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## Fertilization effects on CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> fluxes from a subtropical double rice cropping system

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**Abstract:** A 2-year field study was conducted in a double rice cropping system in southern China to examine the effect of fertilization on CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> fluxes using static opaque chambers and gas chromatographs. Two treatments were set up including conventional fertilization with a rate of 358 kg N/ha per year in forms of compound fertilizer and urea, and a control with no fertilizer application. The results indicated that fertilization did not have a significant effect on CH<sub>4</sub> fluxes and led to a significantly higher cumulative N<sub>2</sub>O emission in the two years of observation period. Fertilization promoted CO<sub>2</sub> fluxes by increasing the autotrophic respiration instead of heterotrophic respiration. By combining the global warming impact of CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub>, it was found that fertilization increased the area-scaled but not the yield-scaled global warming impact. These results indicated that, according to the current amount of nitrogen applied, fertilization may increase the global warming effect of paddy fields in this region. However, the appropriate dose of nitrogen fertilizer application is still a reasonable agricultural management due to the comprehensive consideration of production and environmental impacts.

**Keywords:** nitrogen addition; rice paddy; greenhouse gases; greenhouse gas intensity

Carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) are the most important greenhouse gases (IPCC 2013). Rice paddies have been identified as a major CH<sub>4</sub> source, emitting between 33 and 40 Tg CH<sub>4</sub>/year (IPCC 2013). N<sub>2</sub>O emissions could also be pronounced in paddy fields with frequent drying-wetting alternation (Kudo et al. 2014). Fertilization has been commonly adopted to maintain rice yields, and the application of fertilizer may regulate greenhouse gas emissions (Jassal et al. 2011, Abalos et al. 2014). Therefore, a study on the effect of fertilization on greenhouse gas emissions is imperative for estimation of greenhouse gas emissions and designing possible mitigation strategies in the paddy soils.

The addition of nitrogen fertilizer can raise crop yield and biomass, which may have a positive effect

on soil carbon (C) sequestration as a result of the biomass input into the soil from crop residues and roots (Liu and Greaver 2009). Soil carbon decomposition might be retarded by fertilization when the litter quality and the growth rate of decomposers are both changed. Experimental additions of nitrogen to cropland usually result in increased N<sub>2</sub>O emission (Wu et al. 2017). For CH<sub>4</sub>, different studies got the converse conclusion that fertilization could promote (Shang et al. 2011), inhibit (Yao et al. 2012) or have no significant effect (Dan et al. 2001).

Most of the field measurements of greenhouse gas fluxes were conducted in temperate regions (Shang et al. 2011). However, over 60% of all nitrogen fertilizer will be used in the tropics and subtropics by 2020 (Galloway et al. 2003). China has a large area of paddy fields with

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90% of them being located in the subtropics (Wang et al. 2015). The environmental conditions in subtropical regions are different from those in temperate regions not only in climate but also highly acidic with low base cation concentrations (Mo et al. 2008). Therefore, it is necessary to do more research on the effect of fertilization especially in subtropical region where greenhouse gas emissions remain to be uncertain.

In the present study, two years of measurements were conducted on CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> fluxes from paddy fields in southern China. It is hypothesized that fertilization would significantly increase CO<sub>2</sub> and N<sub>2</sub>O fluxes mainly through promoting autotrophic respiration and applying substrate for the formation of N<sub>2</sub>O.

## MATERIAL AND METHODS

**Site description.** The experimental fields are located at the Qianyanzhou Ecological Research Station (QYZ, 26°44'46"N, 115°04'05"E) in the Jiangxi province, southern China. The site consists of a typical red soil hilly region with a subtropical monsoon climate. The mean air temperature is 18.0°C, and the coldest and warmest months are January and July, respectively. The region receives an average total annual precipitation of 1509 mm. The soils (equivalent to Plinthudults in the US Soil Taxonomy) are predominately sand and loam and were formed from alluvial fans of nearby rivers. The soil had 580, 310 and 110 g/kg sand, silt and clay, respectively. The topsoil (0–10 cm) had an organic carbon content of 9.33 g/kg and a total nitrogen content of 1.00 g/kg, and the soil pH was 4.98 and the bulk density was 1.32 g/cm<sup>3</sup> before the experiment. Double cropping of rice in a year is the primary cropping system in this area.

**Experimental design.** The experimental design consisted of a completely randomized block with four replicates. Each plot had an area of 120 m<sup>2</sup>. Two fertilization levels (conventional fertilization (CF) and no fertilization (NF)) was adopted in this study. Compound fertilizer (N:P:K = 15%:6.6%:12.5%) and urea were applied at a rate of 358 kg N/ha per year to the fertilized plots according to the local typical agricultural management. The fertilizer was spread into rice fields evenly. The time and rates of nitrogen applied to fertilized plots are shown in Table 1. Besides, a non-vegetated subplot of 2 m × 2 m was set up in each plot to determine heterotrophic respiration. The subplots received the same fertilization and agricultural management practices as the other part of the plots.

