FACEing up to Future Uncertainty: Free-air CO_2 Enrichment Experiments in Japanese Rice Paddy Ecosystems

Kentaro Hayashi, Takeshi Tokida and Toshihiro Hasegawa

National Institute for Agro-Environmental Sciences
3-1-3, Kannondai, Tsukuba, Ibaraki, 305-8604, Japan

Abstract

Rice production is essential for humans, but it is now affected by elevated atmospheric carbon dioxide levels (e[CO_2]) and warming climate. Although e[CO_2] has the potential to increase rice production by increasing photosynthesis, this effect depends on other factors such as climate, availability of other nutrients, and perhaps differences between cultivars. In the worst-case scenario, the effect of e[CO_2] on both yield and quality of rice grains might become negative. Meanwhile, rice paddies are an important emitter of atmospheric methane (CH_4), and e[CO_2] also has the potential to stimulate CH_4 emissions by increasing photosynthesis, because photosynthates translocated to the root zone via exudates and root decay are a good source of methanogenesis. The global warming potential of CH_4 is higher than that of CO_2, and the climatic feedback of CH_4 emissions from rice paddies is of future concern. Furthermore, nitrogen (N) availability strongly influences rice production and the carbon (C) cycle, including CH_4 dynamics, and vice versa. N-related processes are also affected by environmental changes. The question is how we can reveal the whole picture of complicated interactions between rice production, C cycle, and N cycle in rice paddies and their responses to environmental changes such as e[CO_2] and climate change. One solution is to perform free-air CO_2 enrichment (FACE) experiments. In this chapter, we summarize the current understanding of responses of rice paddy ecosystems to environmental changes derived from experiments at two Japanese FACE facilities, and outline further studies.

Keywords: climate change, crop production, elevated CO_2 levels, FACE, methane, nitrogen, paddy rice

1. Introduction

1) Rice Paddy Ecosystem and Environmental Change

Rice is a staple cereal crop; its worldwide production (740,902 Gg) is second to that of maize (1,018,112 Gg) and higher than that of wheat (715,909 Gg) (FAOSTAT). In 2013, 90.6% of rice was produced in Asia (FAOSTAT). The production of major cereal crops will need to increase by 70% by 2050 to meet the demand growing mostly as a result of the increasing world population (Bruinsma, 2009).

The atmospheric concentrations of carbon dioxide (CO_2) have increased since 1750 as a result of human activity (IPCC, 2013). In 2011, the CO_2 concentration was 391 ppm and exceeded the pre-industrial level by about 40% (IPCC, 2013). Elevated atmospheric CO_2 levels (e[CO_2]) potentially enhance photosynthesis (so-called CO_2 fertilization effect, Parry et al., 2004), which would increase crop production. However, the fertilization effect observed in laboratory experiments is not necessarily applicable to actual fields under varying environmental conditions, as shown by the difference between growth-chamber and open-field experiments (Hasegawa et al., 2015). Furthermore, ongoing climate change (long-term warming and changes in precipitation and increased frequency and magnitude of the extremes such as hot spells, heat waves, heavy rainfall, drought, tropical cyclones, storm surges, and lack of sunlight, IPCC, 2013) makes sustainable crop production uncertain at present and in the future. The aforementioned increasing demand in crop production must be achieved under the changing environmental conditions, i.e., e[CO_2] and climate change, so any programs to improve crop production must take these changing conditions into account (Hasegawa et al., 2013).

Rice paddies are a source of atmospheric methane (CH_4), which boosts climate change because CH_4 is a more potent greenhouse gas than CO_2. The global warming potential is defined as the time-integrated radiative forcing due to a pulse emission of a given component relative to a pulse emission of an equal mass of CO_2; the 100-year global warming potential of CH_4 is estimated as 34 (Myhre et al., 2013). The mean global CH_4 emission from rice paddies in 2000–2009 has been estimated as 33–40 Tg CH_4 yr^{-1} (9–13% of the anthropogenic CH_4 emission) (Ciais et al., 2013). Elevated [CO_2] enhances photosynthesis, production of photosynthates, and transfer of fresh organic matter from roots to soil by rhizodeposition (root exudates and decayed roots) (Tokida et al., 2010). The current-season photosynthates are labile and are good substrates for methanogens, and the transfer of photosynthates into soil consequently increases CH_4 emissions from rice paddies (Tokida et al., 2010).

Environmental changes also influence the nitrogen (N) cycle in rice paddies, and conversely, changes in N dynamics in rice paddies might affect the environment via such processes as atmospheric emission of nitrous oxide (N_2O), which is another potent greenhouse gas and a stratospheric ozone-depleting substance, emission of ammonia (NH_3), which is involved in atmospheric chemistry and atmospheric deposition, emission of various gases and particles from open-field burning of rice residues (Hayashi et al., 2014a), and N loading to groundwater, rivers, and lakes via leaching or runoff. Changes in the N cycle affect the carbon (C) cycle and might result in a different ecosystem status because of their close relationship via biological activities of plants and microbes.

Much is still unknown about how e[CO_2] and climate change affect rice production and C and N cycles in paddy ecosystems, and about the feedback from the perturbed C and N cycles in paddy ecosystems to environmental changes. This is a source of a large uncertainty in future predictions. This uncertainty must be reduced to enhance the reliability of current assessments and future predictions and to develop effective countermeasures to mitigate the effects of climate change and adapt rice production accordingly. Thus, substantial efforts are necessary to elucidate the mechanisms of the responses of paddy ecosystems to environmental changes under the actual environment.

2) Free-Air CO_2 Enrichment Experiments

Previously, greenhouses and artificially controlled environment chambers were used to investigate the effects of e[CO_2] on plant growth and production. However, research on plant responses in a community or at vegetation scale under actual field conditions is necessary to elucidate how crop production and C and nutrient cycles are affected by changing environmental conditions in the real world. The first free-air CO_2 enrichment (FACE) experiment, i.e., cultivation of cotton in an open field while increasing atmospheric CO_2 levels without the use of an enclosure, was conducted in Arizona, U.S.A., in 1989.

Since then, FACE experiments targeting various types of ecosystems, including cropland, forest, and grassland, have been conducted in many parts of the world. Early FACE experiments in crop production were mainly aimed at verifying the CO_2 fertilization effect in an open-field system and quantification of the effect of e[CO_2] on yield. Meta-analysis of early studies showed that e[CO_2] in open-field systems increased the yields of major C3 crops (rice, wheat, and soybean) by 10–20% (Kimball et al., 2002; Long et al., 2004; Hasegawa et al., 2005; Morgan et al., 2005). The increase in yield was ascribed to the enhancement of photosynthesis by e[CO_2]. Notably, the increases were much higher in some C3 crops, e.g., 28% in potato (Kimball et al., 2002) and 89% in cassava (Rosenthal et al., 2012). By contrast, in C4 crops such as maize and sorghum, e[CO_2] photosynthesis increased to a lesser extent and resulted in only small increases in yield (Kimball et al., 2002; Leakey et al., 2006). The mechanisms underlying the differences in responses of different crops to e[CO_2] are still unknown. An important aspect is the relative deficiency of macro- and micro-nutrients that are required to match the increased C fixation because of the enhancement of photosynthesis by e[CO_2]. A meta-analysis (Myers et al., 2014) showed that C3 grain crops and legumes have lower concentrations of zinc and iron under FACE conditions than under ambient [CO_2]. In C3 crops other than legumes, e[CO_2] also reduces the total protein content, whereas C4 crops seem to be less affected.

The main target of crop research using FACE facilities has been shifting from studying the effects of e[CO_2] alone on photosynthesis, biomass, and crop yield to studying the combined effects of e[CO_2] and other conditions such as temperature, soil moisture, and N availability, and also to the characterization of crop varieties with respect to their adaptation to climate change. Recently, the quality of harvested crops has become a target of research; understanding the C and N cycles and their interactions in croplands (agricultural ecosystems) is also an important aim of research because croplands are interconnected with other ecosystems in the biosphere.

3) Two Rice FACE Facilities in Japan: from Shizukuishi to Tsukuba

The first rice FACE experiment in the world commenced in 1998 in Shizukuishi, Iwate Prefecture, in northern Japan (39º38'N, 140º57'E, 200 m a.s.l.) (Kobayashi et al., 2006). The Shizukuishi facility, located in a cool-temperate climate (slightly cold for rice production), helped to reveal the effects of e[CO_2] on rice production in cool regions. Each of the FACE plots was formed with an octagonal ring. One ring was 12 m in diameter and was composed of eight horizontal emission tubes, each of which was independently connected to a pure CO_2 source. The target level of e[CO_2] was 200 µmol mol^{-1} above the ambient level. To achieve the target level, an automatic system was developed that controlled the release of pure CO_2 from each emission tube on the basis of the real-time data on wind direction and velocity, and the CO_2 level at the center of each ring (Hasegawa et al., 2007). At the Shizukuishi facility, the main focus was to study the effects of e[CO_2] on rice photosynthesis, transpiration, phenology, biomass, and grain yield. Warming treatment, i.e., heating floodwater by 2 ºC above the ambient temperature under the continuously submerged condition, was used in the experiments aimed at elucidation of the combined effects of e[CO_2] and warming on rice plants. In addition, research on rice paddies as an important emitter of atmospheric CH_4 was also conducted.

