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ORIGINAL ARTICLE

Influences of free-air CO_2 enrichment (FACE), nitrogen fertilizer and crop residue incorporation on CH_4 emissions from irrigated rice fields

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Abstract To investigate the response of methane (CH_4) emissions to an elevated atmospheric carbon dioxide (CO_2) concentration (200 ± 40 μmol mol^{-1} higher than the ambient atmosphere), we performed a 4-year multi-factorial experiment at a subtropical rice paddy that contained sandy loam soil in the Yangtze River Delta from 2004 to 2007 using free-air CO_2 enrichment (FACE) technology. Our results revealed that the elevated atmospheric CO_2 increased the seasonal cumulative CH_4 emissions by 15 % on average during the 4-year period. The increase was insignificant and much weaker than the previous studies, which might be primarily attributed to the absence of a significant difference in the rice biomass between the two CO_2 levels in half of the field treatments. Crop residue incorporation hindered the stimulatory effects induced by the elevated CO_2, which were 37, 14 and 6 % for the fields that were incorporated with none, half or all of the wheat straws that were harvested in the preceding winter wheat season, respectively. Nitrogen fertilizers application also hindered the stimulatory effects of the elevated CO_2 on the CH_4 emissions. The CO_2 stimulatory effect was 39 % for the field without nitrogen fertilizers, and reduced to 17, 7 and 5 % for the field with nitrogen fertilization of 125, 250 and 350 kg N ha^{-1}, respectively. The regulation of nitrogen fertilizers on the CO_2 effects in this experiment does not well agree with the previous studies, which might because the soil type was different from those of the previous studies. Thus, further studies are necessary to evaluate the role of soil properties in regulating the effects of elevated atmospheric CO_2 on CH_4 emissions from managed and natural wetlands. There were no significant interactions between the atmospheric CO_2 and the incorporations of nitrogen fertilizer and crop residue. Appropriate experiments are necessary for better understanding of the interact influences of the elevated CO_2 and farm managements.

Keywords Carbon dioxide · Methane · Emission · Free-air CO_2 enrichment (FACE) · Rice field · Nitrogen · Crop residue

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Introduction

Despite the dedicated efforts of scientists and policymakers in many countries, anthropogenic greenhouse gas emissions still increased at a marked rate over the last two decades (IPCC 2007). Methane (CH_4), which is the second most important greenhouse gas, contributes to approximately 20 % of the anthropogenic global warming effect (Houghton et al. 2001). Paddy rice fields account for approximately 5–19 % of the total global CH_4 emissions to the atmosphere (IPCC 2007). The intensification of rice production is imperative in meeting the increasing demand for rice by the increasing human population (Cassman et al. 1998). This demand will most likely lead to increased CH_4 emissions if no appropriate mitigation strategies are developed and applied. The increasing atmospheric carbon dioxide (CO_2) concentration is hypothesized to amplify the level of CH_4 that is released from not only natural (Dacey et al. 1994; Hutchin et al. 1995) but also managed wetlands, including irrigated rice fields (Inubushi et al. 2003; Tokida et al. 2010; Zheng et al. 2006; Ziska et al. 1998).

Empirical records provide incontestable evidence of global changes. The foremost among these changes is the increasing atmospheric CO_2 concentration, which has been predicted to double by the mid-twentyfirst century compared to the concentration in the pre-industrial era (IPCC 2001). Many studies have demonstrated a positive effect of elevated CO_2 levels on rice biomass (above and below ground) and grain yield (Kim et al. 2001, 2003; Yang et al. 2007). Elevated CO_2 levels also increase the soil microbial carbon levels, and they accelerate the turnover rate of the soil microbial carbon during the middle and later parts of the rice growing season (Cheng et al. 2001; Hoque et al. 2001). The stimulation of rice root biomass and tiller number by elevated CO_2 is expected to indirectly affect the CH_4 production and emission from paddy rice fields. Based on a few experiments that were performed under environmentally controlled conditions, such as in open top chambers (Dacey et al. 1994) or greenhouses (Yagi et al. 2002), an atmospheric CO_2 level that was elevated by 60–100 % above the ambient level significantly increased the CH_4 emission (by 19–300 %) in temperate (Cheng et al. 2006; Yagi et al. 2002) and tropical rice paddies (Allen et al. 2003; Ziska et al. 1998).

Free-air CO_2 enrichment (FACE) techniques have been developed in the past two decades (Kimball 1992; Okada et al. 2001) and have recently been applied to investigate the effects of the atmospheric CO_2 enrichment on the CH_4 emissions from managed (Inubushi et al. 2003; Tokida et al. 2010; Zheng et al. 2006) and natural (Silvola et al. 2003) wetland ecosystems. The FACE techniques can facilitate undisturbed field conditions and produce more reliable measurements in comparison with the use of environmentally controlled facilities (Long et al. 2006; Okada et al. 2001). A long-term FACE study of temperate paddy rice fields in Japan showed increases (26–51 %) in CH_4 emissions as a result of long-term CO_2 enrichment (Inubushi et al. 2003; Tokida et al. 2010). Another FACE study of subtropical paddy rice fields that were subjected to a rice–wheat rotation regime in China showed a significant stimulatory effect (53–188 %) on CH_4 emissions without or with a medium addition rate (1,000 kg C ha^{-1}) of wheat straw, but there was no significant effect when the conditions included a high addition rate (2,000 kg C ha^{-1}) of wheat straw (Zheng et al. 2006). The FACE studies that were simultaneously performed at five sites along a transect of oligotrophic peatland mires in Europe demonstrated no significant CO_2 effects on CH_4 emissions (Silvola et al. 2003). The discrepancy among these studies may be partly due to differences in experimental conditions, including different climates, soil types and farm management. In fact, CH_4 emissions from wetland ecosystems are directly controlled by a series of biochemical and microbial processes that are regulated by soil types and water regimes (Conrad 2002; Schimel 2000).

