~~Investigation on the feasibility of straw incorporation to improve~~ Mechanisms of enhancing soil fertility without obviously ~~increasing~~ elevating global warming potential under an optimal rice straw incorporation rate in a paddy soil

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Abstract. The utility of rice straw as an organic fertilizer has been widely recognized as a promising approach to enhancing soil fertility. However, straw return is currently in a dilemma, as it may also provoke greenhouse gas (GHG) emissions, leading to serious environmental consequences. It is urgent to reveal the feasibility of straw incorporation regarding soil fertility improvement without notable increases in GHG emissions. Here, a soil microcosm experiment was ~~employed~~conducted ~~to investigate the relationships between soil fertility and GHG fluxes and the underlying mechanisms influenced by straw amendments.~~using p~~P~~addy soils ~~were~~ collected from a long-term rice straw incorporation field experiment. The treatments of the field experiment, namely CK, ST1, ST2, and ST3 corresponding to the straw application rates of 0, 50%, 100%, and 150% of the local rice straw yield, respectively, were continued in the microcosm experiment. The dynamics of GHG fluxes and concentrations in soils, and the variations in the abundances of soil microbial communities were systematically determined. The results indicated that ~~continuous rice straw incorporation at half of the harvest (~~ST1~~)~~ treatment obviously improved soil fertility but did not induce significant elevation of global warming potential (GWP) with only 3% increase compared to CK. Although ST2 and ST3 treatments showed greater improvements in soil fertility, they significantly increased GWP by up to 151%. The minimal GWP increase ~~in GWP~~ under ST1 was mainly attributed to the significant reduction in N_2O emission and the slight rise in CH_4 emission compared to ~~straw removal~~CK. ~~The main mechanisms for these consequences were that~~The further investigations revealed that ST1 possessed the highest *nos*ZII abundance and the lowest *nirS*/*nos*ZII ratio, indicating its highest N_2O consumption ability~~;~~ ~~–~~. M~~m~~eanwhile, its CH_4 production ability fluctuated around the soil CH_4 holding capacity (190 mmol·mol^{-1}), and most of the produced CH_4 was consumed by methanotrophs in soil. In conclusion, rice straw can be incorporated into paddy soils at a suitable application rate, which can effectively enhance soil fertility without inducing an additional warming effect.

1 Introduction

Rice cultivation not only provides staple food for nearly half of the global population, but also produces massive rice straw that is estimated to be 800 to 1000 million tons·year^{-1} globally (Yuan et al., 2021; Harun et al., 2022). Rice straw has long been a valuable organic resource that can be incorporated into rice paddies to improve soil productivity (Huo et al., 2024; Zhou et al., 2024). The incorporation can enhance soil carbon (C) sequestration (Chen et al., 2025; Li et al., 2025a). It was estimated that about 15%–25% of the incorporated straw C was converted into soil organic carbon (SOC) (Liu et al., 2023b). Straw return can also provide abundant available nutrients for rice plant growth, including nitrogen (N), phosphorus (P), and potassium (K) (Yin et al., 2018; Van Hung et al., 2020; Wang et al., 2020). Thus, rice straw incorporation has been proposed to be an important agronomic practice for sustainable rice agriculture.

However, rice straw return was also revealed to exacerbate greenhouse gas (GHG) emissions from paddy fields (Shi et al., 2023; He et al., 2024). Rice cultivation contributes around 12% of global agricultural GHG emissions (FaoFAO, 2020) with straw incorporation being one of the key drivers. Numerous studies indicated that compared to straw removal, rice straw incorporation substantially promoted methane (CH_4) emissions (Han et al., 2023; Song et al., 2024; Qin et al., 2025). The increases in CH_4 emissions varied widely across observations, which approximately ranged from 23% to 1192% (Shen et al., 2014; Jiang et al., 2019; Lee et al., 2020; Nan et al., 2022; Song et al., 2024). The Straw incorporation may also elevate carbon dioxide (CO_2) fluxes from rice paddies, for example, by 9% to 67% (Shen et al., 2014; Lee et al., 2020; Han et al., 2023; Song et al., 2024). However, the effect of rice straw incorporation on nitrous oxide (N_2O) emissions from rice fields was inconsistent and controversial across studies (Shen et al., 2014; Wang et al., 2015; Han et al., 2023; Qin et al., 2023). The variations in N_2O emissions were about -73% to 137% in comparison with their controls (Shen et al., 2014; Wang et al., 2015; Han et al., 2023; Qin et al., 2023). The variability in GHG emissions could be affected by several factors, such as soil properties, agricultural management, and climate conditions, with the amount of straw incorporated potentially playing a vital role. Despite these issues, managing Optimizing the straw input rate was suggested to mitigateing the straw-induced GHG emissions (Shen et al., 2014; Chen et al., 2018; Li et al., 2024). For instance, incorporating with half of the harvested rice straw effectively reduced CH_4 emission by 48% in relative to the scenario receiving full of the strawfull straw incorporation (Shen et al., 2014). However, most studies focused either on the influence of straw return on soil productivity (Huo et al., 2024; Zhou et al., 2024) or on GHG emissions (Shi et al., 2023; He et al., 2024), rarely taking both into consideration to reveal the rational straw application. As a result, the balance and underlying mechanisms between soil fertility and GHG emissions are still poorly understood, leaving no clear consensus on how much straw should be incorporated appropriately. Hence, it is of high priorityessential to investigate the suitable feasibility of straw incorporation rate and the mechanisms in not causing noticeable increases in GHG emissions while improving soil fertility while not triggering substantial GHG emissions.