The Shizukuishi facility was closed after the last experiment in 2008, and the experience gained was used at the Tsukuba facility, which was established in late 2009 in Tsukubamirai, Ibaraki Prefecture, in central Japan (35º58'N, 140º00'E, 10 m a.s.l.) and started operation in 2010. In addition to the original aims of rice FACE research (studying aspects of the relationship between rice plants and the C cycle; Fig. 1), the scope of research at the Tsukuba facility was expanded to include the N cycle (Fig. 2) and microbial ecology (see Section 5). In the Tsukuba facility, four rectangular bays at farmers' field are used for experiments. The longer side of each bay is 100 m, and the shorter sides range from 30 to 70 m. A FACE plot is set in each bay, accompanied by an ambient plot. Water management and soil properties in both the FACE and ambient plots are the same within each bay to enable pairwise comparisons (Nakamura et al., 2012). The shape and the basic control technique of FACE rings at the Tsukuba facility are similar to those at the Shizukuishi facility, except that the ring diameter is increased to 17 m to double the area. Since 2011, each FACE plot at Tsukuba has been equipped with two-layered octagonal rings for pure CO_2 release. The CO_2 levels at the four ambient plots are monitored every 5 min, and the lowest level is defined as the reference ambient CO_2 level. The target CO_2

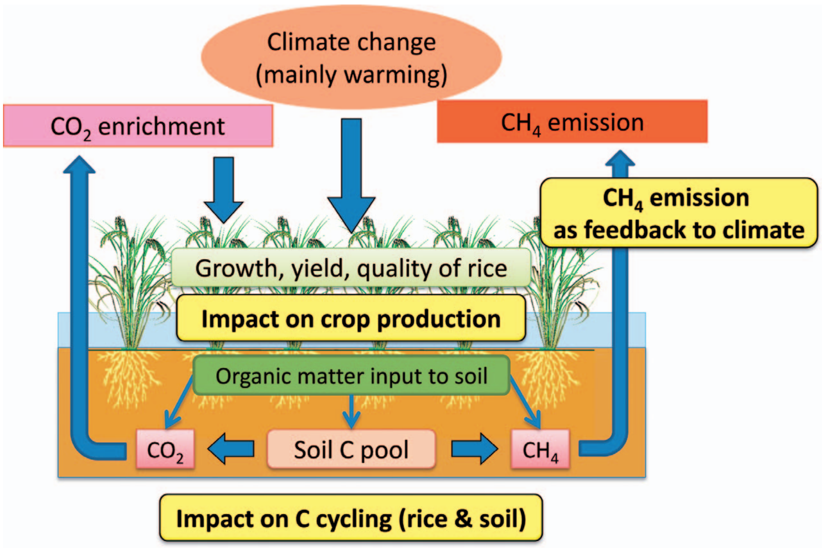

Fig. 1 The research focus of our free-air CO_2 enrichment (FACE) experiments with respect to rice production and the carbon cycle in a rice paddy ecosystem

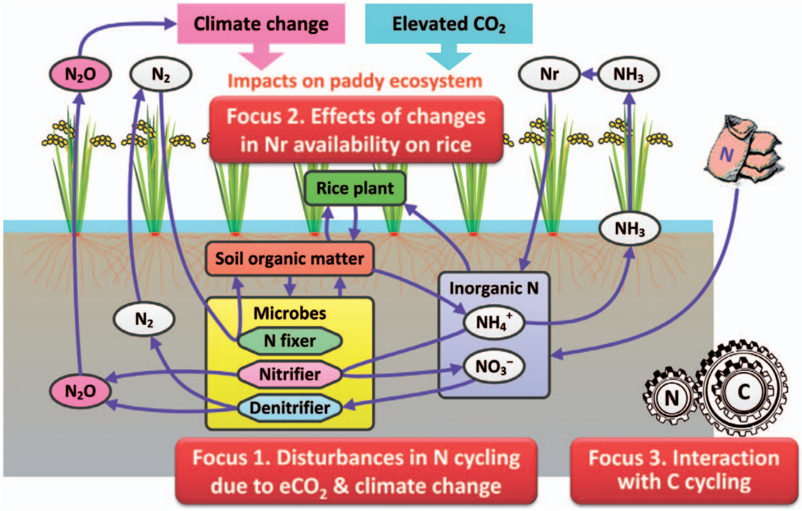

Fig. 2 The research focus of our FACE experiments with respect to the nitrogen cycle in a rice paddy ecosystem

level at the FACE plot is the same as at Shizukuishi, 200 μmol mol^{-1} above the ambient level. The FACE equipment automatically regulates CO_2 release to achieve the average target CO_2 levels (Nakamura et al., 2012). CO_2 enrichment is conducted between sunrise and sunset. The upper ring is fixed at 60 cm above the canopy height of rice plants, whereas the lower one is slightly above the canopy. The CO_2 release from the upper and lower rings alternates in response to wind conditions (the upper ring is used under calm conditions and the lower ring under windy conditions) to ensure that the target CO_2 concentration is achieved. The larger rings at the Tsukuba facility enable the area of subplots for temperature and N availability and the number of rice plants and varieties cultivated to be increased, which encourages participation of various research topics. Basic layout within a ring slightly differs among research years; Figure 3 shows

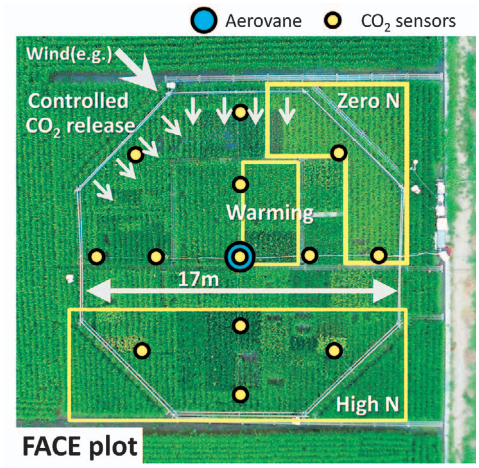

Fig. 3 Layout of a CO_2 enrichment plot at the Tsukuba facility in 2012

the layout used in 2012 as an example.

The purposes of this chapter are to summarize the results of Japanese rice paddy FACE experiments with emphasis on rice production, C cycle (particularly CH_4 emission), and N cycle; and to discuss future challenges for the mechanistic understanding of responses of rice production and rice paddy ecosystems to environmental changes, which is necessary for reducing the uncertainty of future predictions.

2. FACE Experiments to Study Rice Production

In this section, we summarize the results obtained at the two rice FACE facilities concerning the photosynthetic responses to e[CO_2], difference in photosynthetic and yield responses among rice varieties, relationship between yield response to e[CO_2] and seasonal mean air temperature, relationship between e[CO_2] responses and grain N contents, and effects of e[CO_2] on rice quality.

The effect of e[CO_2] on the photosynthesis rate of the rice cultivar Akitakomachi grown at the Shizukuishi facility was consistently positive over three growing seasons, whereas concurrent warming by 2 ºC reduced the photosynthesis rate at the grain-filling stage. As a consequence, at this stage, the photosynthesis rates in warmed plots exposed to e[CO_2] were only 4% higher than those of the control plots without CO_2 or warming. At the grain-filling stage, rice plants grown at e[CO_2] in warmed plots showed photosynthesis rates reduced by 23% in comparison with rice plants grown in the control plots (Fig. 4a). This decrease was attributable to the decreases in the

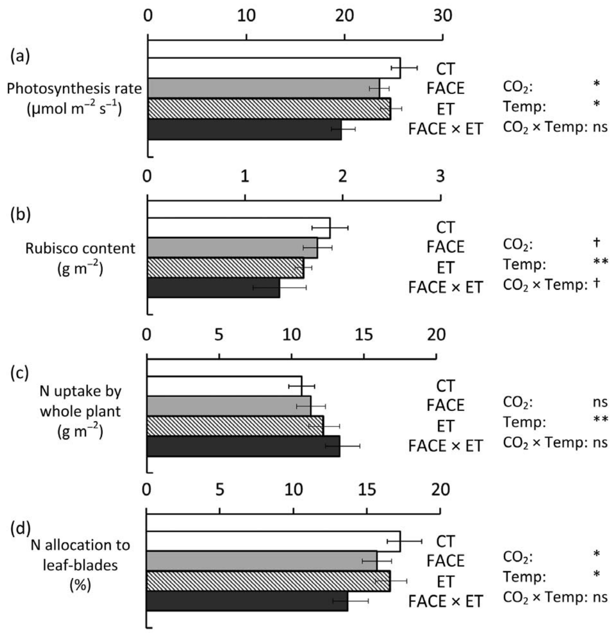

Fig. 4 (a) Leaf photosynthesis rate, (b) Rubisco content, (C) whole-plant N uptake, and (d) N allocation ratio to leaf blades at the mid-grain-filling stage of rice cv. Akitakomachi cultivated at the Shizukuishi facility. **, $p < 0.01$; *, $p < 0.05$; and †, $p < 0.1$

contents of leaf N (data not shown) and Rubisco (Fig. 4b) by the combination of e[CO_2] and warming. Warming alone increased the total N uptake of rice plants (Fig. 4c); the allocation of N to leaves at the grain-filling stage was decreased by the combination of e[CO_2] and warming (Fig. 4d). In line with these observations, the combination of e[CO_2] and warming lowers the leaf N content at late growth stages and therefore reduces the photosynthetic ability (Adachi et al., 2014).