Nitrogenous fertilizers are commonly utilized in rice cultivation to achieve high grain yields. There is an ongoing discussion regarding the possible effects of nitrogen application on the CH_4 emission from rice fields. The application of nitrogen may directly or indirectly influence the processes that regulate CH_4 emission from paddy rice fields, which include CH_4 production, oxidation, and transport from the soil to the atmosphere. Nitrogen application not only stimulates the growth and activity of methane oxidizing bacteria (Bodelier and Laanbroek 2004; Schimel 2000), but also enhance the growth and activity of methanogenic bacteria (Cai et al. 2007). Nitrogen application would also affect CH_4 production and transport through the effect on rice growth because rice plants can provide organic substrates and a major pathway transporting CH_4 from the paddy soil to the atmosphere (Huang et al. 2004; Kludze et al. 1993; Le Mer and Roger 2001). Because of the complexity and counter-balance among the different effects, it is still difficult to assess the net nitrogen effect on CH_4 emissions from rice fields.

Crop residue amendments have been commonly utilized to improve the soil fertility for rice production in China. The incorporation of crop residues provides a source of readily available carbon, which is believed to induce the higher release of CH_4 from rice paddies (Denier van der Gon and Neue 1995; Wang et al. 1999). The decomposition of incorporated organic material is the predominant source of methanogenic substrates in the early stage of the rice-growing season (Huang et al. 2004).

The incorporation of organic matter and nitrogen fertilizer may regulate the effect of an elevated CO_2 on CH_4 emissions from paddy rice fields. Zheng et al. (2006) reported that the impacts of the long-term atmospheric CO_2 enrichment on the CH_4 emissions from nitrogen-rich paddy rice ecosystems correlated positively with the nitrogen addition rates but negatively with the addition rates of decomposable organic carbon; both of the correlations were related to the soil nitrogen availability. However, studies that focus on the regulation of different farm management practices on the effects of elevated atmospheric CO_2 and the CH_4 emissions from rice fields are still lacking.

Currently, only three FACE studies of paddy rice ecosystem have been reported. All of the previous studies were conducted in clay paddies that utilized flooding water during the rice growing period, with the exception of one or two explicit mid-season drainages (Inubushi et al. 2003; Tokida et al. 2010; Xu et al. 2004; Zheng et al. 2006). Only one of the research projects performed a preliminary study of the simultaneous influences of elevated atmospheric CO_2, nitrogen fertilizer and organic matter amendments on CH_4 emissions. Furthermore, the response of CH_4 emissions from paddy rice ecosystems to the elevated CO_2 likely varies for different soil types. However, experimental evidence is still lacking. To enhance our understanding of the response of CH_4 emissions to elevated CO_2 conditions and different farm management practices, we performed a 4-year multi-factorial experiment using a FACE platform on a subtropical paddy rice field that was subjected to a rice–wheat rotation. This experimental site was located on a sandy loam soil in the Yangtze River Delta. Here, we report the results of this experiment.

Materials and methods

Experimental site and rice cultivation

The experimental platform of free-air CO_2 enrichment (FACE) was established over a rice-winter wheat rotation ecosystem located in a suburb (32°35′N, 119°42′E) of Jiangdu county in Jiangsu province, which is located in the Yangtze River Delta region. The crop rotation pattern accounts for approximately 60 % of the regional paddy area (Zheng et al. 2000), and it is typical for other regions that have a similar climate, such as in eastern, central and southwestern China (Zheng et al. 2000; Zheng et al. 2006). This region displays a north subtropical monsoon climate. The annual precipitation is 918–978 mm, and 60 % of the precipitation occurs from June to September. The annual mean air temperature is 14.9 °C, and the annual frost-free period is approximately 220 days. The soil at the experimental site is Shajiang-Aquic Cambosols (CRGCST-Cooperative Research Group on Chinese Soil Taxonomy, 2001) with a sandy loam texture. The relevant soil properties at a 0–15 cm depth are as follows: tillage layer depth 13.0 cm, bulk density 1.16 g cm^{-3}, soil organic carbon 18.4 g kg^{-1}, total nitrogen 1.45 g kg^{-1}, total phosphorous 0.63 g kg^{-1}, total potassium 14.0 g kg^{-1}, available phosphorous 10.1 mg kg^{-1}, available potassium 70.5 mg kg^{-1}, sand (0.02–2 mm) 57.8 %, silt (0.002–0.02 mm) 28.5 %, clay (<0.002 mm) 13.7 % and pH 7.9 (water).