Soil GHG emissions are predominantly regulated by the microbially mediated production and consumption processes. CH_4 production and oxidation are catalyzed by methyl-coenzyme M reductase of methanogens and particulate methane monooxygenase of methanotrophs, with their active subunits encoded by *mcrA* and *pmoA* genes, respectively (Gao et al., 2020; Liu et al., 2025). The potential for N_2O production in paddy soils is closely associated with nitrite reduction, with nitrite reductases encoded by *nirK* and *nirS* genes (Chen et al., 2010; Chen et al., 2024; Kong et al., 2024). N_2O reduction is typically mediated by two distinct clades of N_2O reductase, encoded by *nosZ*I and *nosZ*II genes, respectively (Jones et al., 2013; Hallin et al., 2018; Liu et al., 2023a; Yang et al., 2024). While straw incorporation generally enhanced CH_4 production, its effects on CH_4 oxidation and N_2O production and reduction remain controversial. Some studies reported negligible effects of straw application on *pmoA* abundance (Wang et al., 2018), but others found increases in *pmoA* abundance (Jiang et al., 2019; Zhou et al., 2020a). For instance, long-term straw amendment increased *pmoA* abundance by 59% (Yang et al., 2022). Likewise, straw incorporation led to inconsistent responses from N_2O-related genes (Wang et al., 2018; Wang et al., 2021). The abundances of *nirK*, *nirS*, *nosZ*I, *nosZ*II were reported to increase, decrease, or remain unchanged following straw application (Wang et al., 2018). Taken together, the microbial mechanisms underlying GHG responses to straw incorporation remain insufficiently understood, particularly how key functional microbial communities respond to different straw application rates and thereby regulate GHG emissions.

Here, we hypothesized that there would be a moderate straw incorporation rate, which could well balance improving soil fertility and minimizing the increase in GWP. Considering that field experiments can hardly avoid the influences of climate and other factors, and the advantages of microcosm experiment including controlled environment and high operability in frequent gas and soil sampling, a soil culture experiment was employed to continue the field experiment with 5-year rice straw incorporation at different rates. The incubation was set with flooding and drying periods. The dynamics of GHG emissions and concentrations in soils were determined, and the variations in the abundance of soil microbial communities involved in GHG production and consumption were measured. This study aimed to explore whether there is a suitable straw application rate that can boost soil fertility without causing elevated GHG emissions, and the related mechanisms reveal the mechanisms of boosting soil fertility without elevating GWP under an optimal rice straw incorporation rate.

2 Materials and methods

2.1 Soil collection and rice straw preparationSite description and experimental design

Soils were collected from aA long-term straw return field experiment was initiated in 2017, which was located at the Taoyuan Agroecosystem Research Station (28°55′ N, 111°26′ E), Hunan, China. The region has a subtropical monsoon climate with a mean annual rainfall of 1440 mm and a mean annual temperature of 16.5°C. The soil was derived from quaternary red clay and classified as Ultisol according to USDA soil taxonomy. The experiment contained various rates of rice straw amendments with three replications in a randomized block design. Each plot was 30 m^2 (7.5 m × 4 m) and the cropping was single rice. Rice straw cut into 10 cm segments was evenly spread on the surface of the corresponding plots and turned over into the plow layer (0–20 cm) soils in each early May, which was about one month ahead of rice seedling transplantation. Besides, uUrea, superphosphate, and potassium chloride were annually applied to all treatments at 112.60 kg

$N\cdot ha^{-1}$, 46.33 kg $P\cdot ha^{-1}$, and 135.46 kg $K\cdot ha^{-1}$, respectively. All treatments were subjected to the same water management. During the rice season, all plots were continuously flooded except for two drying events. The first drying event occurred at the end of the tillering stage and lasted for one to two weeks. The second drying event happened one week before harvest. In the fallow season, all plots remained unirrigated.

