Biogeosciences

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

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Abstract. Paddy fields are an important source of atmospheric CH$_4$, the second most important greenhouse gas. There is a strong concern that the increasing atmospheric CO$_2$ concentration ([CO$_2$]) and global warming are further stimulating CH$_4$ emissions, but the magnitude of this stimulation varies substantially by study, and few open-field evaluations have been conducted. Here we report results obtained at a Japanese rice free-air CO$_2$ enrichment (FACE) site under water and soil temperature elevation during two growing seasons. Our objectives were to evaluate the effects of high [CO$_2$] (ambient + 200 µmol mol^{-1}) and elevated soil temperature (+ 2 °C) on CH$_4$ emissions under completely open-field conditions. We found about 80% enhancement in total seasonal emissions by the additive effects of FACE and warming, indicating a strong positive feedback effect of global warming. The enhancement in CH$_4$ emission from the FACE-effect alone (+ 26%) was statistically non-significant (P = 0.19). Nevertheless, observed positive correlations between CH$_4$ emissions and rice biomass agreed well with previous studies, suggesting that higher photosynthesis led to greater rhizodeposition, which then acted as substrates for methanogenesis. Soil warming increased the emission by 44% (P < 0.001), which was equivalent to a Q$_{10}$ of 5.5. Increased rice biomass by warming could only partly explain the enhanced CH$_4$ emissions, but stoichiometric analysis of the electron budget indicated that even a moderate enhancement in organic matter decomposition due to soil warming can cause a large increase in CH$_4$ production under conditions where Fe(III) reduction, which was little affected by soil warming, dominates electron-accepting processes. At later rice growth stages, advanced root senescence due to elevated temperature probably provided more substrate for methanogenesis. Our stoichiometric evaluation showed that in situ Fe reduction characteristics and root turnover in response to elevated temperature should be understood to correctly predict future CH$_4$ emissions from paddy fields under a changing climate. Challenges remain for determination of in situ root-exudation rate and its response to FACE and warming.

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1 Introduction

Paddy fields act as an important carbon cycling interface between the atmosphere and the land. Carbon dioxide (CO$_2$) fixation by photosynthesis provides staple foods to half the world's population, and rice is one of the most important cereal crops, especially in Asian countries (IRRI, 2002). On

the other hand, the paddy-field landscape is one of the major sources of atmospheric CH$_4$, the second most important greenhouse gas. The radiative forcing of CH$_4$ is as much as half that of CO$_2$ if indirect effects are taken into account (stratospheric H$_2$O and tropospheric ozone; Hansen et al., 2005; Denman et al., 2007). Estimate of rice-paddy CH$_4$ emission varies from one study to another, ranging from 31 to 112 Tg yr^{-1}, which are equivalent to 12–26% of the anthropogenic CH$_4$ release and 9–19% of global total emissions (Forster et al., 2007 and references therein).

There is a strong concern that the increasing atmospheric CO$_2$ concentration [CO$_2$] and rising temperature due to global warming are further stimulating CH$_4$ emissions from paddy fields. Most studies have shown greater CH$_4$ emissions under high [CO$_2$] (Inubushi et al., 2003; Xu et al., 2004) and elevated temperature (Ziska et al., 1998; Allen et al., 2003), indicating a positive feedback loop. Generally speaking, a temperature rise stimulates microbial activity in submerged soils, which may lead to higher rate of CH$_4$ production (Fey and Conrad, 2000). Increasing [CO$_2$] enhances photosynthesis of rice (Kim et al., 2001, 2003) and also results in more carbon being available for methane production (Inubushi et al., 2003).

At present, we are still far from being able to predict future CH$_4$ emissions, partly because two issues remain unresolved. First, most studies have been conducted under more or less "closed" conditions, resulting in significant divergence from field conditions, particularly in the case of water and soil temperature regimes in warming experiments, in which air temperature is usually controlled. Under field conditions, not only air temperature but also solar radiation and its interception by the rice canopy and wind speed greatly affect water and soil temperatures (Kuwagata et al., 2008). Second, previous studies have focused primarily on relative changes in CH$_4$ emissions in response to high [CO$_2$] and warming, paying less attention to the mechanisms behind the overall reactions. In fact, considerable enhancement of CH$_4$ emission has been reported in response to elevated air temperatures (+160% by +6°C, equivalent to $Q_{10} = 5.0$; Allen et al., 2003), to a degree not attributable to a single biochemical reaction, which generally shows rather moderate temperature sensitivity ($Q_{10} = 2$–3), indicating the need for further exploration of the mechanisms involved. Because of the large diversity of paddy fields with respect to climatic and edaphic factors as well as management practices, it is not possible to directly extrapolate the responses obtained in a given rice paddy to another. Only by obtaining a comprehensive and systematic understanding can process-based models (e.g. DNDC; Fumoto et al., 2008) be properly validated and given predictive power for future CH$_4$ emissions under various conditions.

The use of free-air CO$_2$ enrichment (FACE) is a promising way to resolve the first issue, because it can simulate elevated canopy [CO$_2$] under open-field conditions (Okada et al., 2001). Further, inclusion of warming treatments in rice FACE experiments might enable us to assess the impact of both high [CO$_2$] and elevated temperature on CH$_4$ emission without altering micrometeorological conditions.

To address the second issue, a basic biochemical understanding of CH$_4$ production in paddy fields is necessary. Methane is the end product of a cascade of organic carbon decomposition, starting with the hydrolysis of polymers (e.g., polysaccharides), followed by primary and secondary (syntrophic) fermentation to produce either hydrogen (H$_2$), C1 compounds, or acetate, which then behave as electron donors (Schink, 1997). Methanogens are responsible only for the final reaction – the conversion of simple compounds (mostly H$_2$ and acetate) to CH$_4$ (Le Mer and Roger, 2001). The absence of an accumulation of electron donors (except at early growth stages, Sigren et al., 1997) indicates that hydrolysis or fermentative processes usually control the rate of CH$_4$ production, not methanogenesis itself (van Bodegom et al., 2000). The organic carbon supply rate primarily determines electron-donor production, and thus the maximum CH$_4$ production potential. Possible carbon sources include soil organic matter (SOM), organic fertilizers (manures), and residues of rice grown in preceding years (straw and stubble) (Chidthaisong and Watanabe, 1997). Photoassimilates of rice under cultivation also serve as important substrates in the form of root exudates and autolysis products of roots (Dannenberg and Conrad, 1999; Watanabe et al., 1999; Lu et al., 2000; Aulakh et al., 2001a, b; Wang and Adachi, 2000). Competition with other final electron acceptors in the soil also strongly inhibits CH$_4$ production because of differences in energy yield, as predicted by thermodynamic theory (Takai, 1961a; Takai and Kamura, 1966). Candidate electron acceptors include O$_2$, nitrate, Mn(IV), Fe(III), and sulfate, among which Fe(III) (iron respiration; Kamura et al., 1963; Balashova and Zavarizin, 1980; Lovley and Phillips, 1988) is dominant (Takai, 1961b), with Fe(III) reduction commonly accounting for half or more of the total electron-donor consumption (Yao et al., 1999; Fumoto et al., 2008).

On the basis of this mechanistic background of CH$_4$ production, we speculated that a comprehensive evaluation of electron-donor supply and their competitive consumption could provide insight into the important mechanisms responsible for changes in CH$_4$ emission in response to high [CO$_2$] and elevated temperature. We further hypothesized that a potential pronounced response of CH$_4$ emission to FACE and soil warming might be explained by the synergistic effects of several processes, even though the response of each single process is rather moderate.

