



Combined Effects of Straw Returning and Chemical N Fertilization on Greenhouse Gas Emissions and Yield from Paddy Fields in Northwest Hubei Province, China

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Received: 6 August 2019 / Accepted: 21 October 2019 / Published online: 5 December 2019
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Abstract

The effects of crop straw returning or chemical N fertilization on greenhouse gas emissions and crop yield have been extensively studied, but their interaction effects remain unclear. So, this study aimed to investigate greenhouse gas emissions and yield from paddy fields as affected by straw returning, chemical N application, and their interactions in the 2017 and 2018 rice-growing seasons in northwest Hubei Province, China. The static chamber-gas chromatography method was used to determine CH₄ and N₂O fluxes. Straw returning had no significant effect on N₂O emissions, but significantly increased CH₄ emissions due to increased *mcrA* abundance and global warming potential (GWP). Chemical N fertilization significantly decreased CH₄ emissions by 7.2–18.8% and GWP by 13.5–17.9%, but increased N₂O emissions by 72.5–311.1% due to increased abundance of AOA-*amoA*, AOB-*amoA*, *nirK*, and *nirS*. Both straw returning and chemical N fertilization significantly increased rice yield. Straw returning significantly increased greenhouse gas intensity (GHGI), while N fertilization obviously decreased the GHGI. Moreover, significant interaction effects of straw returning and chemical N fertilization on CH₄ emissions, GHGI, and grain yield were observed. The combination of 250 kg N ha⁻¹ of chemical N application and no straw resulted in the lowest GWP, second lowest GHGI and relatively high grain yield among all treatments. In conclusion, 250 kg N ha⁻¹ of chemical N application without straw returning may be an ecological and economic practice for rice production in this study. Nevertheless, ecological-friendly methods of straw returning for sustainable agriculture should be further explored in future studies.

Keywords CH₄ flux · Global warming potential · Greenhouse gas intensity · N₂O flux · Straw management

1 Introduction

At present, global warming due to increasing concentration of greenhouse gases (GHG) in the atmosphere has aroused increasing concern. It not only affects crop production, but also

threatens global food production and supply security (Jonathan et al. 2011). GHG emissions from agricultural production are an important part of total global GHG emissions. Agricultural GHG emissions account for approximately 10–12% of global anthropogenic GHG emissions (Smith et al. 2007; IPCC 2013, 2014; Htun et al. 2017). About 1% of carbon dioxide (CO₂), 40% of methane (CH₄), and 60% of nitrous oxide (N₂O) emissions come from agriculture (Zou et al. 2005). In China, agricultural production of GHG emissions accounts for about 17% of global emissions, particularly CH₄ and N₂O emissions, which account for 50% and 25% of the total agricultural GHG emissions in China, respectively Liu et al. 2010a, b).

Crop straw is one of the important organic fertilizers, which can increase available C and N in the soil and improve soil physicochemical properties (Wang et al. 2015; Hoang and Marschner 2019). But straw returning observably affects CH₄ emissions from rice fields (Ma et al. 2009; Zhang et al. 2015). Methyl coenzyme-M reductase (*mcrA*) regulates the last step in all methanogenic pathways and is typically

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selected as the functional gene maker for the analysis of methanogenic communities. Xia et al. (2014) proposed that long-term straw returning could significantly increase CH₄ emissions because it can increase *mcrA* abundance by providing predominant C sources within a rice-wheat rotation system. Hu et al. (2016) reported that different straw-returning methods affect CH₄ emissions from paddy fields through affecting *mcrA* abundance. In addition, straw returning also influences N₂O emissions by providing C and N substrates for nitrification and denitrification. Nitrification and denitrification are key components of soil N cycles (Galloway et al. 2008; Nelson et al. 2019). Nitrification is usually mediated by ammonia oxidation archaeal (AOA) and bacteria (AOB) containing the *amoA* gene (Purkhold et al. 2000). Denitrification is the microbially mediated process converting NO₃⁻ to N₂, in which the conversion of NO₃⁻ to nitric (NO⁻) is the rate-limiting step mediated by the copper-containing nitrite reductase from the *nirK* type denitrifier and the cytochrome *cd1* nitrite reductase from the *nirS* type denitrifier (Braker et al. 2000). Liu et al. (2011) demonstrated that straw returning tends to enhance N₂O emissions due to increases in readily available C and N in soils, while Baggs et al. (2003) pointed out that the microbial degradation of straws returned into the soils could consume the mineral N, thus reducing abundance of nitrification and denitrification related microbes (e.g., AOA-*amoA*, AOB-*amoA*, *nirS*, and *nirK*) and N₂O emissions subsequently. In addition, some studies proposed that straw application has no significant effects on soil N₂O emissions (Malhi et al. 2006). GHG emitted from rice fields is one of the most important sources of GHG emissions in central China (Zhang et al. 2016), and thus, it is highly necessary to consider the influence of the currently prevailing agricultural practices such as straw returning.

N fertilizer is extremely important for increasing crop yields. Thus, farmers tend to increase the application rate of N fertilizer, which not only causes the overgrowth and late maturity of rice to result in a decline in rice yield and N use efficiency, but also leads to agricultural environment source pollution and GHG emissions (Zhu and Chen 2002; Ju et al. 2009; Hou et al. 2012). N₂O emissions are generated from the microbial nitrification and denitrification in soils. N application can increase N₂O emissions by providing N substrates for the nitrification and denitrification. About 1.5 million tons of N₂O are produced each year due to the application of chemical N fertilizer, accounting for 44% of the total amount of N₂O emitted due to human activities (Eichner 1990). At present, high N application rate in rice production is one of the most important contributors to soil N₂O emissions (Ma et al. 2007). Therefore, it is necessary to determine the appropriate rate of N fertilization for sustainable rice production. Moreover, few studies have reported the combined effects of straw returning and chemical N application on global warming potential (GWP) and greenhouse gas intensity (GHGI). Therefore, in

this study, a field experiment was conducted to investigate the effects of different straw-returning rates and N fertilizer levels on CH₄ and N₂O emissions, GWP, rice yield, and GHGI in the 2017 and 2018 rice-growing seasons of northwest Hubei Province, China. We hypothesized that there were significant interactions of straw returning and chemical N fertilization on CH₄ and N₂O emissions, GWP, rice yield, and GHGI.