The early-maturing late japonica rice (*Oryza sativa* L.) cultivar Wuxiangjing 14 was planted in this study. The rice seeds were sown in a separate seedling bed in mid-May and transplanted into the experimental plots in mid-June. The seedlings in the ambient plots were grown under the ambient CO_2 concentration, while those in the FACE plots were grown under the elevated CO_2 concentration. The seedlings were manually transplanted at a density of 3 seedlings $hill^{-1}$ and 24 hills m^{-2} and harvested in mid- to late October. Each rice season was followed by a winter wheat (*Triticum aestivum* L.) growing period, which lasted from mid-November until the following late May. The fields were left fallow for approximately 2 weeks between the two cropping seasons. The farming practices (e.g., water management and the application of pesticides and herbicides) were applied following the local farmers' traditions. The fields were irrigated 2 or 3 days before rice transplanting, and a 1-week long mid-season drainage occurred at approximately 30 days after transplanting (DAT). After the drainage, the fields were intermittently irrigated until the final drainage, which occurred approximately 3–4 weeks before the harvest. No irrigation was adopted after the last drainage, and this lasted until the submergence for the cultivation of rice in the following year.

FACE system

The experiment was organized to facilitate completely randomized replicates with split–split subplots. The atmospheric CO_2 concentration was treated as the main effect. The nitrogen addition was the split-plot treatment, and crop residue was the split–split plot treatment. The rice FACE system has six plots—three plots were randomly allocated for the elevated CO_2 treatments (200 ± 40 μmol mol^{-1} higher than the ambient level, hereinafter referred to as FACE) and three for the ambient treatments (375 μmol mol^{-1} on average). In the FACE plots, the plants were grown within 12 m diameter 'rings' in which pure CO_2 gas was released from peripheral emission tubes set 0.5 m above the canopy. A nominal usable area of each plot was ca. 80 m^2 with a 1 m buffer zone around the emission tubes. The ambient plots had no ring structures, and the plants were grown under ambient CO_2 condition. The FACE treatment was applied for daylight hours in both the rice and wheat growing seasons, and from mid-June 2004 to late October 2007. The FACE treatment was, however, occasionally paused during short fallow periods (approximately 2 weeks) between the two crop-growing seasons. Details of the design, rationale, operation, and performance of the FACE system can be found in previous reports (Liu et al. 2002; Okada et al. 2001). Each CO_2 plot was equally split into two subplots applied with different levels of nitrogen fertilizers. In each nitrogen subplot, three levels of crop residue subplots were randomly assigned.

Nitrogen and crop residue additions

Ammonium-based nitrogen fertilizers (urea plus compound fertilizer—N:P_2O_5:K_2O = 15 %:15 %:15 %) and wheat residues that were harvested in the proceeding crop season were applied in the rice seasons. Four levels of nitrogen addition rates (0, 125, 250 and 350 kg N ha^{-1}, which are hereinafter referred to as N0, N125, N250 and N350, respectively) were applied for both the FACE and ambient treatments in the rice season (Table 1); N125 and N250 were applied in 2004

Table 1 Field treatments of the nitrogen fertilizer (kg N ha^{-1})

Years	Residue codes	Fertilizer codes[a]	Total fertilizer nitrogen	Basal fertilizer[b]	Tillering fertilizer[c]	Earing fertilizer[d]
2004	HR, MR, ZR	N250	250	90	60	100
		N125	125	75	0	50
2005	HR, MR, ZR	N250	250	90	60	69
		N125	125	75	0	24
2006	HR, MR, ZR	N350	350	126	84	140
		N0	0	0	0	0
2007	ZR	N350	350	126	84	140
		N0	0	0	0	0

The organic matter additions and their referring codes

HR high residue rate (2,000 kg C ha^{-1}), *MR* moderate residue rate (1,000 kg C ha^{-1}), *ZR* no residue added

[a] The codes N0, N125, N250 and N350 indicate the nitrogenous fertilizer application of 0, 125, 250 and 350 kg N ha^{-1}, respectively, during the rice season

[b] The basal fertilizer was applied on 15 June (i.e., 1 day prior to transplanting)

[c] The tillering fertilizer was applied on 21 June

[d] The earing fertilizer was applied on 28 July

and 2005, and N0 and N350 were applied in 2006 and 2007. A replicate nitrogen subplot was approximately 40 m^2. The time and amount of nitrogen application in various nitrogen subplots can be found in Table 1. Phosphorous and potassium fertilizers were basally applied in all of the subplots at the rates of 33 kg P ha^{-1} and 62 kg K ha^{-1} in each rice season. The nitrogen fertilizer types were the same in all the experiment years. For N0 subplots in 2006 and 2007, phosphorous fertilizer was $Ca(H_2PO_4)_2$ and potassium fertilizer was KCl. For all the other nitrogen subplots, phosphorous and potassium fertilizers were from compound fertilizers ($N:P_2O_5:K_2O$ = 15 %:15 %:15 %).

For each nitrogen subplot, three wheat residue incorporation rates (0, 1,000 and 2,000 kg C ha^{-1}, hereinafter referred to as ZR, MR and HR, respectively) were applied in 2004–2006. In 2007, however, only ZR was applied. The field area of each residue treatment replicate was at least 1 m^2. The winter wheat stubble, the height of which was approximately 5–10 cm, remained for all of the field plots. One day before submergence, the crop residues (harvested wheat straw from the previous crop season that was cut to 10 cm long) of the MR and HR treatments were incorporated into the cultivation layer of the sub-subplots for all of the nitrogen treatments. In the previous winter wheat seasons, the rice stubble (5–10 cm high) remained in the fields. No additional crop residues or manure from other sources was incorporated in the rice–wheat rotation.

Measurement of the aboveground biomass

At the harvest, the aboveground rice biomass was measured by drying at 80 °C for 48 h.