Here we report results obtained at a Japanese rice FACE site under water and soil temperature elevation. Our primary objective was to evaluate the effects of high [CO$_2$] (+200 μmol mol^{-1}) and elevated soil temperature (+2°C) on CH$_4$ emissions under completely open-field conditions. We also aimed to identify important processes responsible for potential changes in eventual CH$_4$ emissions. To that end, we adopted a stoichiometric approach to evaluate carbon and

electron flow. We investigated CH$_4$ production and Fe(III) reduction in the soil as the dominant electron-accepting processes. As for the electron-donor supply, we quantified SOM decomposition by in situ soil incubation experiments. We also monitored rice growth, including that of the roots, as a proxy for the amount of rhizodeposition.

2 Materials and methods

2.1 Study site, CO$_2$ enrichment, soil warming, and overall weather conditions

The FACE and soil warming experiments were conducted in a rice paddy field at Shizukuishi, Iwate, Japan (39°38′ N, 140°57′ E) during two growing seasons (2007 and 2008). We used the same fields as for our previous rice-FACE experiments, but with three blocks instead of the four blocks used previously (Okada et al., 2001). The soil was an Andosol with a mean organic C content of 77.8 g kg^{-1} DW and a total N of 4.8 g kg DW. Briefly, two paddy fields were assigned to each block; one field had an ambient [CO$_2$] level (hereafter, ambient or Amb) and the other field was CO$_2$-enriched (FACE) with the target concentration of 200 µmol mol^{-1} above Amb achieved by a pure CO$_2$ injection FACE system. The FACE system was essentially the same as that used by Okada et al. (2001), but CO$_2$ fumigation was performed during daylight hours only, whereas previously FACE treatments were performed 24 h a day. The season-long (until the mid-grain-filling stage) daytime average [CO$_2$] was 568 (2007) and 573 (2008) µmol mol^{-1} in the FACE plots and 376 (2007) and 374 (2008) µmol mol^{-1} in the ambient plots. The fraction of time that the 1-min average [CO$_2$] deviated by < 10% or < 20% from the target [CO$_2$] was used to indicate the [CO$_2$] control performance. Averaged over the season and the three FACE rings, the time coverage was 68% (both 2007 and 2008) for a deviation of up to 10%, and 91% (both 2007 and 2008) for a deviation of up to 20%.

We conducted the soil/water temperature treatments in plots within each [CO$_2$] treatment (both FACE and Amb) field using two levels of soil/water temperature: a normal (ambient) temperature (hereafter, NT) plot and an elevated temperature (ET) plot, with the latter targeted at 2 °C above NT. Each plot was 5.5 m × 2.7 m. The locations of the ET plots were exactly the same in 2007 and 2008; hence, in 2008 the ET plot might show a "carry-over" effect of the previous season's warming, although the warming treatment was performed only during the rice growing season. Warming of the surface soil/water was achieved by on-off control of the heating wires, placed on the soil surface between the rows, with the water temperature of both plots being continuously measured. The temperature of the water and plow-layer soil was almost uniformly elevated. The ET plot was enclosed by a corrugated PVC panels to prevent rapid exchange of the paddy water with the surrounding area, including the

NT plots. The soil/water temperature warming treatment was ended about two weeks prior to the harvest (25 September 2007 and 29 September 2008), when water needed for the heating was no longer available because of drainage of the field for harvesting. Until then, the warming facility successfully maintained an increased soil temperature (Table 1). In 2007, the seasonal mean temperature elevation was 1.9 °C for the surface water and 1.8 °C for the soil at 10 cm depth, and in 2008 it was 2.6 °C and 2.4 °C for the water and soil, respectively. The temperature control was less accurate in 2008 than in 2007 because of technical problems. Solar radiation and air, water, and soil temperatures over the course of the growing season in each year are shown in Table 1.

2.2 Rice cultivation and fertilization

We sowed rice seeds (*Oryza sativa* L. cv. Akitakomachi) on 23 April 2007 and on 24 April 2008 in seedling trays, each containing 448 cells (16 mm in diameter and 25 mm in depth, Minoru Pot 448, Minoru Industrial Co. Ltd., Okayama, Japan). The seedlings subsequently used for the Amb and FACE plots were raised in two different chambers, under ambient [CO$_2$] and elevated [CO$_2$] (ambient + 200 µmol mol^{-1}), respectively. Transplanting of seedlings was done by hand on 23 May 2007 and 22 May 2008 with three seedlings per hill. Hills and rows were 17.5 and 30 cm apart, respectively, with a resultant density of 19.05 hills m^{-2}. All fertilizers were applied as basal dressing. Nitrogen was supplied at a rate of 9 g-N m^{-2} (3 g-N m^{-2} as ammonium sulfate and 6 g-N m^{-2} as coated urea; LP-70, Chisso-asahi Fertilizer Co., Ltd., Tokyo, Japan), potassium at a rate of 12.5 g-K m^{-2} (7.5 g-K as KCl and 5.0 g-K as potassium silicate), and phosphorous at a rate of 13.1 g-P m^{-2} as fused magnesium phosphate. Rice straw from the previous year was removed, but the leftover stubble was plowed into the soil about 1 month before transplanting. All agronomic practices were similar to those of local farmers with the exception that midseason drainage was not carried out in the experimental field so that the warming treatment could be continuously maintained.

2.3 Methane flux measurements

Methane flux was measured weekly (2007) or biweekly (2008) until drainage for harvest by a transparent, acrylic, closed-top chamber in a similar manner described in Inubushi et al. (2003). Two mini-plots, each with two hills, were used for repeated measurements of the CH$_4$ flux. Each chamber, 104.5 cm high with a basal area of 30 cm × 35 cm, could cover two hills. Gas samples were collected from the chamber at 0, 15, and 30 min after placement. The samples were injected into pre-evacuated 20 mL glass vials and brought back to the laboratory for analysis. The concentration of CH$_4$ was determined with an automated gas chromatography system equipped with a flame ionization detector (Sudo, 2006). The CH$_4$ flux was calculated from the

Table 1. Solar radiation and mean temperature of air, water, and soil (averaged over 6 fields) for each sampling period and the two cropping seasons (2007 and 2008).

| Year | Period[a] | Solar radiation (MJ d^{-1} m^{-2}) | Air temperature (°C) | Water temperature (°C) | | | Soil temperature (°C)[c] | | |
				Normal, NT	Elevated, ET	Elevation[b]	Normal, NT	Elevated, ET	Elevation[b]
2007	TP-TL (0–26)	18.3	17.0	22.6	24.3	1.7 (< 0.1)	21.3	22.9	1.6 (< 0.1)
	TL-PF (26–55)	16.4	19.4	23.7	25.6	1.9 (< 0.1)	23.2	25.0	1.8 (0.1)
	PF-HD (55–75)	15.4	21.0	23.1	25.0	1.9 (< 0.1)	22.5	24.4	1.8 (0.1)
	HD-MGF (75–104)	13.8	22.0	23.3	25.4	2.0 (< 0.1)	23.1	25.1	2.0 (0.2)
	Season mean	16.0	19.9	23.2	25.1	1.9 (< 0.1)	22.6	24.4	1.8 (0.1)
2008	TP-TL (0–27)	18.5	15.5	20.1	22.5	2.4 (0.1)	19.3	21.4	2.1 (0.3)
	TL-PF (27–54)	17.7	20.1	24.5	27.0	2.5 (0.4)	23.8	26.2	2.4 (0.7)
	PF-HD (54–75)	14.9	22.9	24.5	27.5	2.9 (0.6)	24.2	26.9	2.7 (0.8)
	HD-MGF (75–103)	13.4	21.0	21.9	24.6	2.7 (0.6)	22.0	24.5	2.5 (0.4)
	Season mean	16.1	19.7	22.7	25.3	2.6 (0.3)	22.3	24.7	2.4 (0.5)

[a] TP, transplanting; TL, tillering; PF, panicle formation; HD, heading; MGR, mid-grain-filling. Values in parentheses indicate days after transplanting (DAT) when the roots were sampled.
[b] Standard deviation ($n = 6$) is shown in parentheses.
[c] 10 cm depth.

increase in the gas concentration, the basal area, and the chamber volume.