2 Materials and Methods

2.1 Site Descriptions

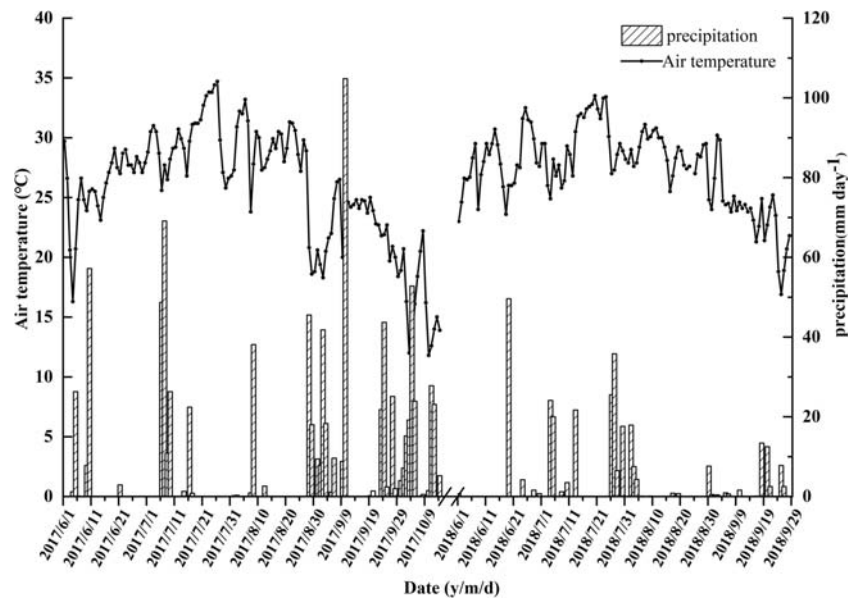
The experimental site is located at an experimental farm of Huazhong Agricultural University in Wudian town, Hubei Province (32°10' N, 112°10' E). The elevation of this site is 150 m above sea level. There is a mid-subtropical monsoon climate, with an annual average temperature of 15.5 °C, annual precipitation of 500–1000 mm, and annual average sunshine time of 260 days. Daily mean precipitation and air temperature during the experimental period are shown in Fig. 1. The major properties of the soil at 0–20 cm are as follows: pH of 6.55 (extracted by H₂O; soil/water = 1:2.5), bulk density of 1.36 g cm⁻³, organic carbon of 18.96 g kg⁻¹, total N of 1.35 g kg⁻¹, nitrate(NO₃⁻) of 6.92 mg kg⁻¹, ammonium (NH₄⁺) of 8.16 mg kg⁻¹, total phosphorus (P) of 0.53 g kg⁻¹, available P of 91.96 mg kg⁻¹ (extracted by NaHCO₃), total potassium (K) of 6.63 g kg⁻¹, and available K of 11.62 mg kg⁻¹ (extracted by CH₃COONH₄).

2.2 Experimental Design

The experiment was designed with a split-plot design of a randomized integral field with preceding wheat straw-returning methods as the main plots and N fertilizer levels as the sub-plots. The main plots included wheat straw removal (C0), returning half of the wheat straws into the fields (C1) and returning all the wheat straws into the fields (C2), whereas the sub-plots included no N fertilizer (N0), 125 kg N ha⁻¹ of N application (N1) and 250 kg N ha⁻¹ of N application (N2) during rice seasons (Table 1). Each treatment was repeated thrice, and each plot was 12 m × 9 m in size. The 30 cm × 40 cm ridges between the plots were covered by black plastic films. Water and fertilizer transference was prevented by burying the lower part of the plastic films 40 cm deep underground. The C/N ratio of wheat straw was 69.

Rice (*Oryza sativa* L., YY4949) was seeded in middle May, transplanted at the rate of 2.22 × 10⁵ hills ha⁻¹ in middle June by hand, and harvested in early October each year. The preceding wheat straws were chopped into 7–10 cm when harvesting, and then subsequently mulched on the soils. The soil was not tilled and the applied fertilizers were surface broadcasted manually. During the 2017 and 2018 rice-

Fig. 1 Daily mean precipitation and air temperature during the 2017 and 2018 rice-growing seasons



growing seasons, N, P and K fertilizers were applied manually. P fertilizers were only used just before transplanting as basal fertilizers, K fertilizers were used at the transplanting (50%) and earing (50%) stages. N fertilizers were applied at the transplanting, tillering, jointing and earing stages, except for N0 treatments. The amounts and time of fertilizer application in different treatments are shown in Table 1. Weeds were controlled by spraying herbicide (36% glyphosate) in June before the field was submerged or by manual weeding during the rice-growing seasons. Whenever the surface water dropped below 4–5 cm, it was irrigated back to the level of about 8 cm.

2.3 Measurements of N₂O and CH₄ Fluxes

The static chamber-gas chromatography method was used to measure N₂O and CH₄ fluxes from the fields (Li et al. 2013). The cylinder chamber with a diameter of 38 cm and a height of 50 cm or 120 cm (depending on rice height) was made by stainless steels. The chambers were temporarily placed on permanent rings installed in each plot in order to create a seal on the gas sampling day. The chamber was wrapped with heat-insulating plastic layer, installed with four circulating fans on the top of the chambers for mixing the air within the chambers, and a thermometer on the top for recording air temperature during sampling within the chambers. A total of four samples were collected at intervals of 10 min each time. The gas samples from headspace in each plot were collected using a 25-mL syringe and transferred immediately to 25-mL vacuum glass containers. The samples were collected at 7–10-day intervals during rice-growing seasons.

CH₄ and N₂O concentrations were assayed using a gas chromatograph meter (Shimadzu GC-14B, Li et al. 2013). A linear regression was performed on the concentration of the four gas samples to obtain a gas discharge rate.

The gas flux was calculated according to the method reported by Zheng et al. (1998) as below.

$$F = \rho \times H \times dC/dt \times 273 \div (273 + T)$$

where F denotes CH₄ or N₂O fluxes flux (mg m⁻² h⁻¹), ρ denotes CH₄ or N₂O density at standard state, h denotes chamber height above the soil-water layer (m), dC/dt denotes CH₄ or N₂O accumulation rate (ppm h⁻¹ and ppb h⁻¹), and T denotes mean air temperature inside the chamber during sampling.

2.4 Rice Grain Yield Measurement

Rice grains were collected randomly using a 2 m × 2 m frames, and then air-dried and weighed. The final rice yields were adjusted to 14% moisture contents.

2.5 Calculation of Cumulative GHG Emissions, GWP and GHGI

Cumulative seasonal emissions of CH₄ and N₂O were calculated for each plot according to the method of Li et al. (2013).

Based on the CH₄ and N₂O emissions, the GWP (kg CO₂-equivalents ha⁻¹) was calculated using the following equation:

$$GWP = CH_4 \times 30 + N_2O \times 268$$

The GHGI (kg CO₂-eq. kg⁻¹ grain yield) was calculated following the method of Shang et al. (2011):

$$GHGI = GWP / \text{grain yield}$$

Table 1 Agricultural managements for different treatments

| Treatments | Fertilizer management | Tillering fertilizer (June 27, 2017; June 28, 2018) | Jointing fertilizer (July 26, 2017; July 27, 2018) | Earing fertilizer (August 10, 2017; August 12, 2018) | Crop straw management | Rice seedling management |
|------------|---|---|--|---|---|--|
| N0C0 | Basal fertilizer (June 13, 2017; June 14, 2018) 643 kg ha ⁻¹ single super-phosphate (14% P ₂ O ₅) + 150 kg ha ⁻¹ potassium chloride (60% K ₂ O) | - | - | 150 kg ha ⁻¹ potassium chloride | No wheat straws | Transplanted in June 14, 2017 or June 15, 2018. |
| N0C1 | 643 kg ha ⁻¹ single super-phosphate + 150 kg ha ⁻¹ potassium chloride | - | - | 150 kg ha ⁻¹ potassium chloride | 2800 kg ha ⁻¹ of wheat straws returned to paddy field. | Transplanted in June 14, 2017 or June 15, 2018. |
| N0C2 | 643 kg ha ⁻¹ single super-phosphate + 150 kg ha ⁻¹ potassium chloride | - | - | 150 kg ha ⁻¹ potassium chloride | 5600 kg ha ⁻¹ of wheat straw returned to paddy field | Transplanted in June 14, 2017 or June 15, 2018. |
| N1C0 | 54 kg ha ⁻¹ urea (46% N) + 643 kg ha ⁻¹ single super-phosphate + 150 kg ha ⁻¹ potassium chloride | 87 kg ha ⁻¹ urea | 87 kg ha ⁻¹ urea | 43 kg ha ⁻¹ urea + 150 kg ha ⁻¹ potassium chloride | No wheat straws | Transplanted in June 14, 2017 or June 15, 2018. |
| N1C1 | 54 kg ha ⁻¹ urea + 643 kg ha ⁻¹ single super-phosphate + 150 kg ha ⁻¹ potassium chloride | 87 kg ha ⁻¹ urea | 87 kg ha ⁻¹ urea | 43 kg ha ⁻¹ urea + 150 kg ha ⁻¹ potassium chloride | 2800 kg ha ⁻¹ of wheat straws returned to paddy field. | Transplanted in June 14, 2017 or June 15, 2018. |
| N1C2 | 54 kg ha ⁻¹ urea + 643 kg ha ⁻¹ single super-phosphate + 150 kg ha ⁻¹ potassium chloride | 87 kg ha ⁻¹ urea | 87 kg ha ⁻¹ urea | 43 kg ha ⁻¹ urea + 150 kg ha ⁻¹ potassium chloride | 5600 kg ha ⁻¹ of wheat straw returned to paddy field | Transplanted in June 14, 2017 or June 15, 2018. |
| N2C0 | 326 kg ha ⁻¹ urea + 643 kg ha ⁻¹ single super-phosphate + 150 kg ha ⁻¹ potassium chloride | 87 kg ha ⁻¹ urea | 87 kg ha ⁻¹ urea | 43 kg ha ⁻¹ urea + 150 kg ha ⁻¹ potassium chloride | No wheat straws | Transplanted in June 14, 2017 or June 15, 2018. |
| N2C1 | 326 kg ha ⁻¹ urea + 643 kg ha ⁻¹ single super-phosphate + 150 kg ha ⁻¹ potassium chloride | 87 kg ha ⁻¹ urea | 87 kg ha ⁻¹ urea | 43 kg ha ⁻¹ urea + 150 kg ha ⁻¹ potassium chloride | 2800 kg ha ⁻¹ of wheat straws returned to paddy field. | Transplanted in June 14, 2017 or June 15, 2018. |
| N2C2 | 326 kg ha ⁻¹ urea + 643 kg ha ⁻¹ single super-phosphate + 150 kg ha ⁻¹ potassium chloride | 87 kg ha ⁻¹ urea | 87 kg ha ⁻¹ urea | 43 kg ha ⁻¹ urea + 150 kg ha ⁻¹ potassium chloride | 5600 kg ha ⁻¹ of wheat straw returned to paddy field | Transplanted in June 14, 2017 or June 15, 2018. |