Measurement of CH_4 flux

The measurements of the CH_4 emissions in situ were performed in the rice seasons of 2004–2007. The methane fluxes from each sub-subplot were measured once every 3–4 days using static, opaque chamber methods in combination with gas chromatography techniques (Wang and Wang 2003; Xu et al. 2004; Zheng et al. 2006). A stainless-steel frame, which covered an area of 0.25 m^2 and six hills of rice, was permanently installed in the center of each sub-subplot. A 0.5 or 1 m high, gas-tight chamber was temporarily installed on the frame when sampling occurred. To determine the CH_4 flux, five gas samples were taken with 60-ml plastic syringes at 5 min intervals. The sampling process was completed between 0800 and 1100 LST, at which time the measured flux was expected to accurately represent the daily mean emission (Zheng et al. 1998). Immediately after taking the fifth gas sample, the chamber was removed from the frame. Within at most 10 h of sampling, the gas samples that were stored in the syringes were analyzed using a gas chromatograph with a flame ionization detector (FID; Wang and Wang 2003; Xu et al. 2004) in a temporary laboratory that was located near the experimental site.

Statistical analysis

For analyses of differences of CH_4 daily fluxes and the aboveground rice biomasses on a single year basis, a split-plot design was used, with CO_2 levels as the main plots, nitrogen levels as the subplots, and crop residue as the sub-subplots. One-way ANOVA was used to evaluate the CO_2 effect on rice biomasses. One-way repeated measures ANOVA was applied to test the CO_2 effect when all data of daily CH_4 fluxes were grouped in 2 categories of FACE and ambient. The regulations of nitrogen fertilization and crop residue incorporation on the CO_2 effects on seasonal cumulative CH_4 emissions were determined by paired *t* test. The levels of $p \geq 0.10$, <0.10, <0.05 and <0.01 were considered to be not significant, slightly significant, significant and highly significant, respectively.

Results

Aboveground rice biomass

In all of the sub-subplots during 2004–2007, with the exception of N0-HR in 2006, the aboveground rice biomass at the time of harvest was enhanced by the elevated CO_2 level (Fig. 1). The mean increase is about 23 %, with a range from 3 to 43 %. Among the totally 20 pairs of treatments, however, only 11 pairs showed significant CO_2-induced increases in aboveground rice biomass (Fig. 1). Both the crop residue and the nitrogen fertilizer incorporation slightly altered the effects of the elevated atmospheric CO_2 on the rice biomass. In the ZR sub-subplots with various nitrogen addition rates, the effect of the elevated atmospheric CO_2 on the rice biomass was 27 % on average, whereas the CO_2 effects in MR and HR reduced to 14 and 13 %, respectively. In N0, N125, N250 and N350, the stimulations of the elevated atmospheric CO_2 on the rice biomass were 12, 16, 24 and 19 %, respectively.

CH_4 flux

The seasonal dynamic patterns of the CH_4 fluxes were very similar under the FACE and ambient conditions, but they varied in different years (Fig. 2). For the HR and MR sub-subplots, the CH_4 fluxes rapidly increased after transplanting and remained high until the mid-season drainage at approximately 30 DAT. During the following intermittent irrigation periods, CH_4 was emitted at relatively high rate until approximately 90 DAT in 2004, 60 DAT in 2005, and 105 DAT in 2006. After this time, the CH_4 fluxes remained close to zero. For the ZR sub-subplots, the CH_4 fluxes were much lower than those of HR and MR, but the seasonal patterns in 2004–2006 were similar to those of HR and MR. In 2007, however, the CH_4 fluxes from the ZR sub-subplots gradually increased until approximately 60 DAT and then gradually decreased. The average CH_4 fluxes in a single year varied largely with the different rates of the organic matter addition, but showed only slight differences between the two CO_2 or nitrogen levels. The ranges of the CH_4 fluxes were 8–15, 5–12, and 2–8 mg CH_4-C m^{-2} h^{-1} for HR, MR and ZR, respectively. The CH_4 fluxes from the MR or ZR sub-subplots showed no significant inter-annual differences in 2005–2007, while the CH_4 fluxes from the HR sub-subplots showed significant inter-annual differences in 2004–2007 rice seasons.

Fig. 2 Methane (CH_4) fluxes that were observed in the plots under elevated (FACE) and ambient (Ambient) CO_2 conditions. The averages of three replicates are provided. The *vertical error bars* represent standard errors. The definitions of N0, N125, N250, N350, ZR, MR and HR are provided in the footnotes of Table 1 and in the text

Effects of the elevated CO_2 level on the seasonal cumulative CH_4 emission

Table 2 presents the seasonal cumulative CH_4 emissions from the paddy rice fields under the FACE and ambient conditions. With various nitrogen fertilizer or crop residue incorporation rates, the seasonal CH_4 emissions varied largely; they ranged from 72 to 445 kg CH_4–C ha^{-1} and 94–499 kg CH_4–C ha^{-1} for the ambient and elevated CO_2 treatments, respectively. Sixteen of the 20 pairs of CO_2 treatments showed increases of CH_4 emissions that were induced by the elevated CO_2, whereas the other data showed slight decreases. Based upon the average measurements of all the sub-subplots, the elevated atmospheric CO_2 increased the seasonal cumulative CH_4 emissions