Leaf photosynthesis and related properties of a high-yielding indica cultivar, Takanari, and a standard japonica cultivar, Koshihikari, were investigated at several growth stages at the Tsukuba facility. In both cultivars, e[CO_2] increased the photosynthesis rates (Fig. 5); however, the responses of Takanari to e[CO_2] were significantly higher than those of Koshihikari at every growth stage. Stomatal conductance and the contents of leaf N and Rubisco were higher in Takanari than in Koshihikari (Fig. 5). These results suggest that Takanari takes up CO_2 at a higher rate owing to its high stomatal conductance and its leaves are relatively rich in N; these characteristics ensure a more active Calvin cycle and result in higher photosynthesis rates under e[CO_2]. These characteristics, which Koshihikari does not have, are perhaps a major factor of high yield in Takanari under e[CO_2] conditions (Chen et al., 20014).

The following data were obtained using Akitakomachi, a common cultivar. Nine-year data (7 cropping seasons at Shizukuishi and 2 at Tsukuba) showed that e[CO_2] increased the average rice

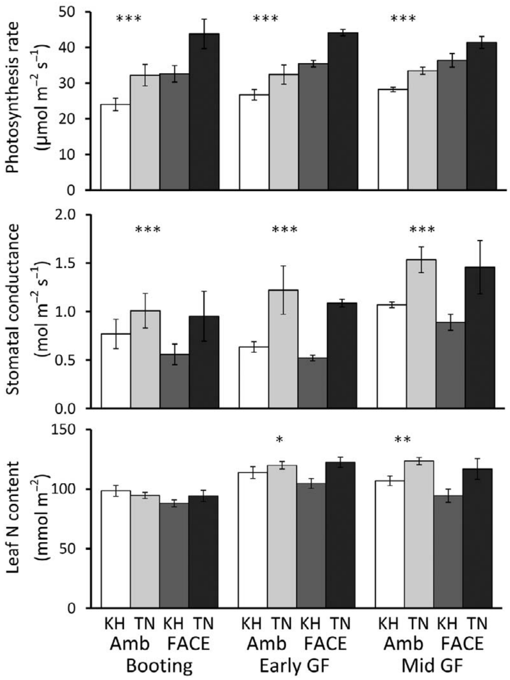

Fig. 5 Effect of elevated CO_2 levels on the photosynthesis rate, stomatal conductance, and leaf N content of two rice cultivars, Koshihikari (KH) and Takanari (TN), cultivated at the Tsukuba facility. Amb, ambient CO_2 levels; FACE, elevated CO_2 levels (ambient + 200 μmol mol^{-1}); ***, $p < 0.001$; **, $p < 0.01$; and *, $p < 0.05$

yield by 13%. However, this effect of e[CO_2] decreased with increasing seasonal mean air temperature, except for one year with a cold summer (Fig. 6). This result implies that the increasing effect of e[CO_2] on rice yield would not be as high as expected in a warmer climate. Furthermore, the data for cultivars with different properties cultivated at Shizukuishi in 2007 and 2008 and at Tukuba in 2010 revealed that the increasing effect of e[CO_2] on rice yield also differed among cultivars (Fig. 7). The e[CO_2]-induced yield increase ranged widely (from 3% to 36%) among 8 varieties cultivated at the Tsukuba facility. Therefore, one can expect that the

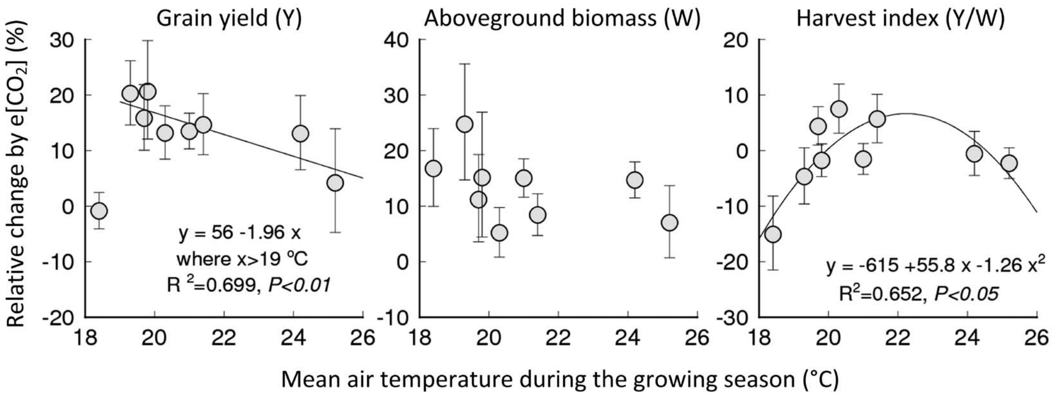

Fig. 6 The effects of elevated CO_2 levels (e[CO_2]) and the mean air temperature during the growing season on grain yield, aboveground biomass, and harvest index of rice cv. Akitakomachi cultivated at the Shizukuishi (7 crop years) and Tsukuba (2 crop years) facilities

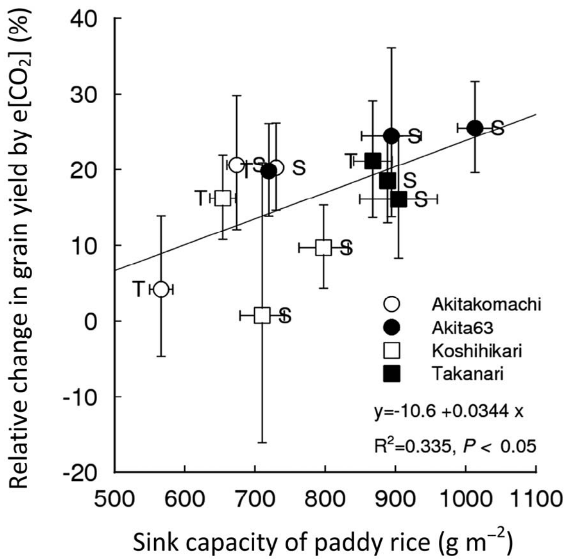

Fig. 7 Relationship between the response of grain yield to elevated CO_2 levels and the sink capacity of 4 rice cultivars. Data were obtained at the Shizukuishi facility (S) in 2007 and 2008 and at the Tsukuba facility (T) in 2010. The sink capacity is defined as the product of the grain number per unit area by the weight of one grain at its maturity

increasing effect of e[CO_2] on rice yield can be enhanced through rice breeding. Overall, these experiments showed that the increasing effect of e[CO_2] on rice yield is strongly influenced by temperature and rice cultivars (Hasegawa et al., 2013).

Enhanced photosynthesis owing to e[CO_2] may result in a relative shortage of other nutrients, typically N, not only in leaves as mentioned above, but also in grains. Rice grains positioned differently in panicles differ greatly in weight and quality. An experiment using the cultivar Koshihirari was conducted at the Tsukuba facility to elucidate the effects of e[CO_2] on grain mass and grain C and N accumulation in spikelets attached to the upper primary rachis branch (superior spikelets; SS) and those attached to the lower secondary rachis (inferior spikelets; IS). Although e[CO_2] increased the yield by 13%, the N concentration in the panicle decreased by 7% on average in two N treatments (no N fertilization and fertilization at a rate of 8 g N m^{-2}) ($P < 0.01$). The difference between the responses of SS and IS to e[CO_2] was particularly noticeable when N fertilizer was applied. For SS, e[CO_2] decreased grain N by 24% ($P < 0.01$) but did not affect grain mass. For IS, e[CO_2] increased grain mass by 13% ($P < 0.05$) without changes in grain N. The reduction in grain N content due to e[CO_2] started at the beginning of grain filling. These results suggest that e[CO_2] stimulates the growth of IS grains, most of which are not marketable due to their small size, at the expense of grain N reduction in SS. Translocation of N from SS to IS may be a mechanism for the reduction in SS grain N (Zhang et al., 2013). In addition to the decrease in grain N content in SS, e[CO_2] also increases grain chalkiness, thus degrading the quality of rice. Changes in SS and IS grain growth were assessed at the Tsukuba facility for three rice cultivars differing in grain size and number, Akita 63, Koshihikari, and Takanari (Zhang et al., 2015). Elevated [CO_2] increased grain yield by 15% across 2 years in all three cultivars. The grain growth responses depended on spikelet position: grain mass increased only in IS, whereas grain N content decreased in SS but slightly increased in IS. The increase in IS grain mass was greatest in Takanari, followed by Koshihikari, whereas Akita 63 showed a slight decrease. Grain chalkiness was increased significantly by e[CO_2], with the greatest damage in Koshihikari, followed by Akita 63 and Takanari (Zhang et al., 2015).