**Measurements of CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> fluxes.** Static chamber and gas chromatograph (GC) method was used to simultaneously measure CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> fluxes. The design of the static chamber was described in our previous article (Yuan et al. 2016). In July 2012, two stainless steel bases were installed in each plot. One base was installed in a place with normal vegetation with five seedlings of rice in it and the other was installed in the middle of the non-vegetated subplot of 2 m × 2 m. Greenhouse gases fluxes were measured in the same frequency in the two static chambers. Five gas samples were collected from each chamber using 100 mL plastic syringes at 10-min interval twice each week between 8:00 AM and 12:00 PM throughout the growing season. In fallow seasons, samples were collected once a week. The gas samples were analysed using a gas chromatograph (GC System, 7890A, Agilent Technologies, USA). The CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> fluxes were calculated from linear or nonlinear changes in the gas concentrations over time (Yuan et al. 2016).

Table 1. Field management practices of the fertilized treatment

Rice growing year	Crop type	Growing season	Fertilization date	Nitrogen application rate (kg N/ha)
The first year (2012–2013)	late rice	2012-7-30–2012-11-14	2012-7-30	71.7 (compound fertilizer)
			2012-8-10	107.5 (urea)
	fallow	2012-11-15–2013-4-23		
The second year (2013–2014)	early rice	2013-4-24–2013-7-23	2013-4-24	71.7 (compound fertilizer)
			2013-5-3	107.5 (urea)
	late rice	2013-7-24–2013-11-2	2013-7-26	71.7 (compound fertilizer)
			2013-8-12	107.5 (urea)
	fallow	2013-11-3–2014-4-24		
	early rice	2014-4-25–2014-7-25	2014-4-19	71.7 (compound fertilizer)
			2014-4-29	107.5 (urea)

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**Auxiliary measurements.** The air temperature and precipitation data were collected from an on-site automatic meteorological station adjacent to the experimental plots. When the fields were flooded, the standing water depth was monitored using a steel ruler. To determine soil chemical properties, soil samples were taken in May 2012, July 2013 and July 2014 from five points of each plot at a depth of 0–10 cm and then combined and mixed thoroughly. Soil pH was measured by a calibrated pH meter (Mettler Toledo, Greifensee, Switzerland) after shaking a soil-water (1:2.5 w:v) suspension for 30 min. Soil nitrate ( $\text{NO}_3^-$ -N) and ammonium ( $\text{NH}_4^+$ -N) were extracted with 1 mol/L KCl (1:5 soil:water ratio) and measured using a continuous flow analyser (Skalar, Breda, the Netherlands). Total carbon (TC) and total nitrogen (TN) were measured with an elemental analyser (Elementar, Vario Max, Hanau, Germany). The results of soil chemical properties can be found in Table 2. To determine biomass and yields, the weights of the rice grain, straw and root were recorded from the average of three 1 m<sup>2</sup> areas at harvest. The grain, straw or root samples, which had masses of approximately 100 g, were oven-dried to a constant weight at 65°C for 24 h after determining the wet weight.

**Data analysis.** The seasonal or annual cumulative emissions of  $\text{CH}_4$ ,  $\text{N}_2\text{O}$  and  $\text{CO}_2$  were computed as the sum of all daily  $\text{CH}_4$ ,  $\text{N}_2\text{O}$  and  $\text{CO}_2$  fluxes, respectively. For those days when measurements were not conducted, the emissions were calculated by linear interpolation using the data from the nearest days. The annual cumulative greenhouse gas emissions were the sum of the two growing seasons (late rice and early rice) and the fallow season.  $\text{CO}_2$  emissions of the vegetated plots were considered to be the ecosystem respiration including autotrophic

respiration (plant-derived  $\text{CO}_2$  efflux from soil) and heterotrophic respiration (microbial respiration).  $\text{CO}_2$  emissions of the non-vegetated subplots of 2 m × 2 m in each plot were considered to be heterotrophic respiration. Autotrophic respiration was calculated by  $\text{CO}_2$  emissions of the vegetated plots minus  $\text{CO}_2$  emissions of the non-vegetated plots.

The combined global warming impact of the three greenhouse gases (GWI) ( $\text{g CO}_2\text{eq/m}^2$ ) =  $\text{CH}_4$  emissions × 25 +  $\text{N}_2\text{O}$  emissions × 298 +  $\text{CO}_2$  emissions (Jiang et al. 2010) as the global warming potentials of 25 for  $\text{CH}_4$  and 298 for  $\text{N}_2\text{O}$  over 100-years (IPCC 2007). The greenhouse gas intensity (GHGI) is calculated by dividing GWI by rice grain yield (Mosier et al. 2006).

All statistical analyses were performed with the SPSS 19.0 (SPSS Inc., Chicago, USA) and Origin 7.0 (Origin Lab Corporation, Northampton, USA) software. The effect of fertilization and growing seasons on  $\text{CH}_4$ ,  $\text{N}_2\text{O}$  and  $\text{CO}_2$  fluxes was determined to be statistically significant using repeated measures of the analysis of variance (ANOVA). An independent samples *t*-test was used to analyse the difference in biomass, yields, cumulative emissions of greenhouse gases, GWI and GHGI between paddy fields with conventional fertilization and no fertilization.