2.2 Soil and straw sampling and preparation

~~The s~~Soils (0–20 cm) were separately taken from CK, ST1, ST2, and ST3 treatments of the field experiment in May 2022 before straw application. The corresponding straw ~~amounts~~ incorporation rates were 0, 3750, 7500, and 11250 $kg\cdot ha^{-1}$, representing 0, 50%, 100%, and 150% of the local rice straw yield, respectively. The soil from ~~one~~ each treatment was collected by taking five points following an "S" pattern in each plot and mixed, and then transported to the lab. The fresh soils were directly flooded and homogenized into slurries to avoid the impact of air–drying on microbial communities. ~~After removal of visible p~~Plant residues and gravel were removed by sieving the slurries through a 2 mm mesh, and the resulting soil~~s~~ slurries were pre-incubated under flooding at room temperature ~~in a greenhouse~~ for 30 days. The soil physicochemical properties were shown in Table 1.

Rice straw was prepared by collecting it from the field in October 2021, followed by air–drying and grinding (< 1 mm) for the microcosm experiment. The sieved straw can be evenly distributed and contacted with soil particles, generating similar decomposition speeds among the pots of each treatment. The rice straw contained 44.14% of C, 0.817% of N, 2.34% of K, and 0.143% of P.

2.~~2~~ 3 Microcosm experiment

The incubation pots were prepared by sealing the bottoms of PVC cylinders (height 15 cm, diameter 15.5 cm). The pots for gas sampling were further equipped as follows (Fig.S1). For gas flux sampling, a hollow rectangular groove (deep 2 cm) was fixed on the top fringe of each pot for water sealing during sampling. The ~~gas sampling was carried out~~gas flux samples were collected using a static chamber (height 29.5 cm, diameter 19.5 cm) fitted with a small electric fan at the inner top. For soil profile gas sampling, two holes (diameter 1.7 cm) across the cylinder were drilled at positions of 5 cm from the bottom, filled with a gas-permeable silicone tube (length 15.5 cm, inner diameter 1.2 cm) closed at both ends with silicone septa. One end of the tube was connected with a three-way stopcock outside the cylinder via a stainless steel tube (diameter 0.2 cm) through the septum. The drilled holes in the cylinder wall were then sealed. ~~For gas flux sampling, a hollow rectangular groove (deep 2 cm) was fixed on the top fringe of each pot for water sealing during sampling. The gas sampling was carried out using a static chamber (height 29.5 cm, diameter 19.5 cm) fitted with a small electric fan at the inner top.~~

The pre-incubated soil~~ slurries~~s drained of surface water from the different field treatments were separately filled into the pots~~, and~~. E~~e~~ach pot was filled with wet soil to 10 cm in height ~~contained~~ containing 1.27 kg ~~(dry weight)~~ of dry soil~~ to reach about 10 cm in depth~~. ~~The incubation followed the field regime and the straw amendment rates were calculated based on the~~

~~field experiment, which were~~The straw addition amounts in the incubation were 0, ~~2.54~~2.00, ~~5.08~~4.00, and ~~7.62~~6.00 g ~~rice~~ straw~~· per pot~~kg^{-1} dry soil for CK, ST1, ST2, and ST3 treatments, respectively, which were converted from the straw incorporation rates of the field experiment. Each treatment had eight pots: three for gas sampling, three for soil sampling, and two for replacing sampling-induced soil ~~cylinders~~ columns to minimize gas exchange from the sampled holes.