Elevated [CO_2] lowers stomatal conductance, which generally increases plant body temperature because of the attendant decrease in transpiration. This might reduce rice quality. An experiment at the Tsukuba facility showed that the proportion of undamaged grains in five standard cultivars (Akitakomachi, Kinuhikari, Koshihikari, Matsuribare, and Nipponbare) was lower in FACE plots (51.7%) than in ambient plots (61.7%). The damage was lower for the heat-tolerant cultivars Eminokizuna, Wa2398, Kanto 257, Toyama 80, Mineharuka, Kanto 259, and Saikai 290 (71.3% of grains were undamaged in the FACE plots and 73.5% in the ambient plots) (Usui et al., 2014). Therefore, our concern is that future warming will reduce rice quality. A 3-year experiment at the Tsukuba facility revealed that e[CO_2] and floodwater warming (by 2 ºC) combined significantly increased biomass and yield of Koshihikari (by 14% on average), mainly owing to increased panicle and spikelet density (Usui et al., 2015). Floodwater warming alone significantly increased biomass but had no effect on yield. Both treatments resulted in a loss of grain appearance quality; e[CO_2] exerted a larger effect than warming did. Moreover, e[CO_2] and warming synergistically reduced grain appearance quality (Usui et al., 2015).

3. FACE Experiments to Study the Rice Paddy Carbon Cycle

Elevated [CO_2] stimulates photosynthesis of rice plants and also increases rhizodeposition of labile organic matter as good substrates for methanogens. Consequently, e[CO_2] boosts CH_4 production in paddy soils and eventually increases emissions of CH_4 to the atmosphere (Inubushi et al., 2003; Liu et al., 2012). The radiative forcing of CH_4 in the Earth's atmosphere, including its indirect effects (e.g., greenhouse effect after oxidation into CO_2), accounts for more than half of that of CO_2 (Shindell et al., 2009). Rice paddies are an important anthropogenic emitter of atmospheric CH_4 (Ciais et al., 2013), and increases in CH_4 emission from rice paddies might considerably influence the global climate system. Thus, the effect of e[CO_2] on CH_4 emissions from rice paddies has been an important research topic of FACE facilities.

A 2-year study at the Shizukuishi facility in 2007 and 2008 showed that a combination of e[CO_2] (200 μmol mol^{-1}) and warming (2℃) stimulated CH_4 emission from rice paddies by approximately 80% in comparison with emission under ambient conditions (Fig. 8). This result revealed a positive climate feedback, namely that climate change would stimulate CH_4 emissions from rice paddies, which would then accelerate climate change (Inubushi et al., 2003; Tokida et al., 2010) . The effect of e[CO_2] by itself on increases in CH_4 emissions was ascribed to the enhanced input of organic matter to the root zone caused by the stimulation of photosynthesis by e[CO_2]. Meanwhile, warming treatment alone increased CH_4 emissions from rice paddies by 40% (Fig. 8). Possible mechanisms of this increase are as follows: i) in the early growth stages, warming stimulated decomposition of soil organic matter without affecting the oxidation capacity of paddy soil (Fig. 9) and ii) in the late growth stages, warming accelerated senescence and decay of rice roots, providing additional substrates for CH_4 production (Tokida et al., 2010).

The next question is how much the current-season photosynthates contribute to CH_4 production in paddy soil and CH_4 emission to the atmosphere. An experiment at the Shizukuishi facility showed that the fraction of C as CH_4 (CH_4-C) emitted to the atmosphere originating from the current-season photosynthates accounted for 30% of total CH_4-C emission at the panicle

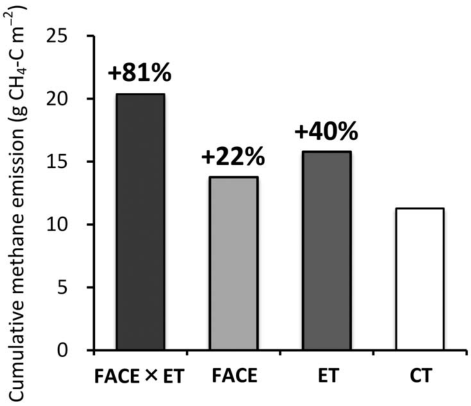

Fig. 8 Increase in CH_4 emissions from rice paddies due to CO_2 levels elevated by 200 μmol mol^{-1} (FACE) and warming by 2 ℃ (ET) in comparison with control (CT)

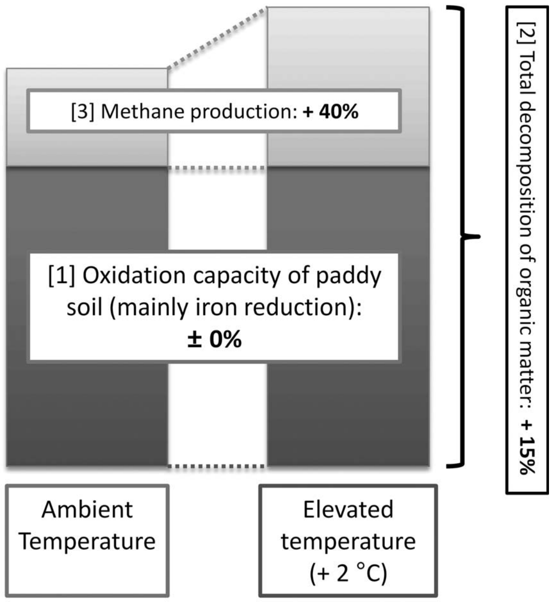

Fig. 9 Mechanism of the large increase in CH_4 emissions by warming

initiation stage and more than 40% after the heading stage (Tokida et al., 2011). These results demonstrate that the current-season photosynthates transferred to the root zone are important substrates for CH_4 production. The increase in CH_4 emissions caused by warming (2 ºC) was accompanied by an increase in the fraction of CH_4-C originated from the current-season photosynthates (Fig. 10). This result shows that the current-season photosynthates are susceptible to enhanced decomposition at warmer temperatures. In addition, a decrease in root biomass after the heading stage supports the hypothesis that warming stimulates root senescence and decay (Fig. 11) (Tokida et al., 2011).

Transport pathways of CH_4 from paddy soil to the atmosphere are also an important research topic. Diffusive CH_4 emission from both the floodwater surface and rice plants is generally considered to be the primary pathway of CH_4 transfer to the atmosphere; however, because of oxidative conditions at the paddy soil surface, 80–100% of CH_4 diffusing through the soil is oxidized before reaching the atmosphere (Hayashi et al., 2015). A large part of CH_4 in paddy soil is partitioned into the soil gas phase owing to its very low water solubility, which results in the formation of bubbles containing CH_4. It has been estimated that the gas-phase CH_4 in paddy soil accounts for 26–45 % of the total CH_4 content at the panicle initiation stage and 60–68% at the heading and grain-filling stages (Tokida et al., 2013). Although stochastic ebullition of bubbles to the atmosphere possibly makes a large contribution to the total CH_4 emission, our knowledge about this process is limited. The bubble-borne CH_4 pool is closely related to the putative rice-mediated CH_4 emissions measured at each stage across e[CO_2] and warming treatments. However, much variation between different growth stages remains unexplained, presumably because the CH_4 transport capacity of rice plants also affects the emission rate. Gas-phase CH_4 in

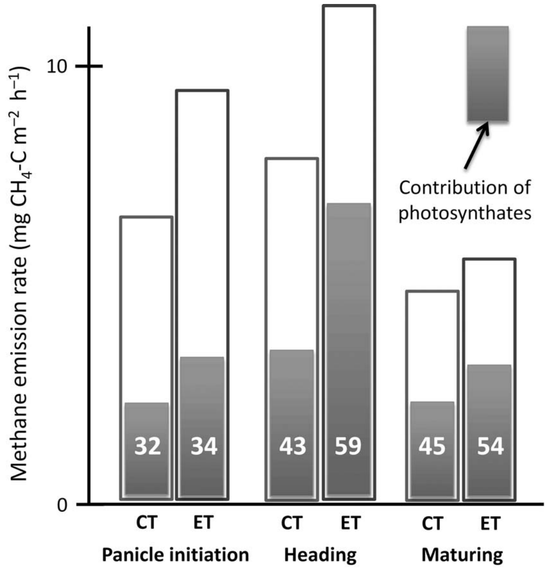

Fig. 10 Effect of warming by 2 ºC on CH_4 emission rates at three growth stages of rice cv. Akitakomachi cultivated at the Shizukuishi facility, and the ratios of C originated from the current-season photosynthates accounting for the total C emitted as CH_4. CT, control; ET, warming by 2 ºC

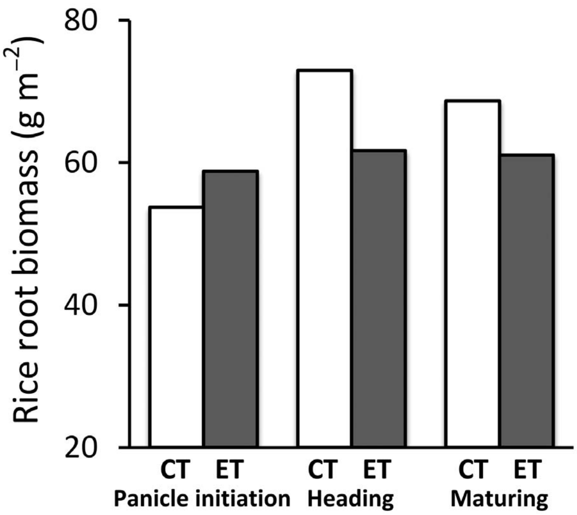

Fig. 11 Effect of warming on root biomass of rice cv. Akitakomachi cultivated at the Shizukuishi facility at three growth stages. CT, control; ET, warming by 2 ºC

soil needs to be considered for accurate quantification of the soil CH_4 pool. Not only ebullition but also plant-mediated emission depends on the gaseous CH_4 pool and the transport capacity of the rice plants (Tokida et al., 2013).