2.4 Plant growth measurements

The number of tillers on the plants in the mini-plots was counted weekly until the final CH$_4$ measurement; then the plants were removed for biomass measurements. A block of plow-layer soil was also taken with the plants so that the root biomass could also be quantified. In order to trace changes in root biomass with time, we also sampled two hills in each plot outside the mini-plots with plants at different growth stages: tillering, panicle formation, heading, or mid-grain-filling stages. Solar radiation and temperature of air, water, and soil corresponding to the periods are shown in Table 1.

2.5 Soil Fe reduction

The time course of Fe(III) reduction was monitored by measuring the in situ Fe(II) concentration as a cumulative product. Soil Fe(II) was determined 4–6 times during the rice growing season in both 2007 and 2008. Fe reduction was also measured in 2003, 2004, and 2006, when crops were grown under similar cultivation practices. Among these growing seasons, we established elevated temperature (ET) plots in 2006, 2007, and 2008, but in 2006, we did not use the FACE

treatment and the soil warming treatment was conducted outside the FACE experimental areas (but within the same fields). In 2006 at one of the FACE fields, a root-exclusion plot was prepared by inserting PVC tubing into surface soil to prevent roots from penetrating inside. Fe reduction was monitored and compared with that of normal plot (soil with root) to see whether or not the presence of root affects soil Fe reduction. Approximately 20 g of wet soil was sampled in triplicate from the upper layer of the soil (0–5 cm depth) in cut-tip polypropylene cylinders. Immediately after sampling, the cylinder containing the wet soil sample was sealed with a butyl rubber stopper and stored under refrigerated conditions (4 °C) to suppress biological redox reactions prior to analysis, which was conducted within four days after the sampling. Soil Fe(II) was extracted following the method of Takai et al. (1958); the cylinder-soil sample was put in a glass beaker and quickly homogenized with a small stick. Subsample (2–3 g) was immediately taken into a plastic bottle and shaken in 45 mL of CH$_3$COONa buffer solution (1 M, pH 3.0). The extract was filtered and analyzed for Fe^{2+} by colorimetry using o-phenanthroline. The moisture content of the remaining wet soil sample was determined gravimetrically, and the Fe(II) content was calculated on a dry-soil basis.

In situ Fe(II) monitoring revealed that Fe reduction was different in different fields (see Sect. 3.3). This is problematic when evaluating the FACE effect on CH$_4$ emission because less Fe reduction means more CH$_4$ emission (Takai, 1961b; Watanabe and Kimura, 1999; Hanaki et al., 2002), if all other things being equal. To overcome this problem, we performed a covariance analysis of two years' worth of data so that we could adjust the seasonal cumulative CH$_4$ emission for the amount of electrons consumed by Fe reduction, which was calculated according to stoichiometric relations described in Sect. 2.7. Units were converted to a per area basis by taking into account the soil bulk density (about 0.7 Mg m^{-3}) and the depth of the plow layer (10.0–13.5 cm) in each field. In the covariance analysis, we assumed that the slope was the same across all experimental units (12 in total), and used the Kenward-Roger method to estimate the degrees of freedom of the denominator (Kenward and Roger, 1997).

2.6 In situ soil incubation for nitrogen mineralization

We performed in situ soil incubation experiments during the growing periods in 2007 and 2008 to measure the soil mineralization rate according to a standard method (Inoko, 1986) with slight modification. Soils for the incubation were collected from the 0–10 cm layer of each experimental plot before fertilization and irrigation for puddling. The soil was sieved (2–3 mm) to remove rice residue from the preceding season and stored at 4 °C without drying until sample preparation. Each soil sample (20 g, on an oven-dried basis) was weighed into a 50-mL polypropylene bottle with 30 mL of distilled water. All bottles were sealed with a screw cap and shaken for an hour and then refrigerated at 4 °C until the onset of in situ incubation. Just after the transplanting, we wrapped the sample bottles with aluminum foil to prevent light penetration and placed them into the plow layer of each experimental plot. Duplicate (2007) or triplicate (2008) samples were retrieved just before the drainage for harvest ($\sim$ 105 days after transplanting, DAT). The collected soil samples were extracted with a 2 M KCl solution using a 1:10 (w:v, soil to solution) ratio. The extract was analyzed either by a continuous-flow auto-analyzer (2007) (TRAACS2000, BRAN+LUEBBE, Norderstedt, Germany) or by manual analysis (2008) to quantify NH$_4^+$, NO$_2^-$, and NO$_3^-$ by the indophenol blue method, the sulfanilamide method, and the copper-cadmium reduction method, respectively. The amount of nitrogen mineralization was computed from the increase in extracted nitrogen. The results indicated that almost all of the mineralized nitrogen was in the form of NH$_4^+$.

2.7 Stoichiometric analyses of soil organic matter decomposition, Fe reduction, and methane production

We evaluated the balance of electrons transferred from electron donors and accepted by electron acceptors from a stoichiometric point of view. For electron donors, we evaluated the amount of electrons supplied through soil organic matter (SOM) decomposition, based on the in situ soil incubation results, as follows: (i) measurement of soil nitrogen mineralization (N$_{min}$) by in situ incubation (see Sect. 2.6); (ii) conversion of N$_{min}$ to carbon decomposition (C$_{dec}$) with the stoichiometry (Cheng et al., 2007),

$$C_{dec} = 5.28 \, N_{min} \text{ (in mass unit)} \tag{1}$$

(iii) further conversion of C$_{dec}$ into electron equivalents, under the assumption that organic carbon with oxidation state zero was the only electron donor (Yao et al., 1999; Fumoto et al., 2008),

$$CH_2O + H_2O \rightarrow CO_2 + 4H^+ + 4e^- \tag{2}$$

For electron acceptors, CH$_4$ production, and reduction of Fe(III) and sulfate (applied as (NH$_4$)$_2$SO$_4$) were taken into consideration because they are the dominant electron-accepting processes (Takai, 1961b; Watanabe and Kimura, 1999; Yao et al., 1999). We here assumed that applied sulfate was completely reduced during the rice growth period. The following stoichiometry has already been experimentally verified (Roden and Wetzel, 1996):

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \tag{3}$$

$$Fe(III) + e^- \rightarrow Fe(II) \tag{4}$$

$$SO_4^{2-} + 8e^- + 10H^+ \rightarrow H_2S + 4H_2O \tag{5}$$
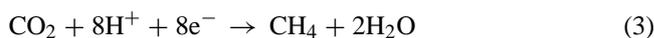
It is noteworthy that combining Eqs. (2) and (3) yields,

$$2CH_2O \rightarrow CO_2 + CH_4 \tag{6}$$

Because acetate (CH$_3$COOH) can be written as 2CH$_2$O, the electron balance shown in Eqs. (2) and (3) also represents CH$_4$ production through acetate fermentation, which is another important pathway of CH$_4$ production (Chanton et al., 2005). In this study, we used the observed CH$_4$ flux as a proxy to evaluate the rate of CH$_4$ production, under the assumption that CH$_4$ consumption (oxidation) between its production and transfer to the atmosphere (e.g., Holzapfel-Pschorn et al., 1985) was negligible and that the produced CH$_4$ was transported to the atmosphere immediately. In fact, the turnover time of acetate, an important CH$_4$ precursor, can be less than a day or even a few hours (Schütz et al., 1989; Sigren et al., 1997). The mean residence time of the produced CH$_4$ in the soil can also be very short, 2–3 h for plant-mediated transport (Byrnes et al., 1995). Because the main focus of this study was CH$_4$, units of SOM decomposition,

Table 2. Seasonal CH$_4$ emission (raw data and adjusted for Fe reduction), Fe reduction, SOM decomposition, and end-of-season rice biomass for different [CO$_2$] and temperature treatments.