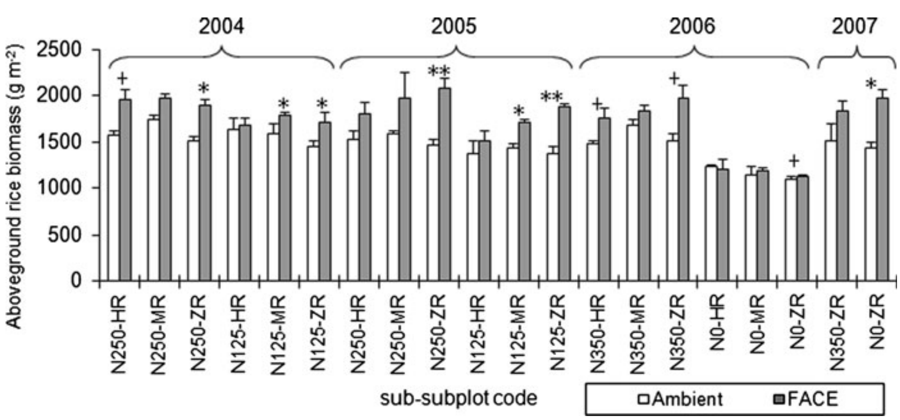

Fig. 1 The aboveground rice biomass (g m^{-2}) at the harvest in 2004–2007. The given data are the means of three replicates under the elevated (FACE) or ambient CO_2 (Ambient) conditions. The definitions of N0, N125, N250, N350, ZR, MR and HR are provided in the footnotes of Table 1 and in the text. The symbols +, *, and ** indicate the significant levels of $p < 0.10$, $p < 0.05$, and $p < 0.01$ respectively, for the differences between the two CO_2 levels, while no symbol indicates no significance ($p \geq 0.10$) in the ANOVA test

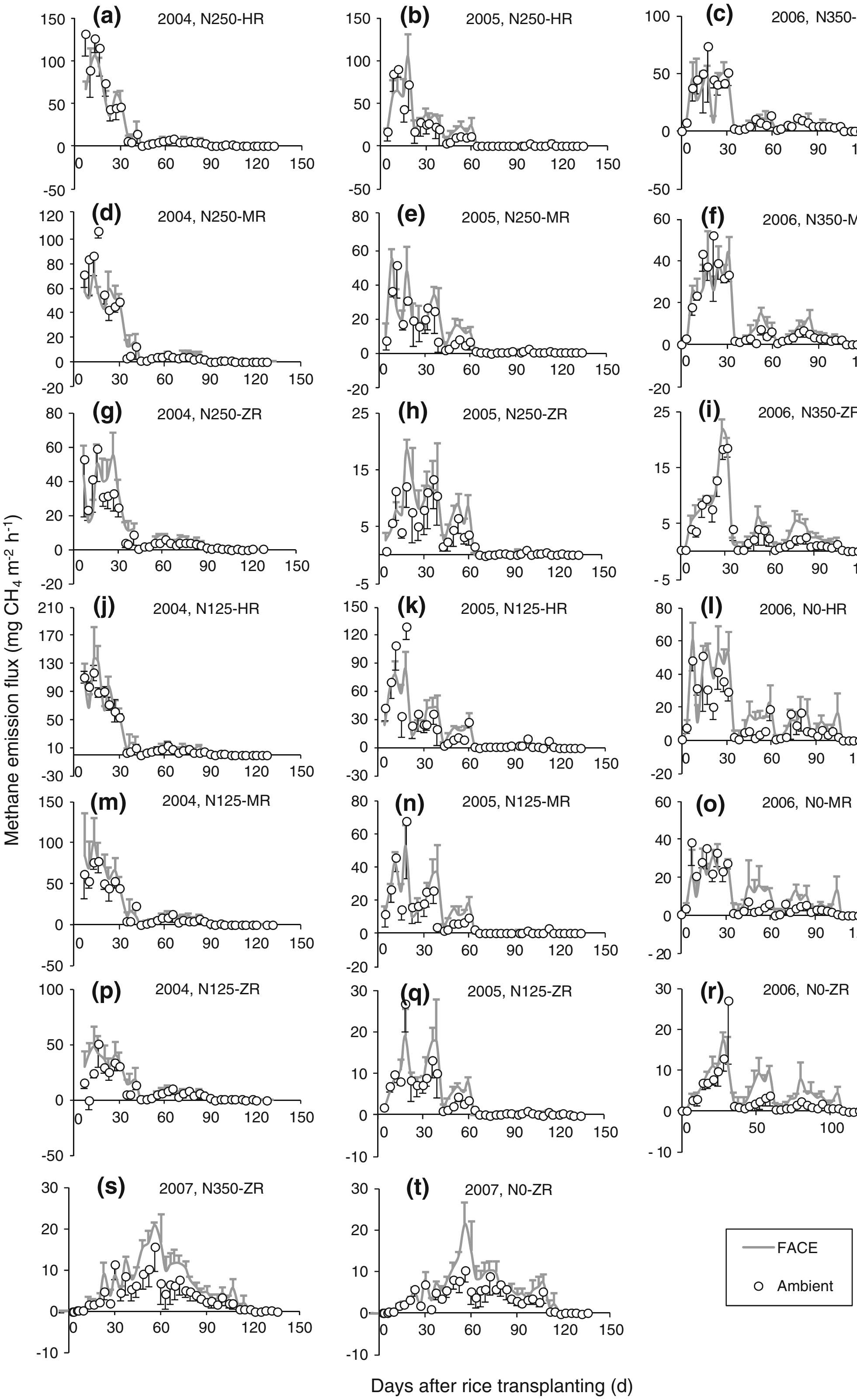
(a) 2004, N250-HR
(b) 2005, N250-HR
(c) 2006, N350-HR
(d) 2004, N250-MR
(e) 2005, N250-MR
(f) 2006, N350-MR
(g) 2004, N250-ZR
(h) 2005, N250-ZR
(i) 2006, N350-ZR
(j) 2004, N125-HR
(k) 2005, N125-HR
(l) 2006, N0-HR
(m) 2004, N125-MR
(n) 2005, N125-MR
(o) 2006, N0-MR
(p) 2004, N125-ZR
(q) 2005, N125-ZR
(r) 2006, N0-ZR
(s) 2007, N350-ZR
(t) 2007, N0-ZR
FACE
Ambient
Methane emission flux (mg CH_4 m^{-2} h^{-1})
Days after rice transplanting (d)