Urea was prepared as a stock solution (12.70 g N·L^{-1}), and 10 mL of this solution was added to each pot, equivalent to 100 mg N·kg^{-1} dry soil. After urea ~~(100 mg N·kg^{-1} dry soil)~~ and straw were applied, the soil was thoroughly mixed immediately to ensure that the straw and N fertilizer can be uniformly distributed in the soil~~thoroughly, and deionized water was added to meet a 3 cm water layer~~. Then, deionized water was added to form a 3 cm surface water layer. T~~t~~he prepared microcosms were randomly positioned and incubated in the dark at 30℃ for 91 days (28-day flooding and 63-day drying). This incubation period was based on our pre-experiment to cover both CH_4 and N_2O emission peaks. During ~~the~~ flooding, a 3 cm top water layer was maintained with deionized water. The drying process was carried out by removing the top water from the microcosm with a syringe on day 28 and then drying by natural evaporation in the room.

2.~~3~~ 4 Gas and soil sampling

Gas samples for ~~both~~ GHG fluxes and soil profile concentrations were taken between 9:00 and 11:00 AM every~~at~~ 1–3 days~~(s) intervals~~. At each sampling event, after the groove was filled with water and covered by the chamber, 30 mL of headspace gas was taken with a syringe separately at 0 and 60 min ~~separately~~ from each gas sampling pot (Wang et al., 2017; Zhou et al., 2020b). The electric fan was running during the sampling period. Immediately after collecting all headspace gas samples~~At the same time~~, 1 mL of soil gas was taken from ~~the~~ each buried silicone tube, ~~followed by~~with an equal volume of helium injected back. The gas from the silicone tube was used to determine the soil CO_2, CH_4, and N_2O concentrations. All the gas samples were stored in 12 mL pre-evacuated exetainer vials (Labco, UK).

Soil samples (0–5 cm) were taken every two weeks via a modified syringe sampler with its head cut off. At each sampling event, three cores (diameter 1.5 cm) were sampled from each pot and mixed, weighing about 35 g wet soil. During flooding, the soil samples were collected directly without removing the overlying water to minimize the disturbance (Cai et al., 2022; Cai et al., 2025). Before starting soil sampling, placing the front end of the syringe sampler as close as possible to the soil surface effectively reduced the collection of the overlying water. A part of the sample was used for soil moisture measurement, and the rest was flash-frozen in liquid nitrogen and stored at -80°C for molecular analysis. After sampling, the holes were immediately ~~replaced by filling~~refilled with the cores taken from the soil replacement pots.

2.~~4~~ 5 Analysis of gas concentrations and soil properties

Gas samples were analyzed using a gas chromatography (7890A, Agilent, USA) equipped with an electron capture detector for N_2O and a flame ionization detector for CH_4 and CO_2. Gas fluxes were calculated based on the Ideal Gas Law (Eq. 1 and Eq. 2), and cumulative emissions by the Trapezoidal Rule (Eq. 3). Global warming potential (GWP) was calculated using cumulative emissions (Eq. 4).

$$F = \frac{\Delta m}{A \times \Delta t}, \quad (1)$$

Where F is the gas flux ($\mu g \cdot m^{-2} \cdot h^{-1}$); Δm is the mass of gas collected in a single sampling event (μg); A is the soil surface area covered by the static chamber (m^{-2}); Δt is the time interval after chamber closure (h).

$$\Delta m = M \times \Delta n = M \times \frac{pV \times \Delta c}{RT}, \quad (2)$$

Where M is the molar mass of gas ($g \cdot mol^{-1}$); Δn is the amount of substance of gas collected in a single sampling event (mol); p is the atmospheric pressure (Pa); V is the active volume of the static chamber during sampling (m^3); Δc is the gas concentration difference (ppm); R is the gas constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$); T is the temperature during sampling (K).

$$CE = \sum_{i=1}^{n} \frac{F_{i+1} + F_i}{1000} \times (t_{i+1} - t_i) \times 24 \times \frac{1}{2}, \quad (3)$$

Where CE is the gas cumulative emission ($g \cdot m^{-2}$); F is the gas flux ($\mu g \cdot m^{-2} \cdot h^{-1}$); 1000 in the denominator is to transform μg to g; t is the sampling time (d); i and i+1 represent the i^{th} and $(i+1)^{th}$ sampling events, respectively; 24 is the transform factor from day to hour; n is the total number of sampling events.

$$GWP = CE_{CO2} + 27.9 \times CE_{CH4} + 273 \times CE_{N2O}, \quad (4)$$

Where the unit of GWP is g CO_2 equivalent·m^{-2}. CE_{CO2}, CE_{CH4}, and CE_{N2O} represent the cumulative emissions ($g \cdot m^{-2}$) of CO_2, CH_4, and N_2O, respectively. The factors 27.9 and 273 are the latest-updated 100-year global warming potentials for CH_4 and N_2O, respectively (Forster et al., 2021).