Quantification of C flows in a soil–rice plant system was once technically difficult to achieve. Recently, the use of a stable isotope of C (^{13}C) has enabled quantitative tracing of C dynamics. The CO_2 used for FACE experiments originates from fossil fuels, which have a low $^{13}C/^{12}C$ ratio compared to that in the atmosphere. This difference can be used as isotope labeling experiments. The aforementioned findings, e.g., that about half of the emitted CH_4 originated from the current-season photosynthates, were obtained by using this technique. As a spin-off of studies, a fully automated high-throughput system for the measurement of $\delta^{13}C$-CH_4 values was developed (Tokida et al., 2014). Our studies have demonstrated the high potential of this system for obtaining $\delta^{13}C$ data useful for process-level understanding of CH_4 biogeochemistry with respect to spatiotemporal variations in CH_4 sources and how these variations are affected by environmental and management factors (Tokida et al., 2014).

It is obvious that reducing atmospheric CH_4 emissions is a mitigation option that will be much more effective than reducing CO_2 emissions, because of the larger radiative forcing but shorter atmospheric lifetime of CH_4 compared to those of CO_2 (Shindell et al., 2012). Although CH_4 emission from rice paddies was already an important research topic at the Shizukuishi facility, much more attention has been paid to this topic at the Tsukuba facility, where a series of studies aimed at revealing the mechanisms of the responses of CH_4 emissions to combined effects of e[CO_2], warming, and rice cultivars has been conducted since 2010. These studies have shown that i) the increasing effect of e[CO_2] on CH_4 emission from rice paddies decreases with rice growth, similar to the downregulation of photosynthesis responses to e[CO_2], and ii) CH_4 emission varies greatly among rice cultivars, among which Takanari, which produces high yield under e[CO_2], is also favorable in terms of its lower CH_4 emission than that of other cultivars. Determination of the mechanisms underlying these differences in CH_4 emission is a subject of future studies.

4. FACE Experiments to Study the Rice Paddy Nitrogen Cycle

Nitrogen is an indispensable macronutrient, in particular for synthesis of proteins such as Rubisco, an essential photosynthetic enzyme. Although e[CO_2] enhances photosynthesis, rice growth does not respond to e[CO_2] sufficiently without N fertilization (Zhang et al., 2013). Early FACE studies paid little attention to the effects of e[CO_2] on the N cycle in rice paddies. However, C-related processes are influenced by N-related processes and vice versa, mostly via biological processes driven by rice plants and various types of microbes in rice paddy ecosystems. It is therefore highly possible that changes in the C cycle caused by e[CO_2] and climate change affect the N cycle, and changes in the N cycle provide feedback to the C cycle.

Many processes are involved in N cycling in rice paddies (Fig. 12), including i) N input: fertilization (chemical fertilizers or manure), application of crop residues from elsewhere, irrigation, biological N fixation (BNF), and atmospheric deposition (wet and dry N deposition); ii) N output: harvesting (grains and residues taken away), overflow, leaching, gas emission accompanied with denitrification, NH_3 volatilization, and open-field burning (emissions of N-containing gases and particles); and iii) internal processes in rice paddies: accumulation and

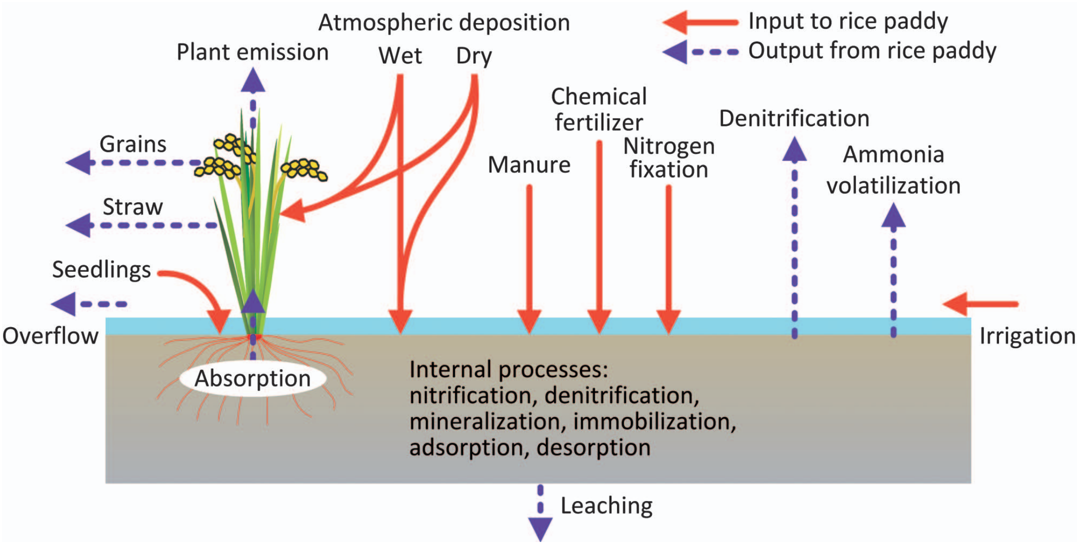

Fig. 12 Schematic view of the N-related processes in rice paddy ecosystems with respect to the N budget

decomposition of soil organic matter (accompanied by N immobilization and mineralization), nitrification, denitrification, and adsorption and desorption of inorganic N, particularly ammonium. In the future, natural N-related processes will be influenced by e[CO_2] and climate change. In this regard, anthropogenic practices, such as N fertilization to promote rice production and residue management, are potential options to control the C and N dynamics (e.g., emissions of CH_4 and N_2O) in rice paddy ecosystems under e[CO_2] and climate change.

A 3-year project focusing on N aspects more than on N as fertilizer (FACE-N) was conducted during 2010–2012 at the Tsukuba facility. This project included 3 topics with 19 subtopics. The 3 topics were exchanges of reactive N (N compounds other than inert molecular N [N_2]) between rice paddies and the atmosphere, effects of e[CO_2] and warming on N processes in a rice paddy ecosystem, and modeling of the N cycle in a rice paddy ecosystem. A subsequent 3-year project during 2014–2016 (FACE-C×N) is underway at the Tsukuba facility; its main objectives are to quantify the relationships between N availability and CH_4 dynamics (production, oxidation, and emission) and to elucidate the mechanisms behind these relationships. Below we summarize our current understanding of the N processes such as BNF, N deposition, and NH_3 volatilization, and hot topics to be addressed in the near future.

Among the natural N-related processes, BNF is responsible for the largest N input to rice paddies, owing to the activity of various types of microbes that possess an N fixing enzyme (nitrogenase), collectively called N fixers. The theoretical maximum total BNF level in rice paddies is estimated to be 170 kg N ha^{-1}, which is the sum of theoretical maximum N fixation by free-living phototrophic (70 kg N ha^{-1}), free-living heterotrophic (60 kg N ha^{-1}), and rhizosphere-associated (40 kg N ha^{-1}) microorganisms (Roger and Ladha, 1992). This potential rate is higher than the current application rate of N fertilizers for single rice cropping in Japan, which is typically 60–80 kg N ha^{-1}. Direct measurement of BNF is difficult because of the difficulty in measuring the downward N_2 flux in the atmosphere containing 78% N_2. Labeling with a stable N isotope (^{15}N) is an excellent approach to measure the N_2 flux directly; however, this approach is practically very difficult to apply in open-field studies such as FACE experiments. Two proxies are used instead, acetylene reduction activity (ARA) and the change in total N content. The former estimates the gross BNF rate by measuring the ethylene production rate using the property of nitrogenase to reduce acetylene to ethylene. The latter approach gives the net BNF rate during relatively long time intervals (a week to a month) by measuring changes in the total N contents of soils.

It is known that e[CO_2] increases BNF rates measured as ARA (Hoque et al., 2001). However, this knowledge was obtained from paddy soils that were destructively sampled, and the in-situ BNF response to e[CO_2] and warming and its mechanisms have not been examined yet. ARA was measured in the laboratory for intact soil cores sampled at the Tsukuba facility in the cropping seasons of 2011 and 2012. Although e[CO_2] significantly increased ARA 96 days after transplanting, this effect was reduced by warming (the increase was 15% in the control plots and 8% in the warmed plots); in this experiment, N fertilizer was applied at 8 g N m^{-2}. Combined effects of e[CO_2] and N fertilization were also investigated for the following treatments: N0, no N fertilizer applied; N8, N fertilizer applied at 8 g N m^{-2}; and N12, N fertilizer applied at 12 g N m^{-2}. The increase in ARA caused by e[CO_2] was 15% (N0), 9% (N8), and 14% (N12) in 2011 and 2% (N0), 25% (N8), and 61% (N12) in 2012. Investigation in 2012 showed that soil cores that did not include rice roots had low ARA values throughout the cropping season, with no e[CO_2] responses. The ARA values of soil cores that included rice roots showed no significant correlations with either root weight per unit volume or the soil labile C content, contrary to a hypothesis that e[CO_2] enhances activity of heterotrophic N fixers by increasing rhizodeposition.