## RESULTS

Air temperature, precipitation, flood water depth, rice biomass and yields. The average temperature was 18.5°C in the first year and 18.6°C in the second year. In the four rice growing seasons, the average temperature was 22.7, 26.0, 24.2 and 25.7°C, respectively. The precipitation of the second year (1397.6 mm) was lower than that of the first year (1518.7 mm), especially in the late rice growing season (Figure 1a).

Table 2. Soil chemical properties of paddy fields with conventional fertilization (CF) and no fertilization (NF) during the observation period

Time	Treatment	pH	$\text{NO}_3^-$ -N	$\text{NH}_4^+$ -N	TC	TN
			(mg/kg)		(mg/g)	
May (2012)	CF	4.92 ± 0.09 <sup>a</sup>	0.32 ± 0.16 <sup>a</sup>	5.78 ± 0.36 <sup>a</sup>	9.23 ± 0.31 <sup>a</sup>	1.01 ± 0.02 <sup>a</sup>
	NF	5.03 ± 0.07 <sup>a</sup>	0.22 ± 0.08 <sup>a</sup>	5.35 ± 0.27 <sup>a</sup>	9.42 ± 0.39 <sup>a</sup>	1.00 ± 0.03 <sup>a</sup>
July (2013)	CF	4.80 ± 0.04 <sup>a</sup>	0.88 ± 0.32 <sup>a</sup>	39.53 ± 0.99 <sup>a</sup>	9.68 ± 0.43 <sup>a</sup>	0.94 ± 0.03 <sup>a</sup>
	NF	4.92 ± 0.06 <sup>a</sup>	0.82 ± 0.41 <sup>a</sup>	14.62 ± 1.05 <sup>b</sup>	9.28 ± 0.27 <sup>a</sup>	0.89 ± 0.03 <sup>a</sup>
July (2014)	CF	5.17 ± 0.04 <sup>a</sup>	0.51 ± 0.15 <sup>a</sup>	27.88 ± 8.61 <sup>a</sup>	11.13 ± 0.45 <sup>a</sup>	1.09 ± 0.03 <sup>a</sup>
	NF	5.34 ± 0.07 <sup>a</sup>	0.22 ± 0.04 <sup>a</sup>	9.39 ± 1.62 <sup>a</sup>	10.27 ± 0.47 <sup>a</sup>	1.04 ± 0.03 <sup>a</sup>

Different letters within the same column between different treatments indicate significant differences between CF and NF at  $P < 0.05$ . Data shown are means ± standard errors of four spatial replicates. TC – total carbon; TN – total nitrogen

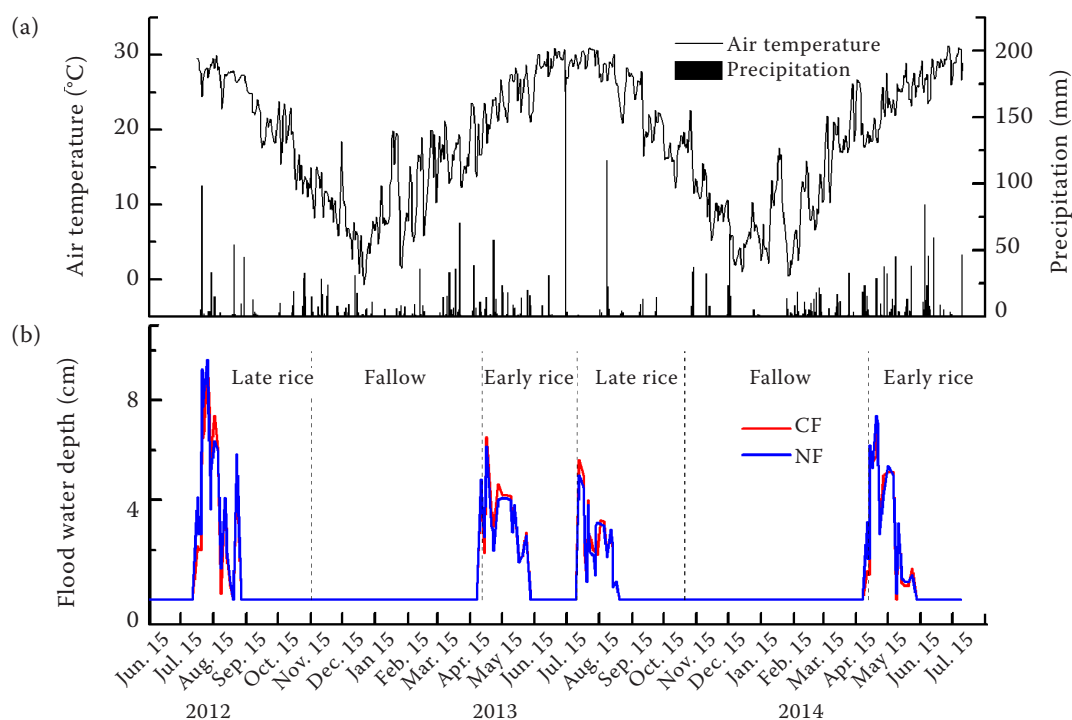


Figure 1. Seasonal variations in daily air temperature and precipitation (a), and flood water depth (b) during the observation period