Soil properties were measured by standard protocols (Bao, 2000). Soil pH was determined using soil slurry at a soil–water ratio of 1:2.5 by a pH meter (FE28–Standard, Mettler Toledo, Switzerland). Soil bulk density (BD) was measured by the ring knife method. Soil cation exchange capacity (CEC) was extracted by 1.66 cM $Co(NH_3)_6Cl_3$ at a soil–extractant ratio of 7:100. Olsen P was extracted by 0.5 M $NaHCO_3$ at a soil–extractant ratio of 1:20. Soil CEC and Olsen P were quantified colorimetrically via a continuous–flow automatic analyzer (AutoAnalyzer 3, SEAL Analytic, Germany). Available N (Av–N) was determined by the alkaline diffusion method. Available K (Av–K) was extracted by 1 M NH_4OAc at a soil–extractant ratio of 1:10 and analyzed via an atomic absorption spectrometer (novAA350, Analytic Jena, Germany). Soil available manganese (Av–Mn), copper (Av–Cu), and zinc (Av–Zn) were simultaneously extracted by 5 mM DTPA–$CaCl_2$–TEA at a soil–extractant ratio of 1:2 and determined by an inductively coupled plasma emission spectrometer (ICP–OES 5110, Agilent, USA). Soil total C (TC) and total N (TN) were analyzed by combustion using an elemental analyzer (Vario MAX cube, Elementar, Germany). Soil total P (TP) and total K (TK) were measured by the ICP–OES after digestion by HNO_3–$HClO_4$–HF.

2.~~5~~ 6 DNA extraction and real-time quantitative PCR

Soil DNA was extracted manually as previously described (Chen et al., 2010). Each sample was performed in triplicate. The quality and concentration of DNA were measured by a NanoDrop ND–1000 spectrophotometer (Thermo Scientific, USA). The abundances of bacterial 16S rRNA, fungal 18S rRNA, *mcrA*, *pmoA*, *nirK*, *nirS*, *nosZ*I, and *nosZ*II were determined by real-time quantitative PCR (qPCR). ~~The genes of *mcrA* and *pmoA* were used as markers for methanogens and aerobic~~

~~methanotrophs, respectively (Liu et al., 2025). The genes of *nirS* and *nosZ*II were selected to reflect N_2O production (Chen et al., 2024; Kong et al., 2024) and N_2O reduction (Jones et al., 2013; Hallin et al., 2018; Liu et al., 2023a; Yang et al., 2024), respectively.~~ The qPCR primers and thermal programs were shown in Table S1. All qPCR reactions were conducted in triplicate on 384-well plates by a LightCycler 480II (Roche, Switzerland). Each reaction (10 μL) contained 1 μL of template DNA (5 ng·μL^{-1}), 5 μL of SYBR Green Pro Taq HS Premix (Accurate Biotechnology, China), and 0.3–1.2 μL of each primer (10 μM). Negative controls were included in each plate by replacing the template DNA with sterile H_2O. Standard curves were prepared by ten-fold serial dilutions of target gene-carrying plasmids. The amplification efficiency was 87%–88% for *nosZ*II and 90%–102% for other genes.

2.~~6~~ 7 Statistical analysis

Statistical tests were performed in IBM SPSS Statistics 25 (NY, USA) and OriginPro 2026 (OriginLab Corporation, MA, USA). Homoscedasticity ($\alpha = 0.05$) was checked with Levene's test. Significant differences between treatments ($\alpha = 0.05$) were tested by one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test when data were homoscedastic or Games–Howell (GH) test when data were heteroscedastic. Spearman correlation analysis was conducted using corrplot package in R v4.5.3 (α = 0.05, 0.01, or 0.001).