	CH$_4$ (gC m^{-2})	CH$_4$-adjusted for Fe (gC m^{-2})[a]	Fe (gC-CH$_4$ equiv. m^{-2})[b]	CH$_4$ + Fe (gC-CH$_4$ equiv. m^{-2})	SOM (gC-CH$_4$ equiv. m^{-2})[c]	Above-ground biomass (g m^{-2})[d]	Root biomass (g m^{-2})[d]
FACE-ET	19.0 (2.9)	20.4 (3.2)	10.9 (1.3)	29.8 (2.6)	19.6 (2.1)	1456 (88)	66 (4)
FACE-NT	12.8 (2.9)	13.8 (3.2)	10.6 (1.3)	23.4 (2.6)	16.5 (2.1)	1354 (88)	70 (4)
Amb-ET	17.0 (2.9)	15.8 (3.2)	9.1 (1.3)	26.0 (2.6)	19.7 (2.1)	1242 (88)	53 (4)
Amb-NT	12.4 (2.9)	11.3 (3.1)	9.1 (1.3)	21.5 (2.6)	17.2 (2.1)	1124 (88)	60 (4)
FACE effect	+ 8%, n.s. ($P = 0.66$)	+ 26%, n.s. ($P = 0.19$)	+ 18%, n.s. ($P = 0.25$)	+ 12%, n.s. ($P = 0.17$)	− 2%, n.s. ($P = 0.19$)	+ 19%**	+ 21%*
Warming effect	+ 42%***	+ 44%***	+ 1%, n.s. ($P = 0.17$)	+ 24%***	+ 17%**	+ 9%**	− 8%† ($P = 0.07$)

n.s., not significant; †, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Pooled analysis results for 2007 and 2008. Values in parentheses indicate the 90% confidence interval. Year × CO$_2$ interaction ($P < 0.05$) for above-ground biomass. No other interaction (Temp × Year, Temp × CO$_2$, Temp × CO$_2$ × Year) was found in any items.

[a] CH$_4$ adjusted for the amount of Fe reduction (as covariate, $P = 0.14$), estimated by a covariance analysis.

[b] The amount of Fe reduction during 0 to about 100 DAT. Units are converted to CH$_4$ equivalent.

[c] Decomposed soil organic matter (SOM) during 0 to ∼ 105 DAT. Units are converted to CH$_4$ equivalent.

[d] Rice was sampled soon after the final CH$_4$ flux measurement, DAT ∼ 105.

Fe and sulfate reduction were transformed and expressed as CH$_4$-equivalent units on the basis of the electron balance described above.

Plant residues are another potential source of electron donors (Yagi and Minami, 1990). We removed most of the rice straw after harvest and only stubble was plowed into the field. To determine the amount of carbon in the form of stubble remaining one month before transplanting in 2008, we collected 20 stubble samples (culm only) from the field and oven-dried them at 80 °C for 72 h. We then estimated total carbon by assuming that the carbon content was 40% of the total dry weight.

We did not measure the rate of rhizodeposition during the growing season, another important electron donor, due primarily to the lack of appropriate method. However, we measured the change in rice biomass of all organs (Sect. 2.4), including that of the roots, which we used as a proxy for the amount of rhizodeposition.

2.8 Statistical analysis

We used a split-plot experimental design arranged in randomized complete blocks. For statistical analysis of the combined data for two years, year was treated as a fixed-effect whole-plot factor, [CO$_2$] as a split-plot factor, and temperature as a split-split plot factor (McIntosh, 1983). The computations were performed with PROC MIXED of SAS v9.2 (SAS Institute Inc.) by the restricted maximum likelihood method (REML with "nobound" option) to test main effects and interactions of fixed effects (Littell et al., 2006). To determine the FACE and warming effects on single-time and single-year measurements, we treated [CO$_2$] as a whole-plot factor and temperature as a split-plot factor.

3 Results

3.1 Plant growth

In 2007, the maximum tiller number was reached at 48–56 DAT, with no apparent difference between temperature treatments. Averaged over ET and NT, the rice tiller number in the FACE treatment tended to be greater than that in Amb, 515 tillers m^{-2} in FACE versus 433 tillers m^{-2} in Amb at 48 DAT and 512 versus 442 tillers m^{-2} at 56 DAT, although the effect was not significant. In 2008, the maximum tiller number was reached at ∼ 53 DAT and was significantly greater in FACE (679 tillers m^{-2}) than in Amb (547 tillers m^{-2}) ($P < 0.05$). Elevated temperature increased the tiller number at 18–39 DAT, but the effect diminished afterwards.

Both FACE and ET accelerated phenological development of the rice. In 2007, the heading date was earlier by 5 days in FACE-ET, by 4 days in Amb-ET, and by 1 day in FACE-NT than in the control plot (Amb-NT, heading at 73 DAT). In 2008, heading was at 76 DAT in Amb-NT, and was 6, 5, and 2 days earlier in FACE-ET, Amb-ET, and FACE-NT, respectively.

Pooled analysis of the data from both years showed that both FACE and soil warming treatments significantly increased the above-ground biomass in the mini-plots ($P < 0.01$, ∼ 105 DAT) without significant [CO$_2$] × temperature interaction (Table 2). The FACE treatment also increased the root biomass ($P < 0.05$). In contrast, root biomass at the grain-filling stage was smaller in ET than in NT ($P < 0.10$).

The time-series investigations conducted outside the mini-plots indicated that the effect of ET on root biomass changed with DAT (Fig. 1). In 2007, dry root biomass was not different at heading (75 DAT) but was smaller in the ET than in the NT plot at mid-ripening (104 DAT) (Fig. 1a). In
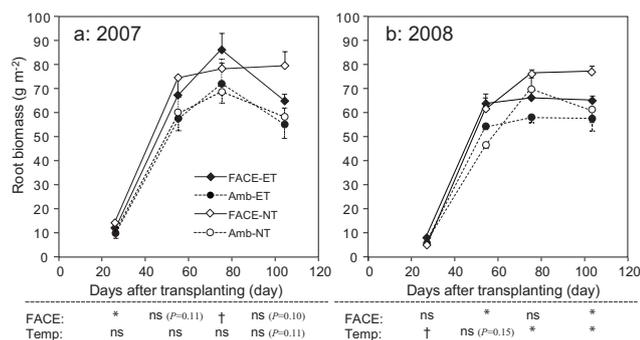
Fig. 1. Time course of root biomass in 2007 **(a)** and 2008 **(b)**. Roots were sampled outside the mini-plots where the CH$_4$ flux was measured. Bars indicate standard errors ($n = 3$). Symbols indicate the level of significance of elevated temperature: n.s., not significant; †, $P < 0.1$; *, $P < 0.05$.

Fig. 2. Changes in CH$_4$ flux from four treatments plots for two levels of [CO$_2$] and two levels of temperature. Symbols indicate the level of significance of elevated temperature: †, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$.

2008, root biomass was greater in ET at panicle formation (54 DAT), presumably owing to advanced plant development, but at flowering (75 DAT), ET plots tended to have a smaller biomass and this tendency remained until the grain-filling stage (103 DAT) (Fig. 1b). Other growth and yield parameters will be presented by Matsunami et al. (2010).