The water-logging period lasted for 37, 43, 35 and 43 days in the four rice growing seasons and the corresponding average flood water depth was 4.53, 3.42, 2.44 and 3.22 cm, respectively (Figure 1b). The application of fertilizer significantly increased biomass and rice yields in the second year but not in the first year (Figure 2a,b). In the second year, the rice biomass and yields in the late rice growing season were 7922 and 3084 kg/ha for NF treatment, while the values were 11 982 and 4316 kg/ha for CF treatment. In the early rice growing season of the second year, the biomass and yields also increased by 52.6% and 57.9% under fertilization. Biomass

yield after fertilization significantly increased  $\text{CO}_2$  consumption during photosynthesis.

Greenhouse gas fluxes and overall global warming impact.  $\text{CH}_4$  fluxes showed significant seasonal variations (Figure 3a). In the rice growing seasons when the fields were water logged, high  $\text{CH}_4$  fluxes were observed. The peak value of  $\text{CH}_4$  fluxes could reach up to 37.9 and 31.1  $\text{mg C/m}^2/\text{h}$  for CF and NF treatments, respectively. In the winter fallow when no rice was grown and standing water was absent,  $\text{CH}_4$  fluxes remained at very low levels and even dropped to negative values sometimes. Among the four growing seasons, the late rice growing season in the second year had the

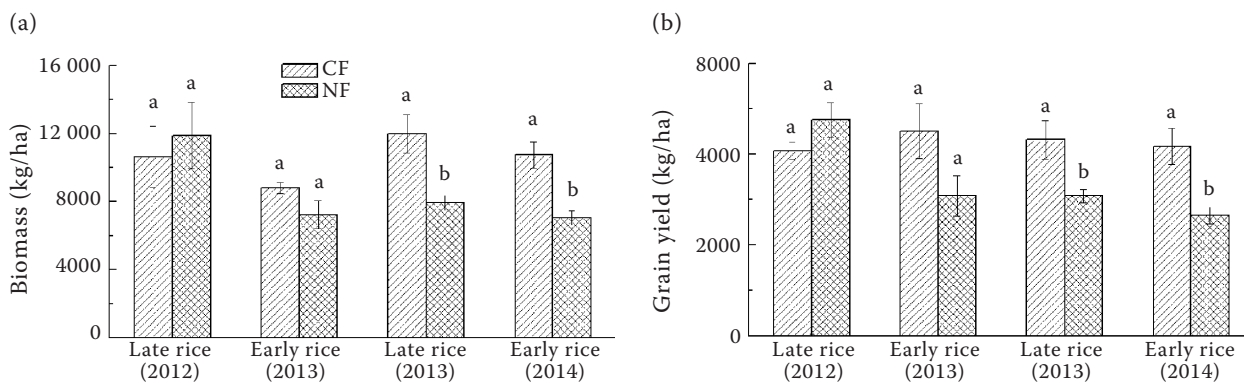


Figure 2. (a) Total biomass and (b) grain yields of paddy fields with conventional fertilization (CF) and no fertilization (NF). The data shown are means of the four replicates for individual treatments. Vertical bars indicate standard errors. Different letters indicate significant differences between CF and NF at  $P < 0.05$