3 Results

3.1 Influence of straw incorporation on soil fertility~~Soil properties~~

Soil physicochemical characteristics were clearly affected by continuous straw incorporation at various rates for five years (Table 1). ~~Although the concentrations of soil TP and TK were not strongly influenced by straw application, the concentrations of TC, TN, available nutrients, and CEC were positively related to the straw incorporation rate and soil BD was negatively related to the rate. When compared with CK, even in the treatment with the least amount of straw (ST1), the TC and TN contents were increased by 4.19% and 2.58%, respectively. ST1 treatment also resulted in obvious increases in Av_N, Olsen P, and Av_K by 6.83%, 15.09%, and 38.52% ($p < 0.05$), respectively. Furthermore, the contents of soil available micronutrients of Mn, Cu, and Zn in ST1 treatment were also increased by 18.48%, 4.57%, and 8.09%, respectively.~~The results indicated that soil BD was negatively and significantly correlated with the rice straw incorporation rate ($p < 0.05$), suggesting the physical character was improved. Concerning the detected soil chemical properties, except for TP, TK, and Olsen P, which were not clearly influenced by straw incorporation, the rest properties were positively related to the straw application rate. Among them, the variations of CEC ($p < 0.05$), AvK ($p < 0.001$), and AvZn ($p < 0.05$) were significantly affected. When compared with CK, even the least amount of straw input (ST1) induced the decrease of BD by 2.76% and the increases of CEC, TC, TN, AvN, Olsen P, and AvK by 3.13%, 4.19%, 2.58%, 6.83%, 15.09%, and 38.52%, respectively. ST1 treatment also resulted in obvious increases in soil micronutrient concentrations in comparison with CK, with the contents of AvMn, AvCu, and AvZn elevated by 18.48%, 4.57%, and 8.09%, respectively.

Table 1: Soil properties after five-year straw incorporation at different rates

	pH (H_2O) (1:2.5)	BD $g\cdot cm^{-3}$	CEC $cmol^{+}\cdot kg^{-1}$	Av–N $mg\cdot kg^{-1}$	Olsen P $mg\cdot kg^{-1}$	Av–K $mg\cdot kg^{-1}$	Av–Mn $mg\cdot kg^{-1}$	Av–Cu $mg\cdot kg^{-1}$	Av–Zn $mg\cdot kg^{-1}$	TC $g\cdot kg^{-1}$	TN $g\cdot kg^{-1}$	TP $g\cdot kg^{-1}$	TK $g\cdot kg^{-1}$
CK	4.90± 0.03 a	1.45± 0.03 a	10.85± 0.15 a	192.79± 5.38 a	13.19± 1.76 a	51.09± 2.70 c	8.28± 0.22 a	1.75± 0.10 a	1.36± 0.07 a	21.74± 0.46 a	2.057± 0.04 a	0.618± 0.03 a	12.16± 0.08 a
ST1	4.98± 0.05 a	1.41± 0.01 a	11.19± 0.23 a	205.96± 3.59 a	15.18± 1.68 a	70.77± 3.62 bc	9.81± 1.50 a	1.83± 0.09 a	1.47± 0.10 a	22.65± 0.59 a	2.110± 0.05 a	0.657± 0.02 a	12.01± 0.11 a
ST2	4.93± 0.02 a	1.34± 0.07 a	11.25± 0.20 a	212.07± 8.15 a	12.57± 1.33 a	82.35± 5.01 ab	11.65± 2.30 a	1.94± 0.07 a	1.70± 0.07 a	23.52± 0.70 a	2.197± 0.07 a	0.610± 0.03 a	11.95± 0.19 a
ST3	5.02± 0.07 a	1.31± 0.03 a	11.45± 0.26 a	209.22± 11.70 a	16.06± 2.22 a	105.36± 11.01 a	16.69± 5.97 a	1.88± 0.05 a	1.76± 0.18 a	23.71± 1.45 a	2.160± 0.12 a	0.648± 0.04 a	12.04± 0.03 a
r	0.45	**-0.67***	**0.63***	0.45	0.17	**0.93*****	0.48	0.30	**0.67***	0.50	0.39	0.11	-0.26

These soils were collected from the plots of a long-term field experiment for the microcosm experiment. CK, ST1, ST2, and ST3 received 0, 50%, 100%, and 150% of the local harvested rice straw, respectively. Different lower-case letters indicate significant differences between treatments (α = 0.05). Data are given in average ± standard error (n = 3). The Spearman correlation coefficient (r) between soil properties and straw rate is presented in the last row. Significant values are shown in bold. *, α = 0.05; **, α = 0.01; ***, α = 0.001. ~~Abbreviations:~~ BD, bulk density; CEC, cation exchange capacity; Av–N, available nitrogen; Olsen P, available phosphorus; Av–K, available potassium; Av–Mn, available manganese; Av–Cu, available copper; Av–Zn, available zinc; TC, total carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium.