C0 wheat straw removal, C1 half of the straws returned into the fields, C2 all the straws returned into the fields, N0 no N fertilizer, N1 125 kg N ha⁻¹ of N fertilization, N2 250 kg N ha⁻¹ of N fertilization

2.6 Abundance Determination of Functional Genes AOA-amoA, AOB-amoA, nirS, nirK, and mcrA

Five soil cores at 0–20 cm depth were randomly sampled by a soil core sampler (inner diameter of 7 cm) in each plot just after the rice was harvested. With the stones and plant debris picked out, the five samples were mixed and homogenized into a composite sample for subsequent biological analysis.

According to the manufacturer's description, the total DNA of soil microorganisms (equivalent to 1.5 g dry soil) was extracted by the Fast DNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). Then, the extracted soil DNA was stored at $-80\text{ }^{\circ}\text{C}$ for testing. The AOB and AOA gene fragments were amplified using primer pairs Arch-amoAF/Arch-amoAR and amoA-1F/amoA2R, respectively (Rotthauwe et al. 1997; Francis et al. 2005). For amplification of amoA gene fragments of nirS and nirK, primer systems nirs-cd3aF/nirs-R3cd and nirK1F/nirK5R were, respectively, used (Braker et al. 1998; Throbäck et al. 2004). The primer used for qPCR of mcrA gene was mlas-mod-F/mcrA-rev-R (Steinberg and Regan 2008).

PCR reactions were conducted in quadruplicate of 20 μL , which were blended to minimize reaction variability. Each reaction mixture contained 10 μL of iTaq™ Universal SYBR green Supermix (BIO-RAD, USA), 0.2 μL of each primer (forward primer and reverse primer), and 1 μL of DNA-diluted template ($15\text{--}20\text{ ng DNA } \mu\text{L}^{-1}$).

Programs of qPCR were carried out by Bio-Rad iQ5 real-time PCR system (BIO-RAD, USA) as follows: 94 $^{\circ}\text{C}$ for 3 min (AOA, AOB, nirS, and nirK) or 4 min (mcrA), followed by 40 cycles of 94 $^{\circ}\text{C}$ for 30 s, 60 $^{\circ}\text{C}$ for 1 min (for AOA) or 50 s (for AOB) or 30 s (for nirK and nirS), and an extension at 72 $^{\circ}\text{C}$ for 1 min (for AOA) or 50 s (for AOB) or 45 s (for nirK and nirS), or followed by 30 cycles of 94 $^{\circ}\text{C}$ for 45 s, 55 $^{\circ}\text{C}$ for 30 s (mcrA), 72 $^{\circ}\text{C}$ for 30 s (mcrA). The standards for qPCR were provided by pEASY-T5 Zero Cloning Kit (TransGen Biotech, China). Every reaction was performed in triplicate, and invalid values were removed. The amplification efficiencies were 98.1–100.3% and the R^2 values were 0.993–1.000. Concentration dilution test was performed by 10-fold, 50-fold, and 100-fold to prove that qPCR assay was not inhibited.

2.7 Data Analysis

A three-way ANOVA analysis was performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA) to analyze the effects of straw returning, N fertilization, study year, and their interactions on seasonal cumulative CH_4 and N_2O emissions, GWPs, GHGI, rice yields, and soil gene abundance. Duncan's multiple range tests were performed to examine whether the differences between the mean values were statistically significant at a significance level of 0.05. Linear regression was conducted to

assess the correlations between cumulative CH_4 and N_2O emissions and soil gene abundance.

3 Results

3.1 Grain Yield

Straw returning, N fertilization, and study year had a significant effect on rice yield (Table 4). Compared with C0 treatment, C1 and C2 treatments significantly increased rice grain yields by 5.6% and 8.0% in 2018, respectively (Table 2). The application of N fertilizer significantly enhanced rice yield (Table 4). Higher rice grain yields under N1 and N2 treatments (31.0% and 40.4% in 2017 and 38.4% and 43.4% in 2018, respectively) than that under N0 treatment were observed. Moreover, there were significant two-way interactions and non-significant interaction (Table 4).

3.2 CH_4 and N_2O Emissions

Similar CH_4 flux patterns were found in all treatments during the rice-growing seasons of 2017 and 2018 (Fig. 2). Two peaks of CH_4 emissions were observed at the stem-elongation and heading stages. The highest fluxes were detected under C2 treatment ($64.63\text{ mg m}^{-2}\text{ h}^{-1}$ in 2017 and $51.40\text{ mg m}^{-2}\text{ h}^{-1}$ in 2018). The flux ranged from 0.29 to $64.63\text{ mg m}^{-2}\text{ h}^{-1}$ in 2017 and from 1.03 to $51.40\text{ mg m}^{-2}\text{ h}^{-1}$ in 2018 across all treatments.

N fertilization was significantly correlated with N_2O emissions, and peaks were found immediately after each N fertilization (Fig. 3). The fluxes from straw-returning treatments ranged from -0.1 to $112.3\text{ } \mu\text{g m}^{-2}\text{ h}^{-1}$ in 2017 and from 9.3 to $99.5\text{ } \mu\text{g m}^{-2}\text{ h}^{-1}$ in 2018; the fluxes from N fertilizer treatments varied from -0.6 to $262.4\text{ } \mu\text{g m}^{-2}\text{ h}^{-1}$ in 2017 and from 13.5 to $235.1\text{ } \mu\text{g m}^{-2}\text{ h}^{-1}$ in 2018. Moreover, significant interactions of straw returning and N fertilizer on CH_4 emissions were observed (Table 2).