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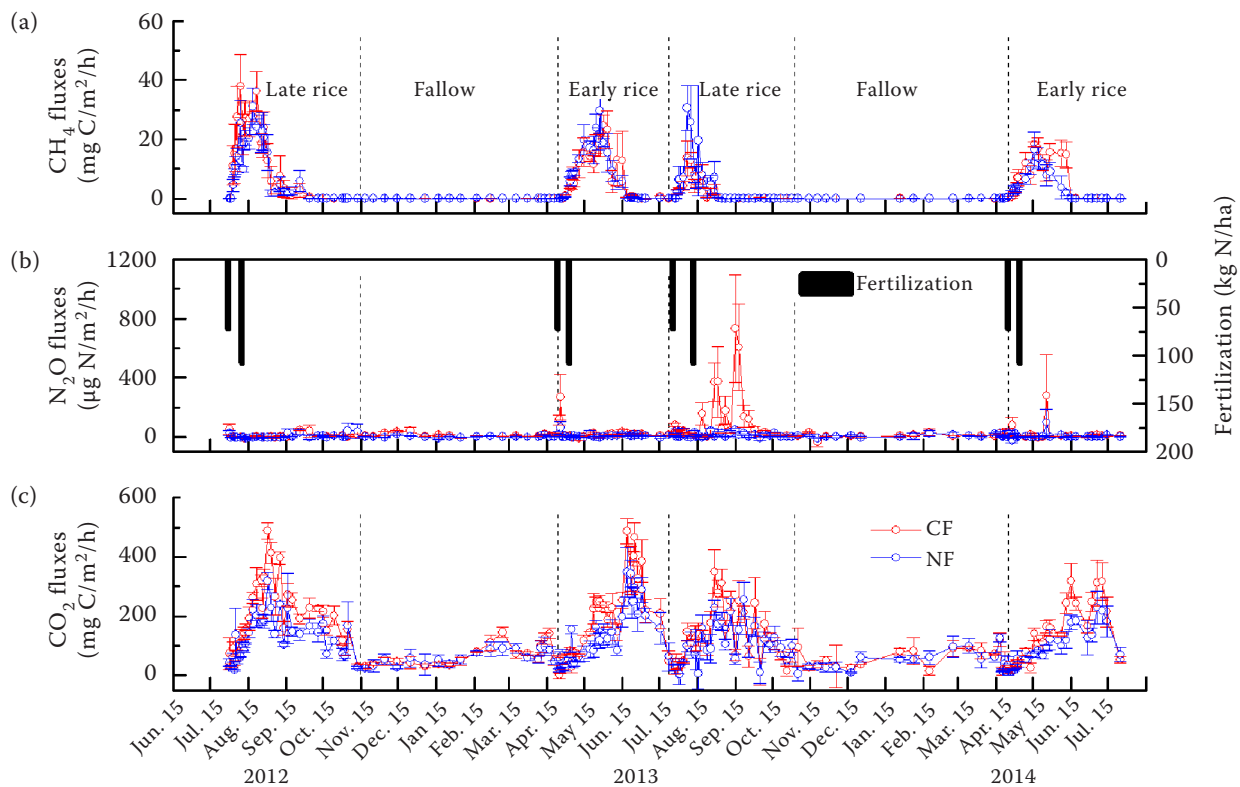


Figure 3. Seasonal variations in (a) CH<sub>4</sub> fluxes; (b) N<sub>2</sub>O fluxes and (c) CO<sub>2</sub> fluxes of paddy fields with conventional fertilization (CF) and no fertilization (NF) and the fertilization time and amount (b). The data shown are means of the four replicates for individual treatments. Vertical bars indicate standard errors

lowest CH<sub>4</sub> emissions both for CF and NF (Table 3). By comparing the cumulative emissions, fertilization was found to have no significant effect on CH<sub>4</sub> emission in any of the four growing seasons (Table 3).

N<sub>2</sub>O fluxes of NF treatment stayed low throughout the entire observation period. N<sub>2</sub>O fluxes of CF treatment were pulse-like and showed apparent seasonal variations (Figure 3b). The peaks were observed after fertilization except in the late rice growing season in the first year when no peak was ever found. The highest N<sub>2</sub>O fluxes were observed in the late rice growing season in the second year with an average value of 105.74 μg N/m<sup>2</sup>/h. Fertilization significantly increased cumulative N<sub>2</sub>O emissions by 2.16 times in the observation period of two years (Table 3). This finding can partially confirm our hypothesis.

CO<sub>2</sub> fluxes showed significant seasonal variations. They were high in the rice growing seasons and relatively low in the winter fallow period (Figure 3c). The average fluxes in the four growing seasons were 187, 190, 128 and 143 mg C/m<sup>2</sup>/h for CF and 132, 139, 98 and 24 for NF. Fertilization increased the cumulative CO<sub>2</sub> emissions by 34.5% and 21.0% in the first year and the second year (Table 3). By di-

viding the ecosystem respiration into autotrophic and heterotrophic respiration, it was found that the autotrophic respiration was significantly higher with fertilization except in the late rice growing season of the second year while the heterotrophic respiration, CO<sub>2</sub> fluxes measured from the non-vegetated subplots, was not affected by fertilization (Figure 4). This is consistent with our hypothesis that fertilization would significantly increase CO<sub>2</sub> fluxes mainly through promoting autotrophic respiration.

GWI was mainly contributed by CO<sub>2</sub> and CH<sub>4</sub> (Table 4). Fertilization significantly increased GWI of the whole observation period of two years. GHGI, the aggregate emissions of the three greenhouse gases expressed on grain yield bases, in two years of the observation period were 5.79 and 5.69 kg CO<sub>2eq</sub> kg/yield for CF and NF treatments. GHGI was not significantly affected by fertilization (Table 4).