The net rates of N fixation by free-living microorganisms at the paddy soil surface (at a depth of 0–1 cm) under submerged conditions were measured at the Tsukuba facility in 2012 (Hayashi et al., 2014b). Open-incubation of soil was conducted in subplots without N fertilizer from 28 May to 18 August (82 days), after which rice plants were transplanted in the same subplot so that soil for incubation was isolated from rice roots using open bottles. The changes in total N in the surface soil compared with the initial total N content. The cumulative net N fixation during the 82-day study period was 47.1 ± 3.7 (mean ± standard error) kg N ha^{-1} in the FACE plots and 43.3 ± 5.8 kg N ha^{-1} in the ambient plots. The difference between the FACE and ambient plots was not significant ($p = 0.05$) (Hayashi et al., 2014b). We expect that e[CO_2] will further affect associative N fixation by heterotrophs by increasing rhizodeposition.

Human activities result in reactive N emission to the atmosphere. The emitted reactive N is eventually deposited on the Earth's surface through atmospheric transport and a variety of chemical reactions in the atmosphere. Atmospheric deposition of reactive N (referred to as N deposition) includes wet deposition with rain and snow and dry deposition of gaseous and particulate reactive N directly to the ground surface. Thus, increases in human-induced emissions of reactive N to the atmosphere result in increased N deposition. The maximum N deposition in Japan is estimated to be about 20 kg N ha^{-1} yr^{-1} (Hayashi and Yan, 2010), excluding extreme cases such as areas adjacent to NH_3 emitters such as livestock facilities. Anthropogenic increases in N deposition may result in N overload in natural ecosystems; in particular, eutrophication of oligotrophic ecosystems and the attendant loss of their biodiversity are of concern. The effects of changes in N deposition on the nutritional status of rice paddy ecosystems seem to be small because of large anthropogenic N input such as fertilization. Even so, quantification of N deposition at rice paddies is useful to evaluate the N budget and to obtain necessary information to be used as the input data for numerical models of the N cycle and benchmark for future predictions. Two-year monitoring of air concentrations and fluxes of reactive N (gases: NH_3, nitric acid, and nitrous acid; particles: particulate ammonium and particulate nitrate) on a weekly mean basis was conducted at the Tsukuba facility (Hayashi et al., 2013b). Special attention is needed to monitor dry deposition of reactive N, because chemically diverse gases and particles with different physicochemical properties relevant for deposition are involved, as well as gas-to-particle conversion or gas particle interconversion. In this regard, ammoniacal N (NH_3 and particulate ammonium) is of particular importance; NH_3 is occasionally emitted from rice paddies to the atmosphere, and conversion between NH_3 and particulate ammonium causes errors in flux measurements (Hayashi et al., 2012; Hayashi et al., 2013a). We should quantify both dry deposition and emission to evaluate the N budget by the atmosphere–rice paddy exchange. The annual net N deposition at the rice paddy was 13.0 (first year from middle September 2010) and 11.7 (second year from middle September 2011) kg N ha^{-1} yr^{-1}. The largest component was wet deposition of ammonium (5.3 and 4.7 kg N ha^{-1} yr^{-1}, respectively) followed by wet deposition of nitrate (4.5 and 3.4 kg N ha^{-1} yr^{-1}). As to dry deposition, the largest component was NH_3 deposition (3.1 and 3.8 kg N ha^{-1} yr^{-1}) but it was counterbalanced by NH_3 emissions (4.3 and 2.0 kg N ha^{-1} yr^{-1}); in total, net emission of 1.2 kg N ha^{-1} yr^{-1} in the first year and net deposition of 1.8 kg N ha^{-1} yr^{-1} in the second year.

Nitrate in submerged paddy soils is easily lost via denitrification activated under anaerobic conditions, and paddy rice is an ammonia-philic plant. Therefore, N fertilizers applied to rice paddies contain ammoniacal N including urea, hydrolysis of which releases NH_3. However, application of ammoniacal N might induce NH_3 volatilization loss. Incorporation of applied N fertilizer into the plowed layer efficiently inhibits NH_3 volatilization, whereas surface application of N fertilizers, urea in particular, induces NH_3 volatilization loss, which is occasionally significant (Hayashi et al., 2006; Hayashi et al., 2008b). Not only CO_2 and water vapor but also NH_3 can diffuse through stomata. Although ammoniacal N that plants uptake is a source of N as a macronutrient, NH_3 emission from plants to the atmosphere takes place when soil is sufficiently rich in N and the atmospheric NH_3 level is lower than the NH_3 level in stomatal cavity (compensation point for NH_3); some data support emissions of NH_3 by rice plants (Hayashi, et al., 2008a; Hayashi et al., 2011). By contrast, a laboratory experiment has shown that the NH_3 compensation point of rice plants (cv. Koshihikari) is considerably lower than that of other crops; rice plants can hardly actively emit NH_3 at ambient NH_3 levels (Miyazawa et al., 2014). Determination of the sign and magnitude of the atmosphere–rice plant NH_3 exchange and elucidation of possible effects of e[CO_2] and climate change on this exchange will be a subject of future studies.

There are other hot topics to be addressed. One concerns the complicated processes of nitrification and denitrification with special emphasis on N_2O production, consumption, and atmospheric emissions (Yano et al., 2014; Hayashi et al., 2015). These processes are entirely due to the activities of microbes interacting with rice plants and paddy soils. The other topic is N mineralization, which provides available N to rice plants and microbes in the form of ammonium. N mineralization accompanies organic matter decomposition in soil, which will be accelerated by warming. Acceleration of N mineralization seems beneficial for rice production in the short term, but would result in a long-term degradation of soil fertility.

5. Future Challenge for Rice FACE Experiments

Studies conducted at the Tsukuba facility aimed at finding not only rice cultivars that increase yield in response to e[CO_2], but also those that maintain high grain quality under e[CO_2] and warming. In addition to changes in average temperatures, climate change also causes extreme weather, which is a serious threat to rice production. Rice sterility due to heat stress is a recent important concern of Japanese rice production. Elevated [CO_2] reduces transpiration by reducing stomatal conductance (Yoshimoto et al., 2005), which increases the plant body temperature. Thus, we are very concerned that a combination of e[CO_2] and heat stress will cause heavy yield losses. Introduction of cultivars tolerant to heat stress is also an important subject.

Vigorous research on microbes in rice paddy ecosystems has been conducted, particularly at the Tsukuba facility (Ikeda et al., 2015; Okubo et al., 2014; 2015). Interdisciplinary studies of microbial ecology and C and N dynamics are also underway; in particular, a study on the responses of CH_4 dynamics to e[CO_2] and N availability with respect to microbe–plant interactions is ongoing. Note that CH_4 we measure at the paddy surface reflects the difference between production (methanogenesis) and consumption (CH_4 oxidation), both of which depend on different microbial groups and are affected by environmental changes via microbe–soil–plant interactions (Hayashi et al., 2015). Thus, elucidating the mechanisms of the responses of methanogenesis and CH_4 oxidation to environmental changes is indispensable for disentangling the enigma of how e[CO_2] and climate change will perturb CH_4 emissions from rice paddies in the future.

Much still remains unexplained about how N-related processes in rice paddy ecosystems respond to environmental changes. Furthermore, even the data on actual conditions are insufficient, particularly for BNF (which provides the largest N input from the atmosphere to rice paddies) and N_2, N_2O, and nitrogen monoxide emissions following denitrification (which are responsible for the largest N loss from rice paddies to the atmosphere). Both BNF and denitrification depend on microbial activity. Similar to the research necessary to understand CH_4 dynamics, future studies to elucidate the mechanisms of the responses of these processes to environmental changes will require focusing on microbe–soil–rice interactions.

Development of numerical models of rice production and CH_4 dynamics in rice paddies is also an important research topic, which is progressing (Fumoto et al., 2008; Li et al., 2015). As a pilot study that should help address future challenges in model development, the FACE-N project verified a process model for rice paddy ecosystems (DNDC-Rice) with respect to improving the description of N-related processes in the model (Katayanagi et al., 2013), improved the model with special emphasis on describing the aspects of atmosphere–land interactions (SOLVEG) relevant to enhancing reproducibility of the reactive N exchanges between the atmosphere and rice paddies (Katata et al., 2013), and analyzed seasonal changes in stomatal conductance of rice plants on a plant-community scale, which should be useful to complement wide-scale observations by remote-sensing techniques (Ono et al., 2013).