Table 2 Seasonal cumulative methane (CH_4) emissions (SME) from the paddy rice fields that were exposed to the elevated carbon dioxide (F) or the ambient (A) condition and their relative changes that were induced by the elevated carbon dioxide

Years	Code	SME(kg CH_4-C ha^{-1})		
		F	A	Changes (%)
2004	N250-HR	392 (40)	425 (45)	−8 (16)
	N250-MR	319 (22)	347 (42)	−8 (12)
	N250-ZR	243 (36)	205 (52)	18 (20)
	N125-HR	499 (113)	445 (26)	12 (34)
	N125-MR	410 (120)	325 (56)	26 (51)
	N125-ZR	261 (47)	180 (51)	45 (69)
2005	N250-HR	379 (41)	316 (76)	20 (16)
	N250-MR	233 (38)	182 (61)	28 (22)
	N250-ZR	94 (17)	72 (24)	30 (42)
	N125-HR	388 (49)	396 (56)	−2 (11)
	N125-MR	237 (30)	193 (45)	23 (29)
	N125-ZR	98 (11)	79 (16)	24 (35)
2006	N350-HR	268 (45)	317 (84)	−16 (38)
	N350-MR	229 (55)	219 (32)	4 (31)
	N350-ZR	92 (5)	74 (8)	24 (17)
	N0-HR	369 (67)	259 (53)	42 (63)
	N0-MR	222 (47)	184 (18)	21 (29)
	N0-ZR	100 (13)	66 (17)	52 (87)
2007	N350-ZR	149 (26)	93 (37)	61 (37)
	N0-ZR	133 (25)	85 (23)	57 (28)

The definitions of N0, N125, N250, N350, ZR, MR and HR are provided in the footnotes of Table 1 and in the text. The values that are outside of the parentheses are the means of three replicates, and those inside the parentheses are the standard errors. The relative changes were calculated by $(F - A)/A \times 100$

by 15 %. However, the difference was not statistically significant. A significant interaction between N fertilization and elevated CO_2 was found in 2007, however, there were no other significant interactions among the atmospheric CO_2, N and crop residue incorporations (Table 3).

Regulation of the crop residue and nitrogen fertilizer addition on the effect of elevated CO_2

The data in Table 2 clearly indicate that the effect of the elevated CO_2 on the seasonal cumulative CH_4 emissions was affected by crop residue incorporation. This regulation is further interpreted in Fig. 3a. The CO_2 effect became weaker with the increasing residue addition rates. On average, the CO_2 effects were 37 ($p < 0.01$), 14 ($p < 0.1$) and 6 % ($p > 0.1$) for ZR, MR and HR, respectively. The significance level of the CO_2 effect also decreased with the increasing residue addition rates (Fig. 3a).

As shown in Fig. 3b, nitrogen fertilizer seemed to inhibit the effect of elevated CO_2 on the seasonal cumulative CH_4 emissions. The highest stimulation of the elevated CO_2 on CH_4 emissions occurred in the N0 subplots, in which the CO_2 effect was 37 % ($p < 0.05$). In the N125, N250 and N350 subplots, the averaged CO_2 effects reduced to 17 ($p < 0.05$), 7 ($p > 0.1$) and 5 % ($p > 0.1$), respectively.

Discussion

High amount of CH_4 emission in 2004 rice growing season

The addition of organic matters such as crop residue is one of the major methanogenic substrates in rice

Table 3 The significance levels (p values) of CO_2, nitrogen (N), residue (R), and their interactions on CH_4 emission from 2004 to 2007

Years	CO_2	N	R	CO_2*N	CO_2*R	N*R	CO_2*N*R
2004	0.643	0.636	0.004	0.392	0.924	0.771	0.967
2005	0.389	0.581	0.000	0.722	0.955	0.879	0.913
2006	0.341	0.992	0.000	0.227	0.997	0.825	0.514
2007	0.170	0.000		0.016			

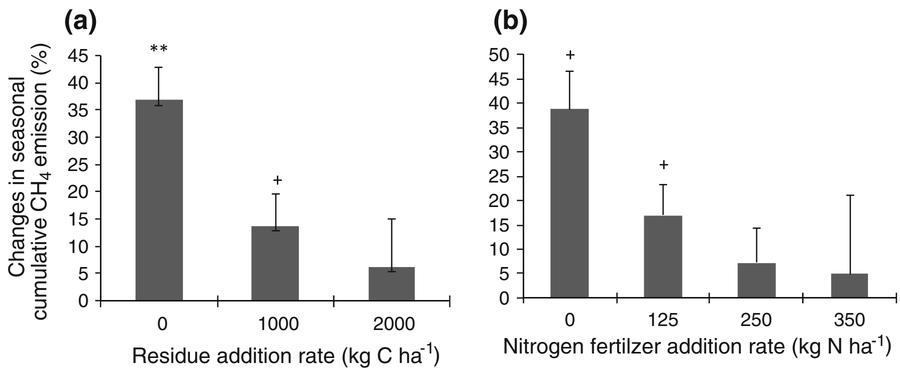

Fig. 3 The relative changes in the seasonal cumulative CH_4 emission that were caused by the elevated CO_2: **a** the crop residue treatment, **b** the nitrogen treatment. The *error bars* represent the standard errors. The symbols + and ** indicate the significant levels of $p < 0.10$ and $p < 0.01$, respectively, while *no symbol* indicates no significance ($p \geq 0.10$) for the differences between the two CO_2 levels