3.2 Effects of FACE and soil warming on CH$_4$ emission

The overall seasonal pattern of CH$_4$ emission was similar between 2007 and 2008 (Fig. 2); the CH$_4$ flux increased from transplanting to panicle formation, reached a peak at the heading (~ 70 DAT) to early grain-filling stages, and decreased afterwards. The seasonal total emission (g CH$_4$-C m^{-2}, standard error shown in parentheses, $n = 3$) was 18.4 (4.3) for FACE-ET, 12.1 (4.6) for FACE-NT, 17.4 (1.6) for Amb-ET, and 13.1 (7.5) for Amb-NT in 2007, and respectively 19.5 (2.9), 13.5 (1.3), 16.5 (3.6) and 11.7 (1.6) in 2008.

Interaction of FACE and soil warming was not significant during either growing season (Fig. 2) nor in the combined data of the two years (Table 2). The effect of FACE on CH$_4$ emission was not significant in any single measurement or in the total seasonal amount, in contrast to previous findings showing substantial increases in CH$_4$ emissions as a result of high [CO$_2$] (Ziska et al., 1998; Allen et al., 2003; Inubushi et al., 2003; Xu et al., 2004; Cheng et al., 2006, 2008; Lou et al., 2008). Pooled analysis of the data from both years also showed a nonsignificant effect of high [CO$_2$] ($P = 0.66$, Table 2, column "CH$_4$").

On the other hand, soil warming increased the CH$_4$ flux under both [CO$_2$] conditions in both years (Fig. 2). This enhancement was more apparent from the panicle formation to the full heading stage than during earlier stages. In terms of the seasonal total, ET increased CH$_4$ emission by $+33\%$ in 2007 and by $+41\%$ in 2008 under ambient [CO$_2$] ($P < 0.05$ for both 2007 and 2008), and by $+52\%$ in 2007 and $+45\%$ in 2008 in FACE plots. Pooled analysis gave a best estimate
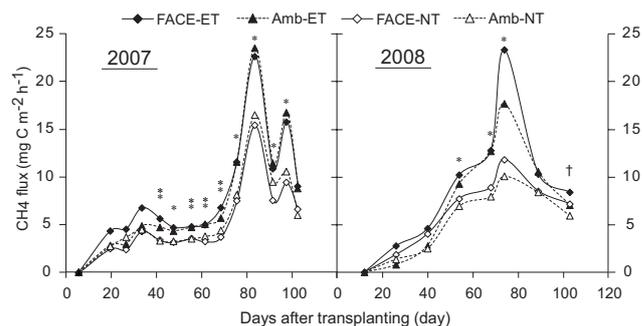
of $+44\%$ enhancement by ET ($P < 0.001$, Table 2). The corresponding Q_{10} values, estimated from the soil temperature difference (Table 1), was 7.1 in 2007, 4.3 in 2008, and 5.5 averaged over both seasons.

3.3 Fe reduction and covariance analysis of CH$_4$ emission

In situ Fe(II) monitoring revealed three distinct characteristics of Fe reduction at this site (Fig. 3). First, the amount of reducible Fe(III) was different among fields; in block-1, more Fe reduction occurred in FACE than in Amb (compare Fig. 3a and b). In the other two blocks, the differences in Fe reduction between the FACE and Amb fields were rather moderate, though small differences seemed to exist (block-2 and block-3 in Fig. 3). Second, the reduction pattern within each field was mostly reproducible over years, despite varying meteorological conditions and rice growth. We further observed that soil Fe(III) reduction occurred in a similar degree regardless of the presence or absence of rice plants ("with or without root" in Fig. 3c). Third, the results revealed no discernible differences between NT and ET plots: soil warming did not accelerate or increase Fe reduction, in sharp contrast to the high-temperature sensitivity of CH$_4$ emission.

We speculated that the absence of a stimulatory effect of FACE on CH$_4$ emission could be attributable, partly, to heterogeneity of the soil within the site (or even within a block); perhaps fields assigned to the FACE plots tended to have inherently larger amounts of reducible Fe(III), leading to greater suppression of CH$_4$ production than those assigned to Amb plots (Fig. 3). To adequately evaluate the effects of the FACE treatment, we performed a covariance analysis of the data for both years (2007 and 2008), treating the amount of Fe reduction as a covariate (see Sect. 2.5). The results indicated that FACE increased the annual CH$_4$ emission by $+26\%$ (Table 2, CH$_4$ adjusted for Fe). Although the effect of FACE was not still statistically significant, the significance
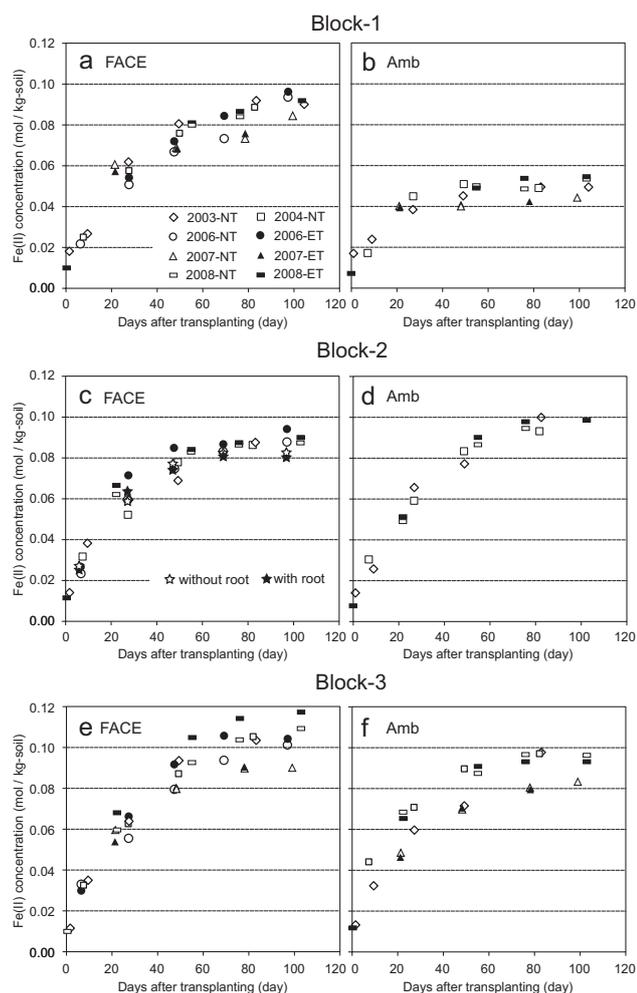
Fig. 3. Seasonal changes in Fe(II) concentration obtained from 3 replication blocks. Different fields are shown separately: **(a)**, **(c)**, **(e)** FACE fields, **(b)**, **(d)**, **(f)** Amb fields. Results from different growing seasons and with different temperature treatments (NT and ET) are shown. In 2006, root-excluded plot was prepared in one of the FACE fields, "without root" in **(c)**.

level increased considerably (P value decreased from 0.66 to 0.19). The significance level of the covariate (the amount of Fe reduction) was $P = 0.14$. The covariance analysis did not change the significant ET effect much; the adjusted enhancement was $+44\%$ ($P < 0.001$), which is comparable to the original value of $+42\%$ ($P < 0.0001$). It is noteworthy that the absence of FACE and ET effects on Fe reduction is an important prerequisite for the covariance analysis. The third characteristic of the Fe reduction exemplified the independence of Fe reduction with respect to temperature. The second characteristic suggested the absence of a FACE effect on Fe reduction.

3.4 Temperature dependence of SOM decomposition

In situ nitrogen mineralization showed a moderate temperature dependence, equivalent to a Q_{10} of ~ 2.0–2.5. As a result, incubation in ET plots produced $+17\%$ more ammonium than that in NT. Because gaseous products (CO_2 and CH_4) increased proportionally to the amount of ammonium (Cheng et al., 2007), the carbon decomposition rate was expected to have a similar temperature dependence (Table 2, "SOM").