Similar to rice grain yield, seasonal cumulative CH_4 emissions were significantly affected by straw returning, N fertilization, and study year (Table 4). Compared with C0 treatment, C1 and C2 treatment resulted in significant increases in the cumulative emissions (by 12.5% and 25.3% in 2017 and by 49.6% and 86.7% in 2018, respectively). The CH_4 emissions under N1 and N2 treatments were significantly decreased by 14.0% and 7.4% in 2017 and by 2.9% and 1.4% in 2018, respectively, relative to N0 treatment. Moreover, significant two-way interactions and non-significant interaction on seasonal cumulative CH_4 emissions were found (Table 4).

Straw-returning treatments did not show significant effects on N_2O emissions (Table 4). However, the N_2O emissions were significantly affected by N fertilizer rates (Table 4). Compared with those from N0 treatment, the seasonal cumulative N_2O

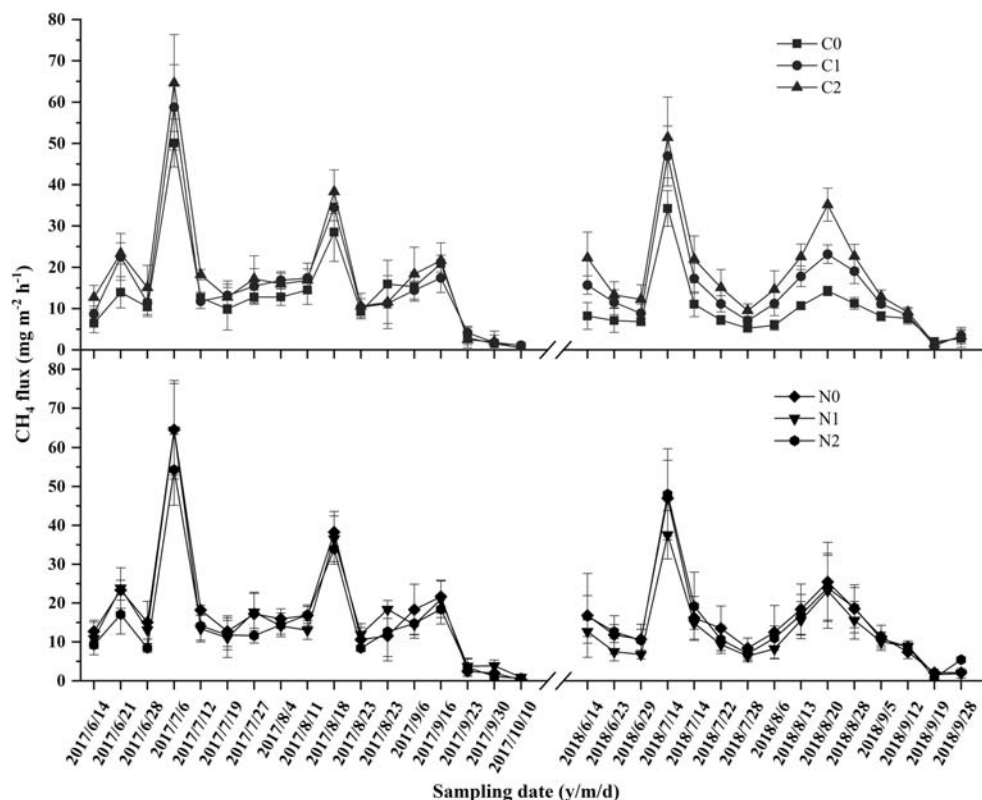
Table 2 Changes in seasonal cumulative emissions of CH₄ and N₂O, grain yield, GWP, and GHGI under different treatments

| Treatments | Cumulative CH ₄ emissions (kg ha ⁻¹) | | Cumulative N ₂ O emissions (kg ha ⁻¹) | | Grain yield (kg ha ⁻¹) | | GWP (kg CO ₂ eq. ha ⁻¹) | | GHGI (kg CO ₂ eq. ha ⁻¹ grain yield) | |
|------------|---|----------------|--|----------------|------------------------------------|---------------|--|----------------|--|---------------|
| | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 |
| C0 | 407.2 ± 25.4 c | 249.0 ± 11.7 c | 0.45 ± 0.25 a | 0.69 ± 0.24 a | 6992 ± 1059 b | 7624 ± 1543 b | 12339 ± 726 c | 7654 ± 400 c | 1.81 ± 0.34 b | 1.03 ± 0.21 c |
| C1 | 458.1 ± 47.8 b | 372.4 ± 46.7 b | 0.46 ± 0.27 a | 0.67 ± 0.25 a | 7441 ± 1148 a | 8054 ± 1317 a | 13865 ± 1420 b | 11350 ± 1411 b | 1.93 ± 0.49 b | 1.41 ± 0.34 b |
| C2 | 510.3 ± 52.6 a | 464.7 ± 60.6 a | 0.48 ± 0.24 a | 0.68 ± 0.24 a | 7329 ± 1267 ab | 8264 ± 1126 a | 15435 ± 1547 a | 14125 ± 1796 a | 2.19 ± 0.59 a | 1.72 ± 0.45 a |
| N0 | 493.6 ± 64.6 a | 387.5 ± 122 a | 0.18 ± 0.04 c | 0.40 ± 0.01 c | 5800 ± 343 c | 6268 ± 572 b | 14856 ± 1938 a | 11733 ± 3669 a | 2.57 ± 0.6 a | 1.80 ± 0.44 a |
| N1 | 424.6 ± 24.4 b | 314.7 ± 64.8 b | 0.47 ± 0.06 b | 0.69 ± 0.04 b | 7770 ± 553 b | 8684 ± 397 a | 12865 ± 742 c | 9625 ± 1945 b | 1.66 ± 0.15 b | 1.10 ± 0.20 c |
| N2 | 457.3 ± 64.9 b | 383.8 ± 97.0 a | 0.74 ± 0.08 a | 0.95 ± 0.04 a | 8191 ± 279 a | 8989 ± 369 a | 13919 ± 1947 b | 11771 ± 2908 a | 1.70 ± 0.23 b | 1.27 ± 0.31 b |
| N0C0 | 417 ± 5 de | 242 ± 8 e | 0.17 ± 0.05 c | 0.40 ± 0.01 d | 5691 ± 245 c | 5641 ± 167 c | 12565 ± 161 f | 7377 ± 240 f | 2.21 ± 0.08 c | 1.31 ± 0.01 d |
| N0C1 | 503 ± 17 b | 398 ± 15 c | 0.18 ± 0.05 c | 0.39 ± 0.01 d | 5949 ± 212 c | 6355 ± 439 c | 15127 ± 506 bc | 12029 ± 450 c | 2.55 ± 0.17 b | 1.90 ± 0.16 b |
| N0C2 | 561 ± 27 a | 523 ± 17 a | 0.18 ± 0.04 c | 0.40 ± 0.01 d | 5760 ± 559 c | 6808 ± 229 c | 16874 ± 811 a | 15793 ± 506 a | 2.95 ± 0.27 a | 2.32 ± 0.10 a |
| N1C0 | 426 ± 21 de | 242 ± 7 e | 0.47 ± 0.02 b | 0.72 ± 0.04 b | 7269 ± 400 b | 8366 ± 364 b | 12894 ± 623 ef | 7460 ± 205 f | 1.78 ± 0.07 d | 0.89 ± 0.02 f |
| N1C1 | 400 ± 8 ef | 312 ± 7 d | 0.42 ± 0.06 b | 0.65 ± 0.02 c | 8153 ± 257 a | 8850 ± 375 ab | 12105 ± 230 fg | 9532 ± 214 d | 1.49 ± 0.04 e | 1.08 ± 0.02 e |
| N1C2 | 448 ± 10 cd | 390 ± 20 c | 0.53 ± 0.03 b | 0.71 ± 0.04 bc | 7890 ± 616 ab | 8834 ± 362 ab | 13596 ± 300 de | 11884 ± 8125 c | 1.73 ± 0.10 d | 1.35 ± 0.11 d |
| N2C0 | 379 ± 16 f | 262 ± 6 e | 0.73 ± 0.09 a | 0.95 ± 0.05 a | 8018 ± 187 a | 8863 ± 582 ab | 11557 ± 491 g | 8125 ± 187 e | 1.44 ± 0.06 e | 0.92 ± 0.04 f |
| N2C1 | 472 ± 20 c | 408 ± 12 c | 0.77 ± 0.10 a | 0.97 ± 0.05 a | 8220 ± 392 a | 8956 ± 319 ab | 14363 ± 589 cd | 12488 ± 355 c | 1.75 ± 0.15 d | 1.40 ± 0.09 d |
| N2C2 | 521 ± 21 b | 482 ± 10 b | 0.72 ± 0.05 a | 0.94 ± 0.03 a | 8336 ± 211 a | 9149 ± 205 a | 15835 ± 635 b | 14699 ± 312 b | 1.90 ± 0.12 d | 1.61 ± 0.05 c |