FACE studies are environmental manipulation experiments under actual open-field conditions. Complementation with growth chamber and laboratory experiments, which allow variation factors to be narrowed down, is indispensable for revealing the mechanism of each key process. We expect that uncertainties in predictive ability of numerical models will be greatly reduced by incorporating the knowledge obtained from these studies into the models in combination with wide-scale monitoring data derived from observations using towers, aircraft, and satellites. We hope that FACE studies will continue to contribute to the establishment of a knowledge base of environmental change impacts and the development of mitigation and adaptation options to achieve win-win-win relationships among the rice production and the C cycle and N cycle aspects of rice paddy ecosystems under future environmental changes.

Acknowledgements

We would like to express our gratitude to all researchers and collaborators involved in experiments at the two rice FACE facilities. Our current understanding would be impossible without their sedulous efforts. Many research grants have supported the rice FACE experiments in Japan; our projects were funded by the Ministry of Agriculture, Forestry and Fisheries, the Core Research for Evolutional Science and Technology (CREST) program of the Japan Science and Technology Agency, and the Grant-in-Aid for Scientific Research (No. 22114515, 22248026, 22880041, 24114711, and 26252061 are related to the topics covered in this chapter) provided by the Japan Society for the Promotion of Science and the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

Adachi, M., Hasegawa, T., Fukayama, H., Tokida, T., Sakai, H., Matsunami, T., Nakamura, H., Sameshima, R., Okada, M., 2014. Soil and water warming accelerates phenology and down-regulation of leaf photosynthesis of the rice plants grown under free-air CO_2 enrichment (FACE), *Plant Cell Physiol.,* 55: 370–380.

Bruinsma, J., 2009. The resource outlook to 2050, by how much do land, water use and crop yields need to increase by 2050, *In:* Technical papers from the expert meeting on 'How to feed the world in 2050', pp. 1–33. FAO, Rome.

Chen, C.P., Sakai, H., Tokida, T., Usui, Y., Nakamura, H., Hasegawa, T., 2014. Do the rich always become richer? Characterizing the leaf physiological response of the high-yielding rice cultivar Takanari to free-air CO_2 enrichment, *Plant Cell Physiol.,* 55: 381–391.

Ciais P, Sabine C, Bala G et al. 2013: Carbon and Other Biogeochemical Cycles. *In:* Climate Change 2013:

The Physical Science Basis, Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, (Eds.) Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

FAOSTAT. Food and Agriculture Organization of the United Nations. Available at: http://faostat.fao.org/

Fumoto, T., Kobayashi, K., Li, C., Yagi, K., Hasegawa, T., 2008. Revising a process-based biogeochemistry model (DNDC) to simulate methane emission from rice paddy fields under various residue management and fertilizer regimes, *Global Change Biol.,* 14: 382–402.

Hasagawa, T., Kobayashi, K., Lieffering, M., Kim, H.Y., Sakai, H., Shimono, H., Yamakawa, Y., Yoshimoto, M., Okada, M., 2005. Impact of increased source capacity on rice yield: Case study with CO_2 enrichment, In: Rice is life: scientific perspectives for the 21st century, (Eds.) Toriyama, K., Heong, K.L., Hardy, B., 124–127.

Hasegawa, T., Shimono, H., Yang, L., Kim, H.Y., Kobayashi, T., Sakai, H., Yoshimoto, M., Lieffering, M., Ishiguro, K,. Wang, Y., Zhu, J., Kobayashi, K., Okada, M., 2007. Response of rice to increasing CO_2 and temperature: Recent findings from large-scale free-air CO_2 enrichment (FACE) experiments. In: Proceedings of the 26th International Rice Conference, 9–12 October 2006, New Delhi, India, (Eds.) Aggarwal, P., Ladha, J., Singh, R., Devakumar, C., Hardy, B.) pp. 439–447.

Hasegawa, T., Sakai, H., Tokida, T., Nakamura, H., Zhu, C., Usui, Y., Yoshimoto, M., Fukuoka, M., Wakatsuki, H., Katayanagi, N., Matsunami, T., Kaneta, Y., Sato, T., Takakai, F., Sameshima, R., Okada, M., Mae, T., Makino, A., 2013. Rice cultivar responses to elevated CO_2 at two free-air CO_2 enrichment (FACE) sites in Japan, *Funct. Plant Biol.,* 40: 148–159.

Hasegawa, T., Sakai, H., Tokida, T., Usui, Y., Yoshimoto, M., Fukuoka, M., Nakamura, H., Shimono, H., Okada, M., 2015. Rice free-air carbon dioxide enrichment studies to improve assessment of climate change effects on rice agriculture, Agronomy Monograph, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, *in press.*

Hayashi, K., Nishimura, S., Yagi, K., 2006. Ammonia volatilization from the surface of a Japanese paddy field during rice cultivation, *Soil Sci. Plant Nutr.,* 52: 545–555.

Hayashi, K., Hiradate, S., Ishikawa, S., Nouchi, I., 2008a. Ammonia exchange between rice leaf blades and the atmosphere: Effect of broadcast urea and changes of xylem sap and leaf apoplastic ammonium concentrations, *Soil Sci. Plant Nutr.,* 54: 807–818.

Hayashi, K., Nishimura, S., Yagi, K., 2008b. Ammonia volatilization from a paddy field following applications of urea: Rice plants are both an absorber and an emitter for atmospheric ammonia, *Sci. Tot. Environ.,* 390: 485–494.

Hayashi, K., Yan, X., 2010. Airborne nitrogen load in Japanese and Chinese agroecosystems, *Soil Sci. Plant Nutr.,* 56: 2–18.

Hayashi, K., Tokida, T., Hasegawa, T., 2011. Potential ammonia emission from flag leaves of paddy rice (*Oryza sativa* L. cv. Koshihikari), *Agric. Ecosyst. Environ.,* 144: 117–123.

Hayashi, K., Ono, K., Tokida, T., Mano, M., Takimoto, T., Miyata, A., Matsuda, K., 2012. Atmosphere-rice paddy exchanges of inorganic aerosols and relevant gases during a winter and a summer weeks, *J. Agric. Meteorol.,* 68: 55–68.

Hayashi, K., Mano, M., Ono, K., Takimoto, T., Miyata, A., 2013a. Four-year monitoring of atmospheric ammonia using passive samplers at a single-crop rice paddy field in central Japan, *J. Agric. Meteorol.,* 69: 229–241.

Hayashi, K., Matsuda, K., Ono, K., Tokida, T., Hasegawa, T., 2013b. Amelioration of the reactive nitrogen flux calculation by a day/night separation in weekly mean air concentration measurements, *Atmos. Environ.,* 79: 462–471.

Hayashi, K., Ono, K., Kajiura, M., Sudo, S., Yonemura, S., Fushimi, A., Saito, K., Fujitani, Y., Tanabe, K., 2014a. Trace gas and particle emissions from open burning of three cereal crop residues: Increase in residue moistness enhances emissions of carbon monoxide, methane, and particulate organic carbon, *Atmos. Environ.,* 95: 36–44.

Hayashi, K., Tokida, T., Matsushima, M.Y., Ono, K., Nakamura, H., Hasegawa, T., 2014b. Free-air CO_2 enrichment (FACE) experiment on net nitrogen fixation at the paddy soil surface under submerged conditions, *Nutr. Cycl. Agroecosyst.,* 98: 57–69.

Hayashi, K., Tokida, T., Kajiura, M., Yanai, Y., Yano, M., 2015. Cropland soil–plant systems control production and consumption of methane and nitrous oxide and their emissions to the atmosphere, *Soil Sci. Plant Nutr.,* 61: 2–33.

Hoque, M.M., Inubushi, K., Miura, S., Kobayashi, K., Kim, H.-Y., Okada, M., and Yabashi, S., 2001. Biological dinitrogen fixation and soil microbial biomass carbon as influenced by free-air carbon dioxide enrichment (FACE) at three levels of nitrogen fertilization in a paddy field, *Biol. Fertil. Soils,* 34: 453–459.

Ikeda, S., Tokida, T., Nakamura, H., Sakai, H., Usui, Y., Okubo, T., Tago, K., Hayashi, K., Sekiyama, Y., Ono, H., Tomita, S., Hayatsu, M., Hasegawa, T., Minamisawa, K., 2015. Characterization of leaf blade- and leaf sheath-associated bacterial community and assessment of their responses to environmental changes of CO_2, temperature, and nitrogen levels under field conditions, *Microbes Environ.,* 30: 51–62.

Inubushi, K., Cheng, W., Aonuma, S., Hoque, M.M., Kobayashi, K., Miura, S., Kim, H.Y., Okada, M, 2003. Effects of free-air CO_2 enrichment (FACE) on CH_4 emission from a rice paddy field, *Global Change Biol.,* 9: 1458–1464.

IPCC, 2013. Summary for policymakers, In: Climate Change 2013: The Physical Science Basis, Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, (Eds.) Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Katata, G., Hayashi, K., Ono, K., Nagai, H., Miyata, A., Mano, M., 2013. Coupling atmospheric ammonia exchange process over a rice paddy field with a multi-layer atmosphere-soil-vegetation model, *Agric. Forest Meteorol.,* 180: 1–21.

Katayanagi, N., Ono, K., Fumoto, T., Mano, M., Miyata, A., Hayashi, K., 2013. Validation of the DNDC-Rice model to discover problems in evaluating the nitrogen balance at a paddy field for single-cropping of rice, *Nutr. Cycl. Agroecosyst.,* 95: 255–268.