3.5 Electron budget

The amount of electrons transferred through CH_4 production, Fe(III) and sulfate reduction was evaluated. Electron consumption by sulfate reduction was ~ 1.3 gC-CH$_4$ equiv. m^{-2} yr^{-1}, which was $< 15\%$ of Fe reduction and one order of magnitude less than the CH_4 production (see Table 2). Thereby we omit sulfate as an electron acceptor in the following stoichiometric analysis.

Fe reduction overwhelmed CH_4 production as an electron acceptor at the beginning of the rice growth period (from transplanting to tillering, TP-TL, Fig. 4). From the tillering stage until the beginning of panicle formation, Fe reduction and CH_4 production were almost equal in their consumption of electrons (Fig. 4, TL-PF). The ET treatment did not stimulate Fe reduction (Fig. 3), but significantly enhanced CH_4 production. After PF (~ 50 DAT), Fe reduction became minor and CH_4 production became dominant. Throughout the growing season, soil warming significantly increased CH_4 production. On a seasonal basis, both Fe reduction and CH_4 production were important (Table 2); Fe reduction accounted for over 40% of total electron consumption ("$CH_4 + Fe$" in Table 2) in NT, whereas its contribution was less ($\sim 35\%$) owing to greater CH_4 production in ET.

We found that SOM decomposition was a quantitatively important source of electron donors; however, SOM alone could not fully account for the electron donors necessary for both Fe reduction and CH_4 production (Table 2). A field survey indicated that the amount of stubble left one month before transplanting was 46.9 (SD = 8.6, $n = 20$) g C m^{-2}. Presuming that all stubble carbon was anoxically decomposed within one growing season, the CH_4-C equivalent of decomposed stubble was 23.5 g C-CH$_4$ m^{-2}, which was almost the same value as that from SOM (Table 2). Apart from SOM and stubbles, rhizodeposition might serve as an important electron donor, especially for CH_4 production (e.g. Watanabe et al., 1999), although we did not quantify it.

4 Discussion

Our open-field experiments provided convincing evidence of a positive feedback effect of climate change on CH_4 emission from a paddy field; $+200\,\mu$mol mol^{-1} [CO_2] combined with 2 °C water/soil warming could cause $+80\%$ enhancement in
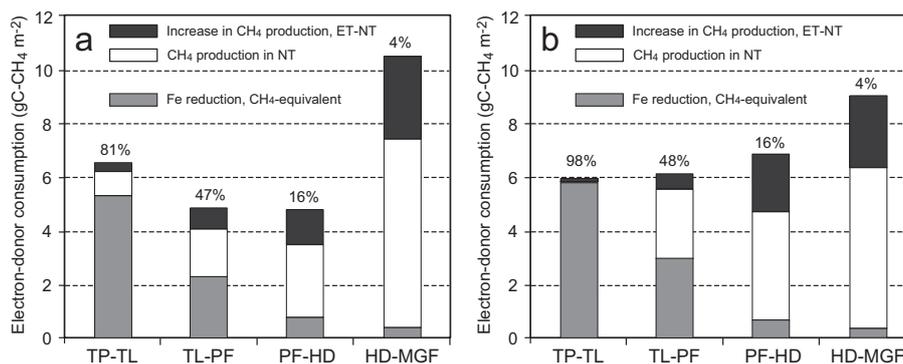
Fig. 4. Electron-donor consumption by Fe reduction and CH$_4$ production during four different growth periods (see Table 1 for definitions of the abbreviations) **(a)** 2007; **(b)** 2008. The CH$_4$ production values (not adjusted for Fe reduction) were averaged over the CO$_2$ treatments. Numbers on top of the bars indicate percentage of Fe reduction relative to total electron consumption in ET. Fe reduction was assumed to be the same for NT and ET and converted into CH$_4$-equivalent units so that direct comparisons with CH$_4$ production could be made. The exact times corresponding to the four growth periods were 0–21, 21–48, 48–78, and 78–99 DAT for Fe reduction and 0–27, 27–48, 48–76, and 76–103 for CH$_4$ production in 2007. For 2008, they were 0–22, 22–55, 55–76, and 76–103 for Fe reduction, and 0–26, 26–54, 54–74, 74–103 for CH$_4$ production.

CH$_4$ emission. Our results further imply that the positive climate feedback has already been taking place owing to past and ongoing rises in [CO$_2$] and temperature, which might partly account for the recent renewed increase in atmospheric [CH$_4$] (Rigby et al., 2008; Dlugokencky et al., 2009). In the following discussion, we seek mechanistic explanations for the observed enhancement of CH$_4$ by the FACE and warming treatments. The effects of FACE and warming are discussed independently, because their interaction was not significant for either CH$_4$ emission or plant growth parameters.

4.1 FACE effect on CH$_4$ emission; comparison with previous high-[CO$_2$] studies

The stimulatory effect of FACE observed in this study (after the covariance analysis) is in agreement with the findings of previous FACE studies (without warming) conducted at the same site (Inubushi et al., 2003) and at another rice FACE site in Jiangsu Province, China (Xu et al., 2004). The positive correlations between CH$_4$ emissions and above-ground or root biomass (Fig. 5) also agree well with the results of previous studies suggesting that greater assimilation of carbon under high [CO$_2$] leads to higher rates of rhizodeposition (root exudation and autolysis products), which is an important source of substrates for CH$_4$ production (Ziska, et al., 1998; Cheng et al., 2001, 2006; Allen et al., 2003; Inubushi et al., 2003; Xu et al., 2004).

The observed relative enhancement of CH$_4$ emission (+26%) was, however, relatively moderate compared with that reported by other studies. Ziska et al. (1998) showed a ~ 50% increase in response to a 300 μmol mol^{-1} [CO$_2$] elevation in open-top chambers at the International Rice Research Institute (IRRI). Allen et al. (2003) observed a ~ 50–100% enhancement by +300 μmol mol^{-1} [CO$_2$] enrichment
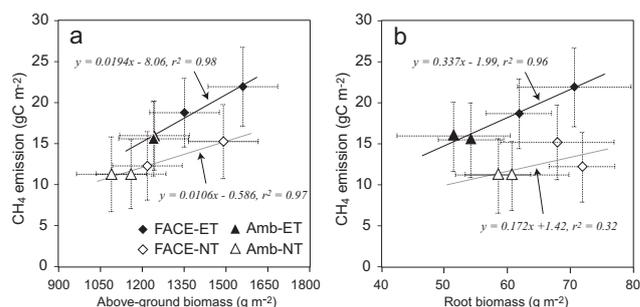
Fig. 5. Seasonal total CH$_4$ emission (adjusted for Fe reduction) plotted against above-ground biomass **(a)** and root biomass **(b)**. Bars indicate 90% confidence intervals. Each point corresponds to a certain treatment plot (e.g., FACE-ET) in either 2007 or 2008.

in soil, plant, atmosphere research (SPAR) chambers in Florida. The reason for this discrepancy is not clear but might be attributable to a difference in the root biomass increase caused by high [CO$_2$]. Ziska et al. (1998) observed a > 50% increase in root biomass at maturity but enhancement of the above-ground biomass was less, resulting in an increased root-to-shoot ratio (Ziska et al., 1997). Allen et al. (2003) found a similar degree of root-biomass stimulation (~ 60%), whereas we observed a smaller increase not only at mid-grain filling (Table 2, +21%) but also over the entire course of the growing season (Fig. 1). Smaller stimulation of root growth may well lead to a smaller substrate supply for CH$_4$ production, because root exudation positively correlates with root biomass (Wang and Adachi, 2000; Aulakh et al., 2001b). Lou et al. (2008) suggested that [CO$_2$] enrichment causes varying degrees of root stimulation in different rice cultivars. At a Chinese rice FACE site (+200 μmol mol^{-1}),

Xu et al. (2004) observed up to +200% enhancement in seasonal CH$_4$ emission, but they did not report its relation to root growth.