## DISCUSSION

**Seasonal and interannual variation of CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> fluxes.** Our results showed that N<sub>2</sub>O fluxes stayed quite low throughout the entire observation



Table 3. Cumulative greenhouse gas emissions of paddy fields with conventional fertilization (CF) and no fertilization (NF) during the observation period

	Treatment	CH <sub>4</sub> (kg C/ha)	N <sub>2</sub> O (kg N/ha)	CO <sub>2</sub> (kg C/ha)
Late rice (2012)	CF	206 ± 58 <sup>a</sup>	0.32 ± 0.10 <sup>a</sup>	5346 ± 145 <sup>a</sup>
	NF	167 ± 32 <sup>a</sup>	0.21 ± 0.09 <sup>a</sup>	3655 ± 313 <sup>b</sup>
Fallow (2012–2013)	CF	0.34 ± 0.17 <sup>a</sup>	0.51 ± 0.14 <sup>a</sup>	2492 ± 179 <sup>a</sup>
	NF	0.85 ± 0.38 <sup>a</sup>	0.14 ± 0.04 <sup>a</sup>	2231 ± 299 <sup>a</sup>
Early rice (2013)	CF	152 ± 35 <sup>a</sup>	0.41 ± 0.03 <sup>a</sup>	4307 ± 258 <sup>a</sup>
	NF	154 ± 23 <sup>a</sup>	0.19 ± 0.02 <sup>a</sup>	3147 ± 391 <sup>b</sup>
Late rice (2013)	CF	43 ± 9 <sup>a</sup>	2.65 ± 0.87 <sup>a</sup>	3197 ± 246 <sup>a</sup>
	NF	88 ± 31 <sup>a</sup>	0.35 ± 0.01 <sup>b</sup>	2613 ± 237 <sup>a</sup>
Fallow (2013–2014)	CF	0.11 ± 0.49 <sup>a</sup>	0.38 ± 0.16 <sup>a</sup>	2487 ± 370 <sup>a</sup>
	NF	−0.20 ± 0.27 <sup>a</sup>	0.35 ± 0.18 <sup>a</sup>	2222 ± 200 <sup>a</sup>
Early rice (2014)	CF	137 ± 24 <sup>a</sup>	0.19 ± 0.05 <sup>a</sup>	3523 ± 164 <sup>a</sup>
	NF	86 ± 16 <sup>a</sup>	0.18 ± 0.10 <sup>a</sup>	2476 ± 312 <sup>b</sup>
The first year (2012~2013)	CF	358 ± 92 <sup>a</sup>	1.24 ± 0.18 <sup>a</sup>	12 146 ± 358 <sup>a</sup>
	NF	321 ± 51 <sup>a</sup>	0.54 ± 0.15 <sup>b</sup>	9033 ± 584 <sup>b</sup>
The second year (2013~2014)	CF	180 ± 31 <sup>a</sup>	3.22 ± 1.01 <sup>a</sup>	9207 ± 695 <sup>a</sup>
	NF	174 ± 30 <sup>a</sup>	0.88 ± 0.20 <sup>a</sup>	7311 ± 505 <sup>a</sup>
Two years	CF	538 ± 120 <sup>a</sup>	4.46 ± 0.89 <sup>a</sup>	21 352 ± 1013 <sup>a</sup>
	NF	495 ± 76 <sup>a</sup>	1.41 ± 0.22 <sup>b</sup>	16 344 ± 1070 <sup>b</sup>

Different letters within the same column between different treatments indicate significant differences between CF and NF at  $P < 0.05$ . Data shown are means ± standard errors of four spatial replicates

period except that several pulse-like emissions were observed after fertilization (Figure 3b). This is consistent with many previous studies (Bronson et al. 1997, Yao et al. 2012) and the low fluxes could be explained by reduction of N<sub>2</sub>O to N<sub>2</sub> through the denitrification

process in the anaerobic environment (Yuan et al. 2016, Smith et al. 2018). The highest N<sub>2</sub>O fluxes were observed in the late rice growing season of the second year when CH<sub>4</sub> fluxes were relatively low (Figure 3a,b). The discrepancy could be caused by the differences in

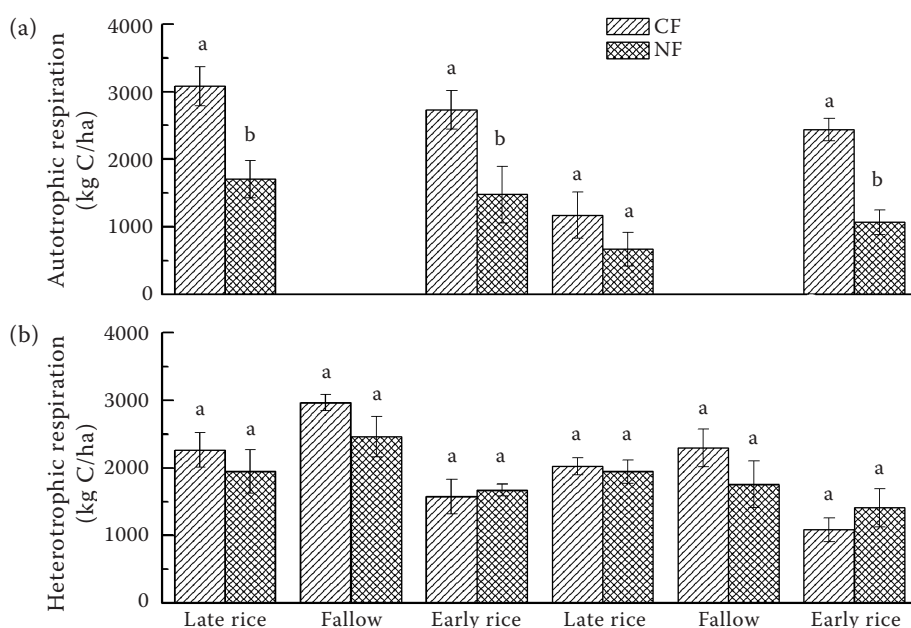


Figure 4. (a) Autotrophic respiration and (b) heterotrophic respiration of paddy fields with conventional fertilization (CF) and no fertilization (NF). The data shown are means of the four replicates for individual treatments. Vertical bars indicate standard errors. Different letters indicate significant differences between CF and NF at  $P < 0.05$