Kimball, B.A., Kobayashi, K., Bindi, M., 2002. Responses of agricultural crops to free-air CO_2 enrichment, *Adv. Agron.,* 77: 293–368.

Kobayashi, K., Okada, M., Kim, H., Lieffering, M., Miura, S., Hasegawa, T., 2006. Paddy rice responses to free-air [CO_2] enrichment. In: Managed ecosystems and CO_2: Case studies, processes, and perspectives, (Eds.) Nösberger, J., Long, S.P., Norby, R.J., Stitt, M., Hendrey, G.R., Blum, H., Ecological Studies, 187, pp. 87–104. Springer, Germany.

Leakey, A.D.B., Uribelarrea, M., Ainsworth, E.A., Naidu, S.L., Rogers, A., Ort, D.R., Long, S.P., 2006. Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought, *Plant Physiol.,* 140: 779–790.

Li, T., Hasegawa, T., Yin, X., Zhu, Y., Boote, K., Adam, M., Bregaglio, S., Buis, S., Confalonieri, R., Fumoto, T., Gaydon, D., Marcaida III, M., Nakagawa, H., Oriol, P., Ruane1, A.C., Ruget, F., Singh, B., Singh, U., Tang, L., Tao, F., Wilkens, P., Yoshida, H., Zhang, Z., Bouman, B., 2015. Uncertainties in predicting rice yield by current crop models under a wide range of climatic conditions, *Global Change Biol.,* 21: 1328–1341.

Liu, G.C., Tokida, T., Matsunami, T., Nakamura, H., Okada, M., Sameshima, R., Hasegawa, T., Sugiyama, S., 2012. Microbial community composition controls the effects of climate change on methane emission from rice paddies, *Environ. Microbiol. Rep.,* 4: 648–654.

Long, S.P., Ainsworth, E.A., Rogers, A., Ort, D.R., 2004. Rising atmospheric carbon dioxide: Plants FACE the future, *Annu. Rev. Plant Biol.,* 55: 591–628.

Miyazawa, S., Hayashi, K., Nakamura, H., Hasegawa, T., Miyao, M., 2014. Elevated CO_2 decreases the photorespiratory NH_3 production but does not decrease the NH_3 compensation point in rice leaves, *Plant Cell Physiol.,* 55: 1582–1591.

Morgan, P.B., Bollero, G.A., Nelson, R.L., Dohleman, F.G., Long, S.P., 2005. Smaller than predicted increase in aboveground net primary production and yield of field-grown soybean under fully open-air [CO_2] elevation, *Global Change Biol.,* 11: 1856–1865.

Myers, S.S., Zanobetti A., Kloog, I., Huybers, P., Leakey, A.D.B., Bloom, A.J., Carlisle, E., Dietterich, L.H., Fitzgerald, G., Hasegawa, T., Holbrook, N.M., Nelson, R.L., Ottman, M.J., Raboy, V., Sakai, H., Sartor, K.A., Schwartz, J., Seneweera, S., Tausz, M., Usui, Y., 2014. Increasing CO_2 threatens human nutrition, *Nature,* 510: 139–142.

Myhre G, Shindell D, Bréon F-M, et al. 2013: Anthropogenic and Natural Radiative Forcing. *In:* Climate Change 2013: The Physical Science Basis, Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, (Eds.) Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Nakamura, H., Tokida, T., Yoshimoto, M., Sakai, H., Fukuoka, M., Hasegawa, T., 2012. Performance of the enlarged rice-FACE system using pure-CO_2 installed in Tsukuba, Japan, *J. Agric. Meteorol.,* 68: 15–23.

Okubo, T., Tokida, T., Ikeda, S., Bao, Z., Tago, K., Hayatsu, M., Nakamura, H., Sakai, H., Usui, Y., Hayashi, K., Hasegawa, T., Minamisawa, K., 2014. Effects of elevated carbon dioxide, elevated temperature, and rice growth stage on the community structure of rice root-associated bacteria, *Microbes Environ.,* 29: 184–190.

Okubo, T., Liu, D., Tsurumaru, H., Ikeda, S., Asakawa, S., Tokida, T., Tago, K., Hayatsu, M., Aoki, N., Ishimaru, K., Ujiie, K., Usui, Y., Nakamura, H., Sakai, H., Hayashi, K., Hasegawa, T., Minamisawa, K., 2015. Elevated CO_2 affect community structure of rice root-associated bacteria, *Front. Microbiol.,* 6: Article 136 (8p).

Ono, K., Maruyama, A., Kuwagata, T., Mano, M., Takimoto, T., Hayashi, K., Hasegawa, T., Miyata, A., 2013. Canopy-scale relationships between stomatal conductance and photosynthesis in irrigated rice, *Global Change Biol.,* 19: 2209–2220.

Parry, M.L., Rosenzweig, C., Iglesias, A., Livermore, M., Fischer, G., 2004. Effects of climate change on global food production under SRES emissions and socio-economic scenarios, *Global Environ. Change,* 14: 53–67.

Roger, P.A., Ladha, J.K., 1992. Biological N_2 fixation in wetland rice fields: estimation and contribution to nitrogen-balance, *Plant Soil,* 141: 41–55.

Rosenthal, D.M., Slattery, R.A., Miller, R.E., Grennan, A.K., Cavagnaro, T.R., Fauquet, C.M., Gleadow, R.M., Ort, D.R., 2012. Cassava about-FACE: Greater than expected yield stimulation of cassava (Manihot esculenta) by future CO_2 levels, *Global Change Biol.,* 18: 2661–2675.

Shindell, D.T., Faluvegi, G., Koch, D.M., Schmidt, G.A., Unger, N., Bauer, S.E., 2009. Improved attribution of climate forcing to emissions, *Science,* 326: 716–718.

Shindell, D., Kuylenstierna, J.C.I., Vignati, E., et al., 2012. Simultaneously mitigating near-term climate change and improving human health and food security, *Science,* 335: 183–189.

Tokida, T., Fumoto, T., Cheng, W., Matsunami, T., Adachi, M., Katayanagi, N., Matsushima, M., Okawara, Y., Nakamura, H., Okada, M., Sameshima, R., Hasegawa, T., 2010. Effects of free-air CO_2 enrichment (FACE) and soil warming on CH_4 emission from a rice paddy field: Impact assessment and stoichiometric evaluation, *Biogeosciences,* 7: 2639–2653.

Tokida, T., Adachi, M., Cheng, W., Nakajima, Y., Fumoto, T., Matsushima, M., Nakamura, H., Okada, M., Sameshima, R., Hasegawa, T., 2011. Methane and soil CO_2 production from current-season photosynthates in a rice paddy exposed to elevated CO_2 concentration and soil temperature, *Global Change Biol.,* 17: 3327–3337.

Tokida, T., Cheng, W., Adachi, M., Matsunami, T., Nakamura, H., Okada, M., Hasegawa, T., 2013. The contribution of entrapped gas bubbles to the soil methane pool and their role in methane emission from rice paddy soil in free-air [CO_2] enrichment and soil warming experiments, *Plant Soil,* 364: 131–143.

Tokida, T., Nakajima, Y., Hayashi, K., Usui, Y., Katayanagi, N., Kajiura, M., Nakamura, H., Hasegawa, T., 2014. A fully-automated high-throughput instrumentation for $\delta^{13}C$ measurement of methane and its application to rice paddy samples, *Rapid Commun. Mass Spectrom.,* 28: 2315–2324.

Usui, Y., Sakai, H., Tokida, T., Nakamura, H., Nakagawa, H., Hasegawa, T., 2014. Heat-tolerant rice cultivars retain grain appearance quality under free-air CO_2 enrichment, *Rice,* 7: 6.

Usui, Y., Sakai, H., Tokida, T., Nakamura, H., Nakagawa, H., Hasegawa, T., 2015. Rice grain yield and quality responses to free-air CO_2 enrichment combined with soil and water warming, *Global Change Biol.,* doi:10.1111/gcb.13128.

Yano, M., Toyoda, S., Tokida, T., Hayashi, K., Hasegawa, T., Koba, K., Yoshida, N., 2014. Isotopomer analysis of production, consumption and soil-to-atmosphere emission processes of N_2O at the beginning of flood irrigation, *Soil Biol. Biochem.,* 70: 66–78.

Yoshimoto, M., Oue, H., Kobayashi. K., 2005. Energy balance and water use efficiency of rice canopies under free-air CO_2 enrichment. *Agr. Forest Meteorol.,* 133: 226–246.

Zhang, G., Sakai, H., Tokida, T., Usui, Y., Zhu, C., Nakamura, H., Yoshimoto, M., Fukuoka, M., Kobayashi, K., Hasegawa, T., 2013. The effects of free-air CO_2 enrichment (FACE) on carbon and nitrogen accumulation in grains of rice (*Oryza sativa* L.), *J. Exp. Bot.,* 64: 3179–3188.

Zhang, G., Sakai, H., Usui, Y., Tokida, T., Nakamura, H., Zhu, C., Fukuoka, M., Kobayashi, K., Hasegawa, T., 2015. Grain growth of different rice cultivars under elevated CO_2 concentrations affects yield and quality, *Field Crop. Res.,* 179: 72–80.