Different letters in a column mean significant differences among treatments at the 5% level

C straw returning method, N fertilization rate, C0 wheat straw removal, C1 half of the straws returned into the fields, C2 all the straws returned into the fields, N0 no N fertilizer, N1 125 kg N ha⁻¹ of N fertilization, N2 250 kg N ha⁻¹ of N fertilization, GWP global warming potential, GHGI greenhouse gas intensity

Fig. 2 Seasonal changes in CH_4 fluxes under different straw-returning and N fertilizer treatments during the 2017 and 2018 rice-growing seasons. Bars represent standard error of the mean. C0, wheat straw removal; C1, half of the straws returned into the fields; C2, all the straws returned into the fields; N0, no N fertilizer; N1, 125 kg N ha^{-1} of N fertilization; N2, 250 kg N ha^{-1} of N fertilization



emissions from N1 and N2 treatments were 2.6 and 4.1 times higher in 2017 and 1.7 and 2.4 times higher in 2018, respectively.

Study year significantly affected N_2O emissions, and there were non-significant two-way or three-way interactions (Table 4).

Fig. 3 Seasonal changes in N_2O fluxes under different straw-returning and N fertilizer treatments during the 2017 and 2018 rice-growing seasons. Bars represent standard error of the mean. C0, wheat straw removal; C1, half of the straws returned into the fields; C2, all the straws returned into the fields; N0, no N fertilizer; N1, 125 kg N ha^{-1} of N fertilization; N2, 250 kg N ha^{-1} of N fertilization

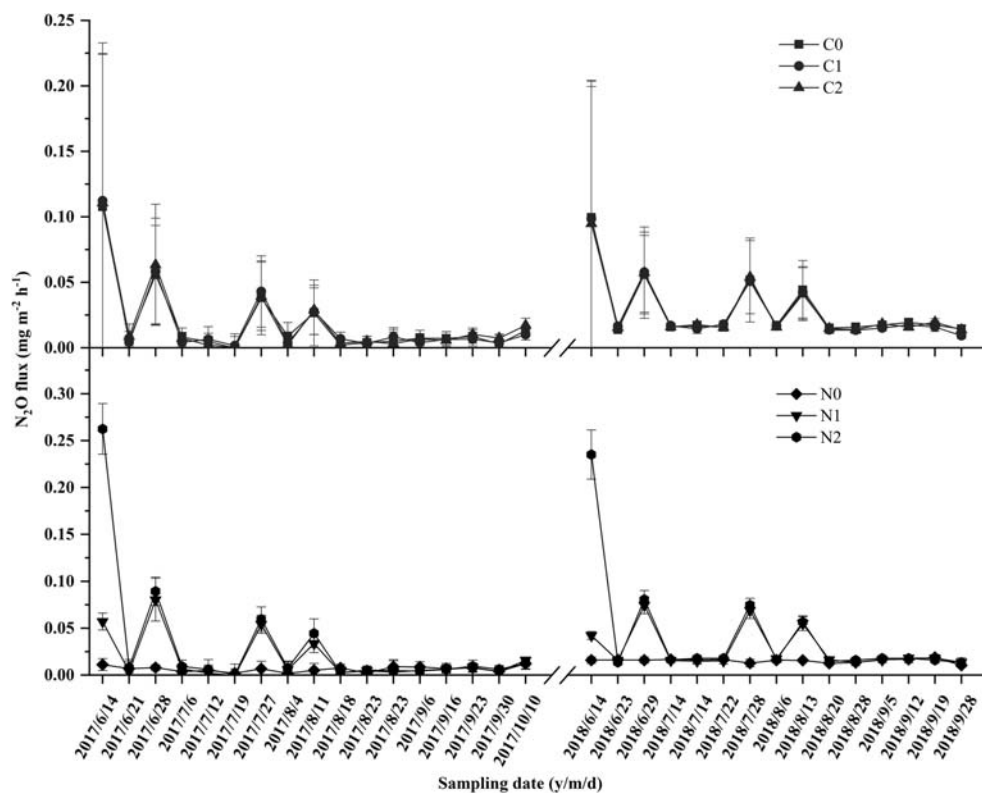


Table 3 Changes in AOA, AOB, nirK, nirS, and mcrA abundance under different treatments