3.2 CO_2, CH_4, and N_2O emissions

Greenhouse gas emissions were obviously influenced by straw incorporation (Fig.1, Table 2). Generally, the CO_2 flux was positively related to the straw incorporation rate throughout the incubation (Fig.1a, Table 3). Compared to CK, ST1 caused a significant increase in the cumulative CO_2 emission by 66% (p < 0.05, Table 2). With the straw amount increasing, the cumulative CO_2 emissions of ST2 and ST3 treatments rose to 2.2 and 2.5 times those of CK (p < 0.05), respectively. A significant difference was also detected between ST2 and ST3 (p < 0.05).

The CH_4 fluxes occurred mainly during flooding (day 0–day 28), and partly during the transition time from flooding to drying (day 29–day 56, ~~) (~~Fig.1b, Table S2). It was observed that CK emitted minimal CH_4, while ST1 emitted a slightly higher CH_4 than CK. Further increasing the straw rate induced significant elevation of CH_4 fluxes. The more straw input, the more CH_4 was emitted. The cumulative CH_4 emission of ST1 was only 4.41 $g\cdot m^{-2}$, while the cumulative CH_4 emissions of ST2 and ST3 were approximately 4 and 8 times higher than those of ST1 (p < 0.05), respectively, reaching about 23 and 38 $g\cdot m^{-2}$ (Table 2).

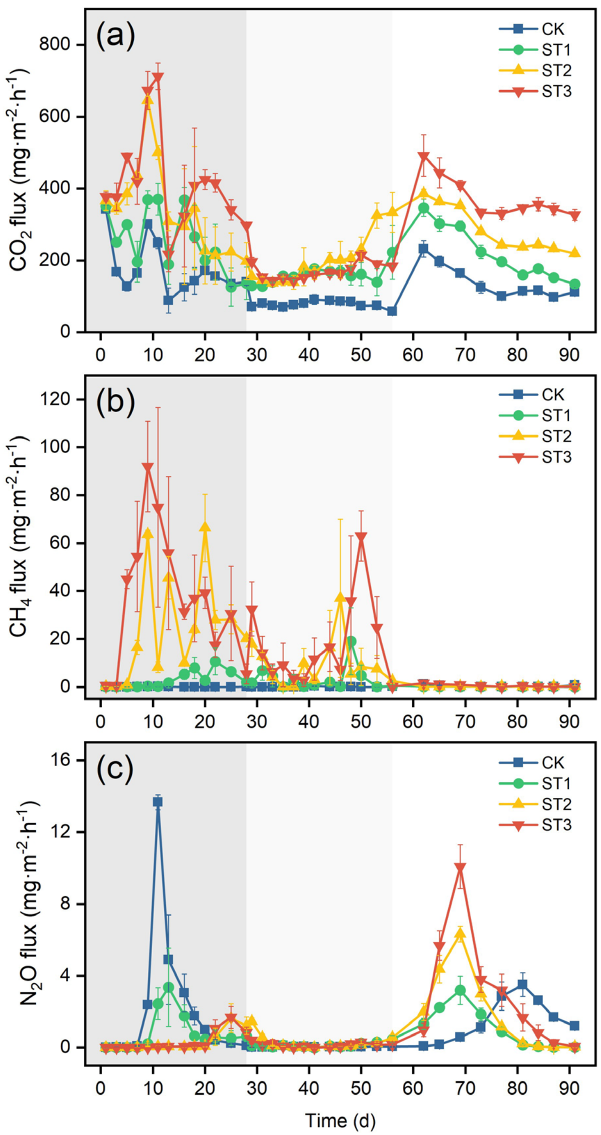

Figure 1: Dynamics of GHG fluxes during the incubation. (a) CO_2; (b) CH_4; (c) N_2O. Data are given in average ± standard error (n = 3). Grey, lighter grey, and white areas indicate flooding, the early drained period, and the late drained period, respectively.

Table 2: Cumulative emissions and global warming potential

		Cumulative emissions		**Global warming potential**
	CO_2 ($g \cdot m^{-2}$)	**CH_4 ($g \cdot m^{-2}$)**	**N_2O ($g \cdot m^{-2}$)**	**(g CO_2 equivalent$\cdot m^{-2}$)**
CK	280.19 ± 4.37 d	0.36 ± 0.02 d	2.61 ± 0.08 ab	1003.49 ± 24.74 c
ST1	465.65 ± 16.07 c	4.41 ± 0.52 c	1.63 ± 0.11 c	1034.95 ± 48.01 c

ST2	605.91 ± 26.84 b	23.22 ± 0.93 b	2.04 ± 0.17 bc	1810.05 ± 56.46 b
ST3	695.71 ± 17.59 a	38.01 ± 0.35 a	2.80 ± 0.20 a	2521.49 ± 80.01 a

Different lower-case letters indicate significant differences between treatments (α = 0.05). Data are given in average ± standard error (n = 3).