paddies (Huang et al. 2004; Zheng et al. 2006). Thus, we checked the inter-annual differences of CH_4 emission with the same residue addition rate. There were no significant inter-annual differences in CH_4 emissions among 2005 to 2007. However, the seasonal cumulative CH_4 emissions in 2004 were larger than the means of the following 3 years by 31, 65 and 136 % for HR, MR, and ZR, respectively. There was a special farm management in 2004 different from 2005 to 2007. The straws of previous winter wheat were removed from the fields before rice transplanting in 2005–2007. However, in 2004, i.e. the first rice season of the FACE study, the straws of previous winter wheat were burned in the field and the ashes were ploughed into soil 2 days before submergence. The in situ combustion of straws may contribute to the high rate of CH_4 emission in 2004. Ma et al. (2008) found a similar result that in situ combustion of straws could enhance CH_4 emission from paddy rice fields by 130 %. They suggested that the ashes from the wheat straws contained much organic carbon which could be utilized by methanogens, resulting in increased CH_4 emission..

Effects of the elevated CO_2 on CH_4 emission from intermittently irrigated rice fields

The emission of CH_4 from irrigated rice fields is controlled by the production of CH_4 as well as oxidation and transport processes, all of which can be affected by elevated atmospheric CO_2. Most of the published environment-controlled or FACE studies have consistently demonstrated that elevated atmospheric CO_2 could significantly stimulate CH_4 emissions from paddy rice fields (Cheng et al. 2006; Inubushi et al. 2003; Xu et al. 2004; Zheng et al. 2006; Ziska et al. 1998). Analyzing both previous studies and their own studies, Zheng et al. (2006) suggested that elevated CO_2 affected CH_4 emission from paddy rice fields primarily by stimulating rice growth.

The 15 % stimulation that was observed in the present study is much weaker than the previous FACE studies (Inubushi et al. 2003; Tokida et al. 2010; Zheng et al. 2006). A previous FACE study that was also conducted in the Yangtze River Delta showed significant stimulation of CH_4 emissions from 53 to 188 % (Zheng et al. 2006). A major difference is that the previous study was conducted in clay paddies, whereas the present study was conducted in sandy loam paddies. The hydraulic conductivity of sandy loam is usually higher than that of clay soil, which requires more irrigation to meet the rice growth demand. In fact, the field in the present study was intermittently irrigated, which might result in higher soil redox potential (Eh) than long-term flooded soil (private communication with Dr. Xiaozhi Wang of Yangzhou University). Soil Eh is one of the most sensitive environmental factors that control CH_4 production. The relatively high soil Eh can remarkably inhibit CH_4 production (Mitra et al. 2002a, b; Wang 2001). Therefore, the different soil type may alter the effect of the elevated CO_2 on the CH_4 emission.

When we statistically tested the effect of elevated CO_2 for each pair of the elevated and ambient CO_2 treatments, it shows the stimulation is not significant. The reasons may include the flowing aspects.

Firstly, as mentioned above, the elevated CO_2 indirectly stimulates CH_4 emission primarily via intensifying rice growth. In the present study, probably due to the uncontrolled rice diseases (ex. rice stripe Tenuivirus) and inconsistent irrigation frequencies and water levels, nearly half of the total 20 pairs of sub-subplots showed no significant differences in the aboveground rice biomass between the two CO_2 levels (Fig. 1). Consequently, the relevant factors or processes (methanogenic substrates, methanogen and methanotroph activity, vascular CH_4 transport) controlled by rice growth may not significantly vary between the two CO_2 levels, which resulted in small and insignificant differences in CH_4 emission from the FACE and ambient fields.

Methane is produced by methanogens under anaerobic conditions, and is oxidized to CO_2 or assimilated into the microbial biomass by methanotrophs under aerobic conditions. Therefore, the populations of methanogens and methanotrophs are key regulators in CH_4 emission (Conrad 1999; Kruger and Frenzel 2003; Kruger et al. 2001; Yao and Conrad 1999). In the FACE study that was conducted in Wuxi in the Yangtze River Delta (Zheng et al. 2006), the methanogen populations were significantly increased by 197 ± 71 %, whereas the methanotrophic populations only changed by 2 ± 16 % (Yue et al. 2007), which contributed largely to the stimulation of CH_4 emission by elevated CO_2 (Zheng et al. 2006). Using the most probable number (MPN) method as Yue et al. (2007), Wang et al. (2006) measured the populations of methanogens and methanotrophs in the fresh soils of four sub-subplots (2 HR and 2 ZR) in the present experimental fields in 2005 rice season. They found that the elevated CO_2 had no significant impacts on populations of both methanogens and methanotrophs, which may be the major microbial reason causing a weaker stimulation of CH_4 emissions by elevated CO_2.