| Treatments | AOA-amoA ($\times 10^8$ copies g^{-1}) | | AOB-amoA ($\times 10^7$ copies g^{-1}) | | nirK ($\times 10^7$ copies g^{-1}) | | nirS ($\times 10^7$ copies g^{-1}) | | mcrA ($\times 10^7$ copies g^{-1}) | |
|------------|--|---------------|--|----------------|--|----------------|--|---------------|--|---------------|
| | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 |
| C0 | 2.69 ± 2.14 b | 3.20 ± 2.53 c | 3.80 ± 2.03 b | 4.33 ± 2.35 b | 3.56 ± 1.66 b | 6.12 ± 2.74 b | 0.34 ± 0.17 b | 0.64 ± 0.28 b | 3.21 ± 0.19 c | 2.07 ± 0.05 c |
| C1 | 3.41 ± 2.13 a | 3.99 ± 2.51 a | 4.38 ± 2.05 a | 4.80 ± 2.12 a | 3.93 ± 1.55 a | 7.10 ± 2.77 a | 0.38 ± 0.16 a | 0.74 ± 0.30 a | 6.32 ± 3.01 b | 4.90 ± 2.24 b |
| C2 | 3.20 ± 2.11 a | 3.62 ± 2.34 b | 4.06 ± 1.94 ab | 4.54 ± 2.14 ab | 3.45 ± 1.66 b | 6.13 ± 2.74 b | 0.34 ± 0.16 b | 0.66 ± 0.30 b | 7.97 ± 1.06 a | 5.42 ± 0.67 a |
| N0 | 1.09 ± 0.06 c | 1.27 ± 0.04 c | 2.17 ± 0.06 c | 2.45 ± 0.07 c | 2.20 ± 0.12 c | 3.82 ± 0.17 c | 0.20 ± 0.01 c | 0.39 ± 0.02 c | 4.99 ± 2.30 b | 3.70 ± 1.54 b |
| N1 | 2.99 ± 1.05 b | 3.48 ± 1.20 b | 3.99 ± 0.89 b | 4.50 ± 0.98 b | 3.37 ± 0.64 b | 6.38 ± 1.49 b | 0.34 ± 0.07 b | 0.68 ± 0.12 b | 7.46 ± 3.52 a | 5.25 ± 2.79 a |
| N2 | 5.23 ± 0.13 a | 6.05 ± 0.09 a | 6.08 ± 0.08 a | 6.72 ± 0.23 a | 5.36 ± 0.07 a | 9.16 ± 0.09 a | 0.52 ± 0.01 a | 0.97 ± 0.03 a | 5.06 ± 2.02 b | 3.44 ± 1.49 b |
| N0C0 | 1.06 ± 0.11 e | 1.32 ± 0.12 e | 2.23 ± 0.22 e | 2.52 ± 0.21 e | 2.23 ± 0.17 d | 3.84 ± 0.37 d | 1.92 ± 0.15 d | 3.86 ± 0.32 d | 2.02 ± 0.20 f | 3.03 ± 0.24 d |
| N0C1 | 1.05 ± 0.09 e | 1.23 ± 0.10 e | 2.15 ± 0.19 e | 2.44 ± 0.22 e | 2.30 ± 0.17 d | 3.97 ± 0.37 d | 1.95 ± 0.14 d | 4.05 ± 0.38 d | 4.05 ± 0.37 d | 4.41 ± 0.41 c |
| N0C2 | 1.15 ± 0.12 e | 1.27 ± 0.14 e | 2.13 ± 0.13 e | 2.39 ± 0.21 e | 2.07 ± 0.21 d | 3.64 ± 0.33 d | 2.11 ± 0.25 d | 3.72 ± 0.21 d | 5.04 ± 0.35 c | 7.52 ± 0.77 b |
| N1C0 | 1.89 ± 0.10 d | 2.21 ± 0.25 d | 3.07 ± 0.24 d | 3.48 ± 0.33 d | 3.02 ± 0.17 c | 5.35 ± 0.46 c | 3.04 ± 0.32 c | 5.85 ± 0.39 c | 2.11 ± 0.23 f | 3.40 ± 0.32 d |
| N1C1 | 3.99 ± 0.28 b | 4.60 ± 0.40 b | 4.84 ± 0.51 b | 5.44 ± 0.42 b | 4.11 ± 0.38 b | 8.09 ± 0.59 b | 4.16 ± 0.32 b | 8.14 ± 0.67 b | 7.44 ± 0.51 a | 9.79 ± 0.72 a |
| N1C2 | 3.08 ± 0.34 c | 3.64 ± 0.31 c | 4.05 ± 0.35 c | 4.57 ± 0.29 c | 2.99 ± 0.27 c | 5.69 ± 0.57 c | 2.95 ± 0.09 c | 6.30 ± 0.62 a | 6.20 ± 0.52 b | 9.17 ± 0.85 a |
| N2C0 | 5.11 ± 0.57 a | 6.07 ± 0.39 a | 6.09 ± 6.16 a | 6.98 ± 0.67 a | 5.42 ± 0.53 a | 9.15 ± 0.87 a | 5.28 ± 0.40 a | 9.43 ± 0.65 a | 2.08 ± 0.16 f | 3.20 ± 0.30 d |
| N2C1 | 5.20 ± 0.50 a | 6.13 ± 0.63 a | 6.16 ± 0.49 a | 6.53 ± 0.60 a | 5.38 ± 0.46 a | 9.25 ± 0.83 a | 5.18 ± 0.40 a | 9.97 ± 0.61 a | 3.21 ± 0.36 e | 4.77 ± 0.43 c |
| N2C2 | 5.36 ± 0.52 a | 5.95 ± 0.36 a | 6.00 ± 0.35 a | 6.67 ± 0.45 a | 5.29 ± 0.53 a | 9.07 ± 0.62 ab | 5.23 ± 0.37 a | 9.64 ± 0.96 a | 5.03 ± 0.47 c | 7.20 ± 0.49 b |

Different letters in a column mean significant differences among treatments at the 5% level

C straw returning method, N N fertilization rate, C0 wheat straw removal, C1 half of the straws returned into the fields, C2 all the straws returned into the fields, N0 no N fertilizer, N1 125 kg N ha⁻¹ of N fertilization, N2 250 kg N ha⁻¹ of N fertilization, GWP global warming potential, GHGI greenhouse gas intensity

Table 4 Cumulative CH₄ and N₂O emissions, GWP, GHGI, yield, and gene abundance as affected by straw returning, N fertilization and study year. F-values are provided for significant effect

| | CH ₄ | N ₂ O | GWP | GHGI | Yield | AOA | AOB | nirS | nirK | mcrA |
|-------|-----------------|------------------|----------|----------|----------|----------|----------|----------|----------|----------|
| C | 501.88** | ns | 9.69** | 505.23** | 109.76** | 22.08** | 9.01** | 11.26** | 12.81** | 364.25** |
| N | 105.40** | 619.80** | 256.51** | 102.15** | 284.33** | 754.75** | 533.49** | 425.70** | 352.95** | 116.50** |
| Y | 550.28** | 278.50** | 53.08** | 531.47** | 337.00** | 28.85** | 21.56** | 655.56** | 458.81** | 179.68** |
| C×N | 34.36** | ns | ns | 34.66** | 16.46** | 21.17** | 14.30** | 6.72** | 8.63** | 45.70** |
| C×Y | 64.34** | ns | ns | 64.40** | 12.37** | ns | ns | ns | ns | 11.26** |
| N×Y | 7.89** | ns | ns | 7.90** | 10.15** | 3.87 * | ns | 34.72** | 23.53** | 4.48* |
| C×N×Y | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

C straw returning method, N N fertilization rate, Y study year, GWP global warming potential, GHGI greenhouse gas intensity, ns not significant
* $P < 0.05$, ** $P < 0.01$

3.3 GWP and GHGI

Both GWP and GHGI were significantly affected by straw returning, N fertilization, and study year (Table 2). Compared with C0 treatment, C1 and C2 treatments significantly increased the GWP by 12.4% and 25.1% in 2017 and by 48.4% and 84.7% in 2018, and the GHGI by 6.6% and 21.0% in 2017 and by 36.9% and 67.0% in 2018, respectively. The GWP and GHGI under N1 treatment were significantly lower than that under N0 treatment. For GWP, there were non-significant two-way or three-way interactions, while significant two-way interactions for GHGI were observed (Table 4).

3.4 Abundance of AOA-amoA, AOB-amoA, nirS, nirK, and mcrA

Straw returning, N fertilization, and their interactions had significant effects on the abundance of AOA-amoA and AOB-amoA (Tables 3 and 4). The abundance of AOA-amoA increased with increasing N fertilizer levels. The treatments of N1 and N2 significantly increased the abundance of AOA-amoA by 31.0% and 40.4% in 2017 and by 38.4% and 43.4% in 2018, respectively. Relative to that under C0 treatment, the AOB-amoA under C1 and C2 treatments was 15.3% and 6.8% higher in 2017 and 10.9% and 4.8% higher in 2018, respectively. Moreover, compared with N0 treatment, N1 and N2 treatments resulted in significant increases in the abundance of AOB-amoA.

The abundance of nirS and nirK was also significantly affected by straw returning, N fertilization, and their interactions (Tables 3 and 4). Compared with C0 treatment, the abundance of nirS in C1 treatment was increased by 11.8% and 15.6% in 2017 and 2018, respectively. No significant difference was found between C0 and C2 treatments. Higher abundance of nirS was found under N1 and N2 treatments than under N0 treatment. The nirK abundance

under C1 treatment was 1.13 times of that under C0 treatment. However, no significant difference was found between C0 and C2 treatments. N fertilization also increased the abundance of nirK. Compared with N0 treatment, N1 and N2 treatments led to significant increase in the abundance of nirK (by 53.2% and 144.0% in 2017 and by 67.0% and 140.0% in 2018).

The abundance of mcrA was also significantly affected by straw returning, N fertilization, and their interactions (Tables 3 and 4). Relative to that under C0 treatment, the mcrA abundance under C1 and C2 treatments was significantly elevated by 137.0% and 162.0% in 2017 and by 96.9% and 148.0% in 2018, respectively. Moreover, compared with N0 treatment, N1 treatment resulted in higher mcrA abundance.