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Table 4. The carbon dioxide equivalents of CH<sub>4</sub>, N<sub>2</sub>O, CO<sub>2</sub> and their global warming impact (GWI) and greenhouse gas intensity (GHGI) of paddy fields with conventional fertilization (CF) and no fertilization (NF) during the observation period

	Treatment	Carbon dioxide equivalents (kg CO <sub>2eq</sub> /ha)			GWI (kg CO <sub>2eq</sub> /ha)	GHGI (kg CO <sub>2eq</sub> /kg yield)
		CH <sub>4</sub>	N <sub>2</sub> O	CO <sub>2</sub>		
The first year (2012~2013)	CF	11 946 ± 3059 <sup>a</sup>	580 ± 84 <sup>a</sup>	44 534 ± 1314 <sup>a</sup>	57 061 ± 3379 <sup>a</sup>	6.76 ± 0.78 <sup>a</sup>
	NF	10 723 ± 1687 <sup>a</sup>	251 ± 69 <sup>b</sup>	33 122 ± 2142 <sup>b</sup>	44 096 ± 3737 <sup>b</sup>	5.68 ± 0.43 <sup>a</sup>
The second year (2013~2014)	CF	5989 ± 1032 <sup>a</sup>	1507 ± 475 <sup>a</sup>	33 758 ± 2547 <sup>a</sup>	41 254 ± 2413 <sup>a</sup>	5.03 ± 0.65 <sup>a</sup>
	NF	5806 ± 989 <sup>a</sup>	411 ± 94 <sup>a</sup>	26 806 ± 1853 <sup>a</sup>	33 023 ± 2355 <sup>a</sup>	5.75 ± 0.19 <sup>a</sup>
Two years	CF	17 936 ± 4004 <sup>a</sup>	2086 ± 417 <sup>a</sup>	78 292 ± 3714 <sup>a</sup>	98 314 ± 4620 <sup>a</sup>	5.79 ± 0.35 <sup>a</sup>
	NF	16 529 ± 2527 <sup>a</sup>	662 ± 102 <sup>b</sup>	59 928 ± 3923 <sup>b</sup>	77 119 ± 6046 <sup>b</sup>	5.69 ± 0.25 <sup>a</sup>

Different letters within the same column between different treatments indicate significant differences between CF and NF at  $P < 0.05$ . Data shown are means ± standard errors of four spatial replicates

water flooding and distribution of precipitation. In the late rice growing season of the second year, the precipitation was sparse (Figure 1a) and the duration of flooding was shorter with lower flood water depth (Figure 1b). The less flood water and precipitation might have caused the lower CH<sub>4</sub> fluxes and the higher N<sub>2</sub>O fluxes. Water condition has been proved to play an important role in regulating CH<sub>4</sub> and N<sub>2</sub>O fluxes (Li et al. 2005). Significant CH<sub>4</sub> emissions could occur when soil is submerged and redox potential stays low. When the flooded layer disappears and the soil becomes aerobic with elevated redox, potential N<sub>2</sub>O emissions could be significant (Hou et al. 2000). Previous studies also found that paddies may have lower CH<sub>4</sub> emissions (Berger et al. 2013) while N<sub>2</sub>O emissions would be pronounced when aerobic environments were formed (Kudo et al. 2014).

CO<sub>2</sub> fluxes were lower in the winter fallow than in rice growing seasons (Figure 3c). CO<sub>2</sub> fluxes included autotrophic respiration and heterotrophic respiration. However, autotrophic respiration was generally zero in the winter fallow since there was no vegetation growing. Another possible reason is temperature, which is one of the most important factors affecting soil respiration (Smith et al. 2018). The low temperature could partially explain the small CO<sub>2</sub> fluxes in winter. CO<sub>2</sub> fluxes were lower in the second year than the first year (Figures 3c and 4a,b). Temperature and plant biomass were good proxies for variations in both autotrophic and heterotrophic capacity for respiration (Flanagan and Johnson 2005, Smith et al. 2018). However, in the present study, both of them did not differ interannually (Figure 1a) and could not be the reason for the interannual variability of CO<sub>2</sub> fluxes. Soil moisture was another

important environmental factor that controlled seasonal and interannual variation in CO<sub>2</sub> fluxes besides temperature (Flanagan and Johnson 2005). In the present study, the precipitation and flood water of the second year were both less than the first year, and this might have caused the difference in CO<sub>2</sub> fluxes between the two years. Ecosystem respiration is controlled by the complex interaction of environmental and biotic factors (Han et al. 2007). Further study needs to be conducted to identify all of the other factors.

