the CO_2 -enriched plants may also have accounted for the lack of an effect of elevated $[\text{CO}_2]$ on the net biomass increase for the period between PI and the maturity (Fig. 2).

The above hypothesis is supported by the findings in a recent sun-lit chamber experiment (Sakai *et al.*, 2001). It was found that canopy photosynthetic rate was not higher in elevated $[\text{CO}_2]$ (667 ppmV) than

that in control $[\text{CO}_2]$ (353 ppmV) after heading, but that grain yield was increased by 22 % due to the CO_2 enrichment. It was also found that loss of leaf area was stimulated by elevated $[\text{CO}_2]$ from the heading stage onwards.

In this study, non-structural carbohydrates (NSC) content was higher under elevated $[\text{CO}_2]$, although no difference was found between the CO_2 levels in the depletion of stored NSCs during grain-filling (Kuroda unpublished data). Sasaki *et al.* (2004) also reported that current and stored photosynthates are transported to filling grains more rapidly under elevated $[\text{CO}_2]$ with a ^{13}C feeding study in a sun-lit chamber experiment.

The hypothesis outlined above is to be tested against observations from the second Rice FACE in 2003 and 2004, and the data from the Rice-Wheat FACE in China.

4. Comparison of the rice yield increase across different studies

The above results from the Rice FACE experiments can be compared with those of earlier studies carried out in chambers with rice under moderate temperature regimes of 24–26°C (Baker and Allen, 1993; Kim, 1996; Ziska *et al.*, 1997) and also with the recent results of the Rice-Wheat FACE in China (Huang *et al.*, 2004). For the sake of comparability across the different studies, we converted the grain yield change in each study to that caused by a 200 ppmV increase in $[\text{CO}_2]$ by assuming a linear yield response to elevated $[\text{CO}_2]$.

When the converted yields are plotted against the amount of N fertilizer applied in the various experiments (Fig. 5), it can be seen that the yield gain caused by 200 ppmV extra $[\text{CO}_2]$ is larger under higher N application rates although the response tends to saturate above 100 kg N/ha. It is also clear that elevated $[\text{CO}_2]$ resulted in a similar yield increase across experiments for a given N level. When 100–150 kg N/ha was applied, for example, the yield gain due to the CO_2 enrichment averages around 13% for the studies. In reviewing FACE results, Kimball *et al.* (2002) found a similar yield increase of 13 % for wheat as an average of four experiments with FACE.

It is notable that the two Japanese varieties: Akitakomachi and Akihikari, showed similar responses to CO_2 enrichment (Fig. 5). While it appears that the tropical varieties IR30 and IR72 exhibited a higher response to $[\text{CO}_2]$ than the Japanese varieties, the temperate variety from China showed a yield response comparable to the tropical varieties. With the sink-mediated mechanism for the yield increase interaction between N availability and elevated CO_2 , a varietal difference is possible. This is because different varieties could respond differently to the increased N accumulation at PI due to elevated $[\text{CO}_2]$, as suggested in Fig. 3.

Fig. 5. A comparison of rice yield increase in response to a 200 ppmV increase in [CO₂] under various levels of nitrogen fertilizer. Crops were grown under elevated [CO₂] with FACE in Shizukuishi (Kim *et al.*, 2003) and Wuxi (Huang *et al.*, 2004), SPAR chambers in Gainesville (Baker and Allen, 1993), temperature-gradient chambers in Kyoto (Kim, 1996), and open-top chambers in Los Banos (Ziska *et al.*, 1997).

References

- Baker, J.T. and Allen, Jr., L.H., 1993: Contrasting crop species responses to CO₂ and temperature: rice, soybean and citrus. *Vegetatio*, **104/105**, 239-260.
- Horie, T., Ohnishi, M., Angus, J.F., Lewin, L.G., Tsukaguchi, T., and Matano, T., 1997: Physiological characteristics of high-yielding rice inferred from cross-location experiments. *Field Crops Res.*, **52**, 55-57.
- Huang, J., Yang, H., Yang, L., Liu, H., Dong, G., Zhu, J., and Wang, Y., 2004: Effects of free-air CO₂ enrichment (FACE) on yield formation of rice (*Oryza sativa* L.) and its causes. *Scientia Agric. Sinica* (in press).
- Kim, H.-Y., 1996: Effects of elevated CO₂ concentration and high temperature on growth and yield of rice. Ph. D. Thesis. Kyoto University, Kyoto, Japan.
- Kim, H.-Y., Lieffering, M., Kobayashi, K., Okada, M., Mitchell, M., and Gumpertz, M., 2003: Effects of free-air CO₂ enrichment and nitrogen supply on the yield of temperate paddy rice crops. *Field Crops Res.*, **83**, 261-270.
- Kim, H.-Y., Lieffering, M., Miura, S., Kobayashi, K., and Okada, M., 2001: Growth and nitrogen uptake of CO₂-enriched rice under field conditions. *New Phytol.*, **150**, 223-229.
- Kimball, B.A., Kobayashi, K., and Bindi, M., 2002: Responses of agricultural crops to free-air CO₂ enrichment. *Adv. Agron.*, **77**, 293-368.
- Okada, M., Lieffering, M., Nakamura, H., Yoshimoto, M., Kim, H.Y. and Kobayashi, K., 2001: Free-air CO₂ enrichment (FACE) with pure CO₂ injection: system description. *New Phytol.*, **150**, 251-260.
- Okada, M., Shimono, H., Yamakawa, Y., Inoue, M., and Kobayashi, K., 2005: Effects of elevated CO₂ on floral sterility of rice plants caused by low temperature. *J. Agric. Meteorol.*, **60**, 000-000.
- Sakai, H., Yagi, K., Kobayashi, K., and Kawashima, S., 2001: Rice carbon balance under elevated CO₂. *New Phytol.*, **150**, 241-249.
- Sasaki, H., Aoki, N., Sakai, H., Hara, T., Ishimaru, K., and Kobayashi, K., 2004: Effect of CO₂ enrichment on the distribution and translocation of carbon at the early grain-filling stage in rice. *Plant Prod. Sci.* (in press).
- Seneweera, S.P., Conroy, J.P., Ishimaru, K., Ghanoun, O., Okada, M., Lieffering, M., Kim, H.-Y., and Kobayashi, K., 2002: Changes in source-sink relations during development influence photosynthetic acclimation of rice to free air CO₂ enrichment (FACE). *Funct. Plant Biol.*, **29**, 945-953.
- Ziska, L.H., Namuco, O.S., Moya, T., Quilang, J., 1997: Growth and yield responses of field-grown tropical rice to increasing carbon dioxide and air temperature. *Agron. J.*, **89**, 45-53.