3.5 Regression Analysis Between CH₄ and N₂O Emissions and the Abundance of Genes

Cumulative N₂O emissions were closely positively related to the abundance of AOA-amoA, AOB-amoA, nirS, and nirK (Table 5). Moreover, there was also significant and positive relationship between cumulative CH₄ emissions and the abundance of mcrA.

4 Discussion

4.1 Effects of Straw Returning and Chemical N Fertilization on Grain Yield

In the present study, straw returning remarkably increased the rice grain yield relative to straw removal (Table 2), possibly because the returned straws can input various nutrient components, such as organic N and available P and K, into the soil (Wang et al. 2007; Bi et al. 2009; Xue et al. 2013). But the grain yield did not differ between C1 and C2 treatments. Though soil organic matter increases as the amount of straw

Table 5 The correlations between CH₄ and N₂O emissions and the abundance of functional genes

| | AOA | | AOB | | nirS | | nirK | | mcrA | |
|------------------|--------|--------|--------|--------|--------|--------|--------|--------|-------|--------|
| | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 |
| CH ₄ | -0.24 | 0.05 | -0.26 | 0.01 | -0.23 | 0.01 | -0.25 | -0.004 | 0.28* | 0.49** |
| N ₂ O | 0.90** | 0.93** | 0.91** | 0.92** | 0.93** | 0.93** | 0.89** | 0.91** | 0.03 | -0.09 |

* $P < 0.05$, ** $P < 0.01$. $n = 54$

returning increases, the decomposition of large amounts of straws returned to the fields may consume soil oxygen content and increase the content of reducing substances, which is not conducive for the growth of rice roots (Bird et al. 2001; Chung et al. 2001; Kumar and Goh 2003).

In this study, N fertilization significantly increased rice grain yield (Tables 2 and 4) possibly due to development of leaf area, enhancement in photosynthetic capacity, high resistance to biotic stress, and improvement of N uptake (Kim et al. 2019). It has been reported that decreased N use efficiency by overuse of N fertilizers can result in economic loss and environmental problems (Huang and Tang 2010; Feng et al. 2013), such as surface water eutrophication and rain acidification (Liu et al. 2016). Therefore, optimum N rate is important to increase rice yield and protect the environment against pollution for rice production (Kim et al. 2019). In general, rice grain yield increased with increasing N fertilization rate (Table 2). However, the increase in grain yield did not rise linearly as the amount of N applied increased in the present study, suggesting that N use efficiency might not increase with increasing N rates. Our results indicate significant interaction between straw returning and N fertilization on grain yield (Table 4). Under N fertilization conditions, straw returning can increase organic carbon and release micronutrients to the soil, thus improving soil fertility and promoting rice growth (Wang et al. 2018).

4.2 Effects of Straw Returning and Chemical N Fertilization on CH₄ and N₂O Emissions

In the present study, CH₄ and N₂O emissions were affected by study year (Table 4), in which CH₄ emissions were higher in 2017 season than in 2018 season, but it was the opposite for N₂O emissions (Table 2). The differences in CH₄ and N₂O emissions between both years may be ascribed to more precipitation in 2017 than in 2018 (Fig. 1), especially from September to October. Continuous flooding at the late stage (from September to October in 2017) of rice growth caused the reduction of soil redox potential, and thus promoted CH₄ emissions. Moreover, continuous flooding is not conducive to microbial nitrification and denitrification to form N₂O (Liu et al. 2010a, b; Liu et al. 2016).

Double CH₄ flux peaks were observed at the tillering and heading stages (Fig. 2). In the tillering stage, the vigorous roots can excrete sufficient substrates. Meanwhile, the air temperature of the period is suitable for the growth of methanogens (Fig. 1). At the heading stage, rice stems grow vigorously, and thus, more CH₄ can be released through the stems. However, it is worth noting that another peak in CH₄ emissions was observed in September 2017 (Fig. 2). In September 2017, the large-scale heavy precipitation (Fig. 1) could have caused an anaerobic environment for methanogens in the soil, which in turn produced more CH₄ (Win et al. 2013).

Straw returning evidently increased CH₄ emissions (Tables 2 and 4), which is in agreement with increased mcrA abundance (Tables 3 and 5). Our result is consistent with previous studies (Guenet et al. 2012; Yuan et al. 2014; Tang et al. 2016). In the present study, C2 treatment resulted in the highest CH₄ emissions among all straw returning treatments (Table 2). The decomposition of wheat straws returned into fields can provide large amounts of available substrates for methanogenic bacteria and thus promote methanogenic bacterial growth (see Table 3), further increasing CH₄ emissions (Wassmann et al. 2000; Naser et al. 2007). However, some studies have found that when the straw was placed on the soil surface, the top of the straw is exposed to the air, which could reduce the activity of methanogenic bacteria, as they would be inhibited by O₂ (Chareonsilp et al. 2000; Harada et al. 2005; Ma et al. 2009). These inconsistent findings highlight that further research is important to reveal the relationship between crop straw returning and CH₄ emissions from paddy fields in order to propose a reasonable strategy for sustainable agriculture.

Many studies have reported that both CH₄ production and oxidation are influenced by N fertilizers, but the magnitude and direction of this response vary (Bodelier and Laanbroek 2004; Liu and Greaver 2009). Our results indicated that though N fertilization resulted in high abundance of mcrA relative to no N fertilizer (Table 3), the application of N fertilizer decreased CH₄ emissions (Table 2). The high abundance of mcrA (Table 3) may be attributed to enhanced rice growth and increased C substrates from rice roots due to N fertilization (Sun et al. 2018). But CH₄ and

ammonium (NH_4^+) can be oxidized by CH_4 monooxygenase (Bodelier and Laanbroek 2004). N fertilizer applied into the soil can produce a large amount of NH_4^+ , which will act as an inhibitor to reduce CH_4 oxidation by competing for CH_4 monooxygenase (Castro et al. 1994). Moreover, N uptake by crops under the reduction of N input may reduce the concentration of NH_4^+ in the soil, and thus the inhibition on the activity of the CH_4 monooxygenase enzyme may be attenuated (Whalen 2005), thereby promoting the oxidation of CH_4 . Moreover, N fertilization also promotes the growth of roots and tillering of rice plants, and thus more oxygen adheres to the surface of the root, which is beneficial to the activity of the methanotropic bacteria near the root, and then promotes CH_4 consumption (Baruah et al. 2010). Thus, CH_4 emissions are caused by the activities of both methanogenic archaea and methanotropic bacteria, and the balance between the two types of bacteria determines the net flux of CH_4 (Ahn et al. 2014). There are debates regarding the N fertilization effects on CH_4 emissions from paddy fields. Zhang et al. (2019) showed that N fertilization could lead to more litters of crops into the soil, increase C sources, and thus promote the activity of methanogens and CH_4 emissions subsequently. The inconsistent results suggest that it is necessary to study the mechanism of the effects of N fertilization on CH_4 emissions from paddy fields.