Effects of fertilization on CH<sub>4</sub>, N<sub>2</sub>O and CO<sub>2</sub> fluxes. The application of fertilizer did not significantly affect CH<sub>4</sub> fluxes (Table 3) and this is in line with previous studies (Dan et al. 2001) although other studies also found that the application of fertilizers would either promote (Liu and Greaver 2009) or decrease (Yao et al. 2012) CH<sub>4</sub> emissions in paddy fields. The effects of N fertilization on CH<sub>4</sub> production, oxidation and transport are complex, involving many biochemical processes (Schimel 2000). N fertilization promotes plant growth and provides more substrates for CH<sub>4</sub> production, but this will also relieve the methanotrophs and promote CH<sub>4</sub> oxidation in the intermittent flooded paddy fields, especially in the drainage period (Banger et al. 2012). Dan et al. (2001) found that N fertilization stimulated both CH<sub>4</sub> production and CH<sub>4</sub> oxidation and resulted in no significant effect on CH<sub>4</sub> emissions.

Our findings are in line with many other studies that fertilization would promote N<sub>2</sub>O emission (Millar et al. 2010), and this might be caused by applying substrate for nitrification and denitrification (Azam et al. 2002, Yao et al. 2012). However, a significant promotion effect of fertilization on N<sub>2</sub>O emissions was found only in the late rice growing season of the

second year with less flood water and precipitation. Soil might have a chance to be aerobic when the paddy fields were logged with lower water depth for shorter periods. As it was well known that  $\text{N}_2\text{O}$  could be reduced to  $\text{N}_2$  through the denitrification process in the anaerobic environment in many previous studies (Bronson et al. 1997, Yao et al. 2012, Smith et al. 2018), this study speculated that most of the nitrogen fertilizer might have been transformed to  $\text{N}_2$  by denitrification in the other three growing seasons as the fields were flooded (Hayatsu et al. 2008). This result suggests that water condition associated with fertilizer application, plays an important role in regulating  $\text{N}_2\text{O}$  fluxes in paddy fields.

As for  $\text{CO}_2$  emitted by ecosystem respiration, fertilization showed a significant promotion effect through promoting autotrophic respiration other than heterotrophic respiration (Figure 4). This indicated that fertilization affected ecosystem respiration through promoting crop growth. Although the biomass of the fertilized treatment was not significantly higher in the first year, the root activity and respiration may have been promoted. A study by Xu and Wan (2008) also found that soil respiration was greater in the fertilized plots than in the unfertilized plots and this was attributable to stimulated plant growth, root activity and respiration. It should be noted that both phosphate and potash were also applied into the paddy fields, but their effects on greenhouse gas fluxes were not examined in the present study.

Effects of fertilization on rice biomass, yields and the integrated greenhouse effects of  $\text{CH}_4$ ,  $\text{N}_2\text{O}$  and  $\text{CO}_2$ . Fertilization significantly increased rice yields of the second year but not those of the first year (Figure 2b). It was noticed that all the fields had been fertilized for years before the experiment was conducted. Previous studies found that most of the intensive agricultural production in China was over-fertilized (Vitousek et al. 2009). The strong retention of external nitrogen inputs could remain available for the subsequent crops (Ju et al. 2009). The retention of nitrogen might have maintained the high rice yields for NF in the first year. This indicates that it is feasible to reduce nitrogen application rates in the excessive nitrogen fertilization agro-ecosystem without sacrificing crop yield.

To assess the combined climatic impacts from the three greenhouse gases as affected by fertilization, the carbon dioxide equivalents of  $\text{CH}_4$ ,  $\text{N}_2\text{O}$  and  $\text{CO}_2$  were calculated. It was found that fertilization increased the area-scaled global warming impact but did not change the yield-scaled global warming impact (Table 4). This

is because fertilization promoted greenhouse gas emissions and increased crop yields, simultaneously. Other factors affecting the environment, such as mineral nitrogen leaching and ammonia volatilization (Griggs et al. 2007, Zhao et al. 2009) should be included in the further research to provide a more comprehensive evaluation of fertilization in paddy fields.

Both  $\text{N}_2\text{O}$  and  $\text{CO}_2$  emissions were significantly promoted by the application of fertilizer, while  $\text{CH}_4$  had no difference between treatments with and without fertilization in a paddy field in southern China. Fertilization increased  $\text{CO}_2$  fluxes by promoting the autotrophic respiration instead of heterotrophic respiration. Fertilization increased the area-scaled global warming impact of  $\text{CH}_4$ ,  $\text{N}_2\text{O}$  and  $\text{CO}_2$  in the two years of observation period while the yield-scaled global warming impact did not differ significantly with fertilization. For NF, stopping fertilizer use did not reduce rice yields immediately in the first year as the retention of external nitrogen inputs in the previous growing seasons might have maintained the rice yields. An appropriate amount of nitrogen fertilization should be an effective way to maintain crop yields without increasing environmental impact.

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