Obviously, more studies are needed to understand the underlying mechanisms responsible for the large variation in high-[CO$_2$] effects from study to study. It is noteworthy that Schrope et al. (1999) even reported a negative impact of high [CO$_2$] on CH$_4$ emission, despite a substantial increase in root (up to +83%) and above-ground dry weight (up to 35%). High [CO$_2$] possibly improved the O$_2$ supply to below-ground parts of the plant, hindering CH$_4$ production in the vicinity of the roots, and in turn resulting in very small emissions (peak < 10 mg CH$_4$ m^{-2} d^{-1}).

4.2 Effect of soil warming on CH$_4$ emission: possible mechanisms, comparison with previous studies, and implications for the real warming world

4.2.1 Synergistic effects with Fe reduction

The covariance analysis did not show important consequences of Fe reduction on the ET effect, which was +44% when adjusted for Fe, comparable to the unadjusted value (+42%) (Table 2). Rice in ET plots might provide a greater amount of organic carbon to methanogens than NT because the rate of root exudation might positively correlate with total biomass, including both above-ground components, which was significantly increased by ET (Table 2), and root weight (Aulakh et al., 2001b). However, even for the same amount of above-ground biomass, CH$_4$ emission from the ET plot was overwhelmingly larger than that from the NT plot (Fig. 5a, compare the two regression lines). How can we explain this difference?

We propose here one possible amplification mechanism that might explain the high temperature sensitivity of CH$_4$ production, especially during the early stages of rice growth. Figure 6 schematically illustrates how a moderate enhancement of organic matter decomposition (here defined as electron-donor production through hydrolysis and fermentation) can give rise to a large increase in CH$_4$ production when the Fe reduction rate is independent of temperature. Suppose the amount of electron-donor production is 5 and Fe reduction accounts for 60% (3/5) of the total electron-donor consumption in NT. Then, the remaining portion, 2, is available for CH$_4$ production. In ET plots, electron-donor production may increase by 20% (equivalent to a Q_{10} of ~ 2.5, as indicated by the in situ soil incubation results), whereas the same amount of Fe reduction occurs as in NT because of the temperature insensitivity of Fe reduction (Fig. 3). Thus, another 1 can be utilized for CH$_4$ production with a resultant enhancement of +50% compared with NT.

Our field observations suggest that this stoichiometric explanation is applicable to the enhancement of CH$_4$ in the early stages of rice growth, that is, from transplanting to panicle initiation (up to ~ 50 DAT), when Fe reduction dominates
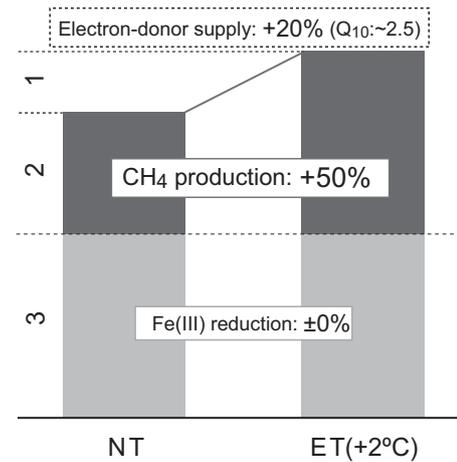
Fig. 6. A schematic illustration showing how (i) moderate enhancement of organic matter decomposition (= electron-donor production) combined with (ii) temperature insensitivity of Fe reduction can cause a large increase in CH$_4$ production (see text for detailed explanation).

electron-donor consumption. The moderate yet significant increase in SOM decomposition in ET plots (Table 2) also supports this hypothesis. In early stages of rice growth, SOM (together with stubble) should be the main source of electron donors. From then onward, however, Fe reduction became a minor electron acceptor (Fig. 4), so this mechanism probably does not explain the large increase in CH$_4$ emission from ET plots (see Sect. 4.2.2). Further research on the temperature sensitivity of residue degradation is necessary. It is noteworthy that we here assumed that the CH$_4$ production potential itself is unlikely to be a limiting factor, because methanogens can rapidly metabolize substrates as long as they are available (e.g., Sass et al., 1990). Modeling studies have also indicated that the accurate description of substrate availability is more important than kinetic microbial parameters for the prediction of CH$_4$ production (van Bodegom et al., 2000; van Bodegom and Scholten, 2001).

4.2.2 Greater amounts of substrate through enhanced root decay

An important question that has remained unanswered is "how can we explain the large effect of warming on CH$_4$ emission in later growth stages?" One possible explanation is that enhanced root degradation provided more substrates for methanogenesis in the ET plots. Indeed, the time course of root biomass change suggests accelerated root decay in the later part of the season (Fig. 1). A simple stoichiometric evaluation further supports this hypothesis. For illustrative purposes, we here assumed that soil warming caused root degradation to be increased by ~ 10 g m^{-2} in ET compared with NT plots (based on the results shown in Fig. 1). If the entire amount of root loss was subjected to anoxic carbon

decomposition (we assumed a root carbon concentration of 40%), another 2 g of CH_4-C m^{-2} should have been produced in ET plots, an amount that can account for a considerable portion of the increased CH_4 released from panicle formation onward. Therefore, accelerated root decay by warming was of quantitative importance for the increased electron-donor availability for CH_4 production. Our conjecture is consistent with the results of a pot study using ^{13}C-labeled rice straw, which showed that organic material from rice plants plays a key role in supplying substrate for methanogenesis after the heading stage (Chidthaisong and Watanabe, 1997).

4.2.3 Comparisons with results of previous studies

To our knowledge, this is the first study focusing on both water and soil temperature elevation under open-field conditions. Previous reports have focused primarily on effects of air temperature elevation. For example, in an open-top chamber study, Ziska et al. (1998) found no enhancement in CH_4 flux with an experimental soil temperature elevation of < 0.5 °C, although air temperature was increased by 4 °C.

Rice grown in pots in a SPAR chamber showed significantly higher emission at warmer temperatures, especially under high-[CO_2] conditions (+160% by 6 °C air temperature elevation, $Q_{10} = 5.0$) (Allen et al., 2003). Although no attempt was made to control water temperature, it was raised by ∼ 3 °C as a result of the air temperature warming (Baker et al., 1994, their Figs. 3 and 4). As in our study, root biomass tended to decrease with increasing temperature later in the season (Allen et al., 2003, their Table 4). The similarities between their results and ours strongly support the hypothesis that accelerated root decay contributed to the enhanced CH_4 production in warming plots at later growth stages.

However, the underlying mechanisms responsible for the CH_4 enhancement may partly differ between the two studies. First, in our study, enhanced decomposition of rice stubble might be another cause of greater CH_4 emission, whereas residues seemed almost absent in the SPAR chamber study (Allen et al., 2003). Another important difference is the presence or absence of a carbon-allocation change in relation to heat-induced low fertility. With air temperature warming, the rice yield was drastically decreased by 65–80% (Baker et al., 1994), but a similar decrease did not occur with our water/soil temperature elevation. Allen et al. (2003) inferred that a greater amount of root exudation as partitioning of photoassimilates to seed production decreased was the main cause of enhanced CH_4 emissions. Their hypothesis is supported by the findings of another study in which restriction of carbon storage in seeds (by clipping of the spikelets) stimulated CH_4 emission (Denier van der Gon et al., 2002). In other studies, a high nighttime temperature (32 °C) during the reproductive stage also increased CH_4 emission, accompanied by an increase in total biomass and a reduction in grain yield, compared with a low nighttime temperature (22 °C) (Cheng et al., 2008, 2009). In our study,

however, soil warming did not negatively affect grain yield (Matsunami et al., 2010), indicating that carbon allocation was not altered much.