In this study, straw returning significantly affected the abundance of AOA, AOB, nirK, and nirS (Table 3); moreover, significant relationship between N_2O emissions and this gene abundance was found (Table 5). However, straw returning did not affect N_2O emissions in the present study (Table 4), which might be related to the C/N ratio of the returned straws (Mosier et al. 1998; Huang et al. 2004; Zou et al. 2005; Shan and Yan 2013). Zhang et al. (2015) showed that when the C/N ratio of straws was between 20 and 75, the fixation of mineral N and soil available N released from straw decomposition was in a state of dynamic equilibrium. In our study, the C/N ratio of the applied wheat straws was 69. Thus, almost no N was converted to N_2O emissions into the atmosphere. Moreover, straw returning can undoubtedly provide readily labile carbon and nitrogen substrates for nitrification and denitrification, thus stimulating growth of nitrification- and denitrification-related microorganisms (Table 2) and the potential of nitrification and denitrification subsequently (Burford and Bremner 1975; He et al. 2007; Chu et al. 2009). The improvement of nitrification due to increased reaction substrates from the decomposition of returned straws might promote N_2O emissions. However, though straw returning can

increase the readily labile organic carbon concentration, high concentration of labile organic carbon can decrease $\text{N}_2\text{O}/\text{N}_2$ ratios, resulting in decrease in N_2O emissions (Weier et al. 1993; Giles et al. 2012). Moreover, the decomposition of straws can consume soil oxygen and facilitate soil anaerobic conditions (Wang and Luo 2018), and thus increase denitrification, causing a large quantity of N_2O converted to N_2 . Therefore, no significant effects of straw returning on N_2O might be observed in this study.

Many studies have shown that N fertilization could provide substrates for microbial nitrification and denitrification, which in turn promote N_2O emissions (Yao et al. 2013a, b; Yang et al. 2017). Our results indicated that the application of N fertilizers increases N_2O emissions (Table 2). Nitrification involves two processes: ammonification and nitrite oxidation (He et al. 2007). The ammonification process is the rate-limiting step of nitrification process (Arp et al. 2002) and is catalyzed by ammonia monooxygenase (amo), which is mainly composed of AOA and AOB (Schleper 2010; Monteiro et al. 2014). The excess product hydroxylamine (NH_2OH) in the intermediate step undergoes incomplete oxidation under the action of reductase to produce N_2O (Poth 1986). Denitrification involves a variety of microorganisms, such as nitrite reductase (nir), which produces N_2O during the reduction of nitrite to N_2 . Nitrification and denitrification usually occur simultaneously, which together lead to the production of N_2O (Hu et al. 2015). Thus, more substrates are provided to the nitrifying and denitrifying microorganisms due to the application of more N fertilizers, which can be supported by higher abundance of AOA, AOB, nirK, and nirS under N fertilization (Table 3). Therefore, N fertilization increased N_2O emissions (Table 2).

We observed that the combination of straw returning and N fertilization significantly increased CH_4 emissions (Tables 3 and 4). Similar results were reported by Xia et al. (2014), which demonstrated that under the application of chemical fertilizer, returning of more straws into the fields could result in higher CH_4 emissions. Htun et al. (2017) indicated that the combination of straw and N fertilizer could significantly affect CH_4 uptake in soil. Meanwhile, our results indicated that there were no significant interaction effects of straw returning and N fertilization on N_2O emissions (Table 2), which is inconsistent with previous studies (Huang et al. 2017; Htun et al. 2017). Microbial nitrification and denitrification are the main processes producing N_2O in soil (Liu et al. 2011), but they are significantly affected by the soil conditions such as water and temperature (Siciliano et al. 2009). The

combination of straw and N application may affect soil moisture, temperature, organic matter content, inorganic nitrogen content, and soil oxidation potential, which may affect the release of N₂O in the soil (Xu et al. 2015; Grave et al. 2018). However, whether the combination of the two practices has a significant impact on N₂O emissions depends on local specific environment (Butterbach-Bahl et al. 2013). Thus, their interactions can be better understood when examined under different ecological conditions.

The GWP is usually used as a reference gas to convert CH₄ and N₂O emissions into CO₂ equivalents to comprehensively assess the potential effects of CH₄ and N₂O emissions on the climate system. In this study, the GWP of the rice growing seasons ranged from 7654 kg CO₂-eq. ha⁻¹ to 15435 kg CO₂-eq. ha⁻¹, which are lower than those reported by Zhang et al. (2015). Zhang et al. (2015) estimated that the GWP in the rice seasons was 16,245–21,422 kg CO₂-eq. ha⁻¹ in the central plains of China. The lower GWP in this study may be due to lower mean annual air temperature (15.5 °C) of the current study than that (17.8 °C) of Zhang et al. (2015). Higher air temperatures can promote microbial activity and cause the associated microbes to produce more CH₄ and N₂O (Khalil et al. 1998; Skiba et al. 2009). Moreover, in this study, straw returning also significantly increased GHGI (Table 2). Similar results were observed in previous studies (Sander et al. 2014; Hu et al. 2016). On the contrary, the application of N fertilizers reduced the GWP and GHGI (Table 2). These results indicated that CH₄ produced by straw returning is one of the important sources of GHGs emissions. In our study, the emitted CH₄ accounted for more than 99% of rice period non-CO₂ GWP (Table 2), suggesting that CH₄ is the major contributor to non-CO₂ GWP in rice-growing season. Similar results have been reported by Yao et al. (2013a, b) and Wang et al. (2018). Therefore, straw removal might be an ecological way to reduce GHGs emissions in the present study. However, when the crop straws are not required to be returned into the fields, the farmers in China usually burn the crop straws to save time and labor, which will cause environmental pollution. In contrast, straw returning can increase soil carbon sequestration and improve soil fertility (Khosa et al. 2010). Therefore, ecological straw treatment methods should be adopted. Ma et al. (2009) studied a variety of straw returning methods and found ditch mulching and strip mulching can significantly reduce CH₄ emission from rice fields with no adverse effect on grain yield. Dong et al. (2013) found that carbonized crop straws returned into the soil can increase soil stability carbon storage, improve soil

physical and chemical properties and microbiological properties, and inhibit or reduce CH₄ and N₂O production and emissions. Khosa et al. (2010) reported reduction of CH₄ emissions from rice paddies by addition of straw compost and increase in soil fertility and crop productivity relative to uncomposted straw returning. Hence, effective straw treatments can be developed to mitigate GWP, improve soil fertility, and increase or maintain crop yields in rice fields.

5 Conclusions

This study investigated the effects of straw returning and chemical N fertilization on greenhouse gas emissions from rice fields in central China. Straw returning significantly increased CH₄ emissions due to the increase in *mcrA* abundance, but had no significant effect on N₂O emissions. N fertilization significantly increased N₂O emissions due to increased abundance of AOA-*amoA*, AOB-*amoA*, *nirS*, and *nirK*, but decreased CH₄ emissions. Moreover, significant interaction effects of straw returning and N application on CH₄ emissions, greenhouse gas intensity, and grain yield were observed. Our results showed that 250 kg N ha⁻¹ of N application with straw removal could produce the lowest global warming potential, second lowest greenhouse gas intensity, and relative high grain yield. Hence, this approach can be used as a sustainable way to increase the agricultural economic and environmental benefits of rice fields in central China. Nevertheless, when the straw is not returned to the field, the organic carbon of the soil will not be replenished, and the treatment of straws in the field is still an important issue to be addressed. Our results suggest that the government should encourage the farmers to adopt environment-friendly approaches to treatment crop straws.

Acknowledgments We sincerely thank the editor and anonymous references for their valuable suggestions and critical comments for the improvement of the original manuscript.

Authors' Contributions Quanyi Hu, data analysis of postharvest experiments, and writing of the manuscript; Tianqi Liu, Songsong Jiang, Bin Chen, and Junbo Liu, conducting experiment in field; Cogui Cao and Chengfang Li, planning, design, and data analysis of postharvest experiments.

Funding Information This work is funded by the National Key Research and Development Project of China (2018YFD0301303 and 2017YFD0301403), the National Natural Science Foundation of China (31671637 and 31670447), the Natural Science Foundation of Hubei Province (2016CFA017 and 2018CFB608), and the Fundamental Research Funds for the Central Universities (2662019FW009).

Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interests.

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