Similar to our results, a recent FACE study in Japan found that the enhancement (+26 %) in CH_4 emission resulting from the elevated atmospheric CO_2 (ambient + 200 μmol mol^{-1}) was statistically insignificant (Tokida et al. 2010). They speculated that the absence of a significant stimulatory effect of FACE on CH_4 emission could be attributable partly to the heterogeneity of the soil within the experimental sites. Obviously, more studies are needed to understand the underlying mechanisms responsible for the large variation in the effects of elevated CO_2 on CH_4 emissions from study to study.

The statistical power of ANOVA in this study was very low. Three replications of FACE rings only gave a degree of freedom for all effect of 1 or 2 for the error. Therefore, there was a high risk of type II error—falsely rejecting the null hypothesis (no difference between FACE and ambient), i.e., in our study an actual simulating effect of elevated CO_2 on CH_4 emission from rice paddies was probably disregarded by the test. Although the statistical test failed to reject the null hypothesis, but never proved that there was no difference between FACE and ambient treatments. In general, increasing the sample size can reduce type II error. We collectively tested the effect of elevated CO_2 by splitting all data into two categories, FACE and ambient, as treating the sub treatments as repeated measurements in different conditions. This analysis increased the effect size to 120 (20 pairs F-A with 3 repetitions) and consequently indicated that elevated CO_2 significantly stimulated CH_4 emission. However, in experimented fields, the effort to increase sample size generally may not be feasible. Therefore, many of the effect, even though having a rather high effect size, were not significant because of type II error, like our split-plot ANOVA test.

Impacts of the crop residue and nitrogen fertilizer incorporation on CH_4 emission in response to elevated CO_2

To our knowledge, there is only one published report that focuses on the impacts of crop residue and nitrogen fertilizer incorporation on CH_4 emissions in response to elevated CO_2 (Zheng et al. 2006). The present study showed that crop residue incorporation appeared to inhibit the stimulatory effect of elevated CO_2 on CH_4 emission. An important reason for this result may be related to the different elevated CO_2-induced increases of rice biomass in various crop residue treatments. In the ZR sub-subplot, the elevated CO_2 stimulated the aboveground rice biomass by 26 %, which could provide a large increase in the methanogenic substrates. However, the increments of the rice biomass induced by the elevated CO_2 dropped to 12 % in the MR and HR sub-subplots, where the increased proportions of the methanogenic substrates would be lower than in ZR, thus resulting in weaker stimulations of CH_4 emissions by elevated CO_2.

Zheng et al. (2006) also reported that the significant stimulation of CH_4 emissions by the elevated CO_2 correlated negatively with the addition rates of the organic matter. They suggested that this result might be due to an increased C/N ratio in the incorporated wheat straw that was produced under the elevated CO_2. The increased C/N ratio would limit the decomposition of the residue, and it would therefore result in a decrease of the methanogenic substrates. Liu et al. (2009) reported an incubation study that focused on the decomposition process of crop residue that was mixed with a paddy soil. The authors used winter wheat and rice straw that was produced under ambient and elevated CO_2 conditions in the same FACE station as reported by Zheng et al. (2006). They found that there were no significant differences in the straw-induced CH_4 emissions of the incorporated wheat straw that were produced either under ambient or under elevated CO_2 conditions. It should be noted that the soil in the incubation was not taken from the same FACE station but rather from an experimental station of the International Rice Research Institute; furthermore, the incubation experiment was not performed under elevated CO_2 conditions. Consequently, the incubation results may vary largely from the in situ FACE experiment. Therefore, appropriate experiments are necessary to understand the elevated atmospheric CO_2 influence on the decomposition of organic matter that is incorporated into soil.

Zheng et al. (2006) reported that the significant stimulation of CH_4 emissions by the elevated CO_2 correlated positively with nitrogen addition rates. They suggested that low soil nitrogen availability can counteract the stimulatory effect of elevated CO_2 on CH_4 emissions from nitrogen-poor paddy rice ecosystems, whereas high soil nitrogen availability can further amplify the stimulatory effect of elevated CO_2 on CH_4 emission from nitrogen-rich paddy rice ecosystems. In the present study, the elevated CO_2 significantly stimulated CH_4 emissions from the fields that lacked nitrogen fertilizer, and the addition of nitrogen fertilizer seemed to hinder the stimulatory effects of the elevated CO_2 on the CH_4 emission. However, the significant regulation of the nitrogen incorporation occurred only in the N125 treatment. In different nitrogen treatments, there were no significant differences in the elevated CO_2 effects on the aboveground rice biomass, which probably resulted in the similar elevated CO_2-induced methanogenic substrates and vascular transport capacities. Consequently, the CO_2-induced CH_4 emissions in the various nitrogen treatments were similar. Wang et al. (2006) reported that there were no significant differences in the CO_2-induced methanogen or methanotroph population changes in the N125 and N250 treatments, which may also support the insignificant differences in the elevated CO_2-induced CH_4 emission between the two nitrogen levels.

Conclusion

Our results revealed that an elevated atmospheric CO_2 concentration (200 ± 40 μmol mol^{-1} higher than the ambient atmosphere) stimulated the seasonal cumulative CH_4 emissions by an average of 15 % from the paddy rice fields with a sandy loam texture. The stimulatory extent was smaller than those of previous studies, which might result from different soil types or farm management practices. In addition, the present study also implied that the stimulatory effect of the elevated CO_2 on CH_4 emission tended to be inhibited by the incorporation of nitrogen fertilizer and crop residue. To accurately evaluate the effects of increasing atmospheric CO_2 concentration on CH_4 emissions from wetland ecosystems, other factors such as soil types and farm managements must be considered.

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