4.2.4 Implications and future research needs: experimental warming versus real global warming

Our open-field study clearly showed that rice paddies can be expected to respond positively to global warming. The warming effect may have an analogy for inter-annual variation in CH_4 emission observed under ambient temperature conditions. In a natural wetland (Hudson Bay Lowland), a large Q_{10} value of 7, similar to our warming effects, was observed from the relation between CH_4 emission and annual air temperature (Worthy et al., 2000). However, we are aware of no rice paddy studies which gave quantitative analysis on changes in long-term CH_4 emission in response to changing ambient temperature. We further conjecture that the increment in CH_4 emission would be even more severe if the storage capacity were decreased by heat stress (e.g., by increased sterility) caused by an air temperature increase. Under the presumption that global warming will eventually increase both air and water/soil temperature, it is desirable to conduct ecosystem-scale warming experiments, where both air and water/soil temperatures are elevated, in order to mimic the physiological responses of rice more realistically.

On the other hand, our experimental soil warming (stepwise warming) might have resulted in overestimation of CH_4 production from SOM, because the amount of decomposable SOM was identical, by definition, between the NT and ET plots at the start of warming treatment (especially in the first year). Under real global warming, temperature increases gradually, so the SOM content would be decreased by enhanced decomposition. Long-term monitoring of SOM is necessary to address this experimental problem.

4.3 Temperature insensitivity of Fe reduction

We found that synergistic effects can amplify the temperature sensitivity of CH_4 emission: that is, (i) the temperature insensitivity of Fe reduction coupled with (ii) moderate enhancement of organic matter decomposition (Fig. 6). The independence of Fe reduction with respect to temperature was surprising because higher temperatures usually accelerate Fe reduction until a certain optimal temperature is reached (Yao and Conrad, 2000). Different from our field observations, van Bodegom and Stams (1999) found a moderate temperature sensitivity of Fe(III) reduction ($Q_{10} = 2.4$) in well-homogenized soil slurries. What then accounts for the discrepancy between their results and ours?

We speculated that the electrons necessary for Fe(III) reduction were sufficient at our site even in the NT plots, whereas they were not in the incubation studies of van Bodegom and Stams (1999). If the reduction is regulated by the

electron donor supply rate, it should depend on temperature, because higher temperature leads to a higher rate of organic matter decomposition. In the present study, however, the electron donor supply might have been sufficient, presumably because of a larger amount of labile organic carbons (e.g., plant residue) than were present in the incubation studies (sieved soil is usually used). Instead, some physicochemical factors might be rate-limiting. Roden and Zachara (1996) showed that Fe(III) reduction is controlled by the available surface area of Fe(III) oxides. They also showed compelling evidence that the Fe(II) produced (mostly siderite; Kyuma and Kawaguchi, 1966; King, 1998; Ratering and Schnell, 2000) precipitates on the surface of Fe(III) oxides, preventing further reduction. More studies are necessary to judge whether the temperature independence is unique to this site, or whether it is more or less prevalent under field conditions.

4.4   Simultaneity of Fe reduction and CH$_4$ production

It is worth emphasizing the concurrency of Fe reduction and CH$_4$ production found in this study, because most earlier works found that CH$_4$ production is almost completely suppressed during Fe(III) reduction (Takai, 1961b; Takai and Kamura, 1966; Achtnich et al., 1995; Peters and Conrad, 1996). Our stoichiometric analyses clearly indicated that CH$_4$ production overlapped Fe(III) reduction over considerable periods of time (Fig. 4). This apparent contradiction compared with previous studies can be explained by the different experimental conditions. In the present study, the paddy soil was not homogeneous; therefore, the presence of plant debris and the rhizosphere might have provided varying redox conditions with various spatial scales. On the other hand, previous works used laboratory soil incubation techniques in which debris was removed from the soils, which were well homogenized before use, leading to uniform soil reduction.

To date, few data are available on the kinetics of Fe(III) reduction under field conditions. We suggest that measurements of Fe reduction along with CH$_4$ emissions are highly desirable, not only because Fe(III) is the dominant alternative electron acceptor but also because the competition pattern might differ from that observed in pure-soil incubations.

4.5   Uncertainties in the stoichiometric analyses

We showed that stoichiometric approach was useful for quantitatively evaluating the effects of FACE and/or warming on CH$_4$ production (Table 2, Figs. 4 and 6). Among many processes, Fe reduction should be underscored because the reducible Fe(III) content and the absolute rate of reduction and its sensitivity to temperature are important factors that can significantly affect the response of CH$_4$ production to climate change.

However, we made important assumptions in the stoichiometric analyses described above. The most important one is

that we ignored CH$_4$ oxidation, which would lead to underestimation of electrons utilized by CH$_4$ production, especially at early periods. In situ measurements of CH$_4$ oxidation rates have suggested that CH$_4$ consumption by methanotrophs can be significant ($\sim$ 40%) at the beginning of the season (Krüger et al., 2001), although it is of only minor importance ($<$ 7%) during later periods (Groot et al., 2003).

Electrons consumed by Fe reduction might also have been underestimated because we assumed no reoxidation of reduced Fe(II). Reoxidation of reduced Fe has been shown to be significant in the rhizosphere, where O$_2$ is transferred through the aerenchyma of plants (Roden and Wetzel, 1996; Frenzel et al., 1999). This process might be important because produced Fe(III) might, in turn, inhibit CH$_4$ production. At this point, we cannot quantitatively estimate this process.

We showed indirect evidence that root turnover provided substrates for CH$_4$ production, especially at later parts of the season; however, we could not quantify the amount of exudation materials from living roots, which might serve as electron donors for CH$_4$ production substantially (e.g. Aulakh et al., 2001a, b). For more comprehensive and rigorous stoichiometric analysis, it is necessary to quantify the absolute rate of root exudation, as well as its response to FACE and warming.

5   Conclusions

Free-air CO$_2$ enrichment ($+$ 200 µmol mol^{-1}) in combination with soil warming ($+$ 2 °C) increased CH$_4$ emission considerably, $\sim$ 80%, with no interaction between the two factors. This result indicates that increasing [CO$_2$] stimulates CH$_4$ emission from rice paddies, which in turn results in further global warming by increased atmospheric [CH$_4$].

The effect of FACE was not statistically significant in this study ($P = 0.19$); however, the tendency of higher CH$_4$ emission under FACE ($+$ 26%) was in agreement with the findings of previous FACE studies. Positive correlations between CH$_4$ emissions and above-ground and root biomass suggest that greater photoassimilation under high [CO$_2$] leads to higher rates of rhizodeposition, which provides important substrates for CH$_4$ production. The observed relative enhancement was, however, relatively moderate compared with values reported previously. Further studies are needed to better understand the mechanisms underlying year-to-year and site-to-site variations in the enhancement of CH$_4$ emission by high [CO$_2$].

Soil warming increased the seasonal CH$_4$ emission by $\sim$ 44% (pooled analysis result, $P < 0.001$) consistently over the [CO$_2$] treatments and the years. The resulting mean Q_{10} value was 5.5. The increased above-ground biomass could only partly explain the enhancement. We proposed that synergistic effects can amplify the temperature sensitivity of CH$_4$ emission: that is, (i) the temperature insensitivity of

Fe reduction, coupled with (ii) moderate enhancement in organic matter decomposition. This mechanism works as long as Fe reduction dominates the electron-accepting processes. At later growth stages, enhanced root senescence is likely to provide more substrates for methanogenesis under warmer conditions.

Finally, our results indicate that stoichiometric approach is useful to quantitatively understand the mechanisms responsible for the enhanced CH$_4$ emissions in response to FACE and/or warming. Challenges remain, however, on the determination of in situ root-exudation rate and its response to elevated [CO$_2$] and increasing temperature.

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