Table 3: Spearman correlation between straw rate, cumulative GHG emissions, and microbial gene abundances

	CO_2	CH_4	N_2O	Bacteria	Fungi	*mcrA*	*pmoA*	*nirK*	*nirS*	*nosZ*I	*nosZ*II
Straw rate	**0.97*** **	**0.97*** **	0.22	**0.91*** **	**0.97*** **	**0.97*** **	**0.78** **	**0.78** **	**0.82** **	**0.91*** **	0.28
CO_2				**0.87*** **	**0.95*** **	**0.96*** **	**0.76** **	**0.76** **	**0.79** **	**0.89*** **	0.30
CH_4				**0.85*** **	**0.95*** **	**0.96*** **	**0.78** **	**0.74** **	**0.75** **	**0.87*** **	0.25
N_2O				0.26	0.21	0.28	0.17	**0.64***	0.13	0.16	-0.50

Significant values are shown in bold. *, α = 0.05; **, α = 0.01; ***, α = 0.001.

~~The dynamics of N_2O emission during the experiment exhibited two peaking periods: one occurred just after nitrogen fertilization and the other happened during drying process, but the variation patterns of the flux peaks between the treatments within the two peaking times were obviously different (Fig.1c)~~The dynamics of N_2O emission exhibited two distinct peaks, with one occurring shortly after fertilization and the other during the late drying period (Fig.1c). However, the variation patterns of the two peaks differed markedly both among treatments and between periods. During the first peak~~ing period~~, CK emitted most N_2O, followed by ST1, while the N_2O emitted from ST2 and ST3 were negligible. However, this order reversed in the latter drying period, where the highest N_2O flux was from ST3, followed by ST2, whereas the N_2O fluxes of ST1 and CK were similarly low, despite that the peak of CK appeared about 10 days late. The cumulative N_2O emission indicated that ST1 emitted significantly lower N_2O than CK ($p < 0.05$) with only 1.63 $g \cdot m^{-2}$ (Table 2). Meanwhile, ST2 also generated about 22% lower N_2O than CK, while the treatment receiving the highest amount of straw (ST3) possessed a similar cumulative N_2O emission to CK.

Overall, ST1 displayed a slightly higher GWP than CK without a significant difference, but both ST2 and ST3 showed significantly higher GWPs than CK ($p < 0.05$).

3.3 Dynamics of CO_2, CH_4, and N_2O concentrations in soils

The soil concentrations of the three GHGs were deeply affected by the amendments of rice straw but exhibited distinct patterns (Fig.2). Straw incorporation ~~significantly increased~~boosted the soil CO_2 concentration compared to the continuously declining CK, with the peaking time of straw–amended treatments occurring around 10 days of the incubation (Fig.2a). The outstanding characteristic ~~of the dynamics of CO_2 concentrations~~ was that the increases were proportional to the straw incorporation rates. For example, at the peak~~ing time~~, the increments ~~of~~ in ST1, ST2, and ST3 relative to CK were approximately 50%, 100%, and 150%~~ against CK~~, respectively.

The soil CH_4 concentrations indicated that straw incorporation resulted in sharp increases since day 3 (Fig.2b). On average, the CH_4 concentrations of ST1, ST2, and ST3 were about 45, 57, and 58 times ~~those~~ that of CK ~~during~~ between day 1 and day 56. It was also observed that the CH_4 concentrations of ST1 fluctuated around 200 $mmol \cdot mol^{-1}$. ~~The treatments of~~ ST2 and ST3 further promoted soil CH_4 concentrations but there was no ~~significant~~ obvious differences between them.

The soil N_2O concentrations were only detectable between days 62 and 87 under drying phase (Fig.2c). The unique feature was that the N_2O contents of CK and ST1 were quite similar despite the peaking time in CK was delayed. It was observed that the N_2O concentration of ST2 was almost two times that of ST1. Although the peak of ST3 was not as high as that of ST2, ST3 maintained high N_2O concentrations for longer in the soil.

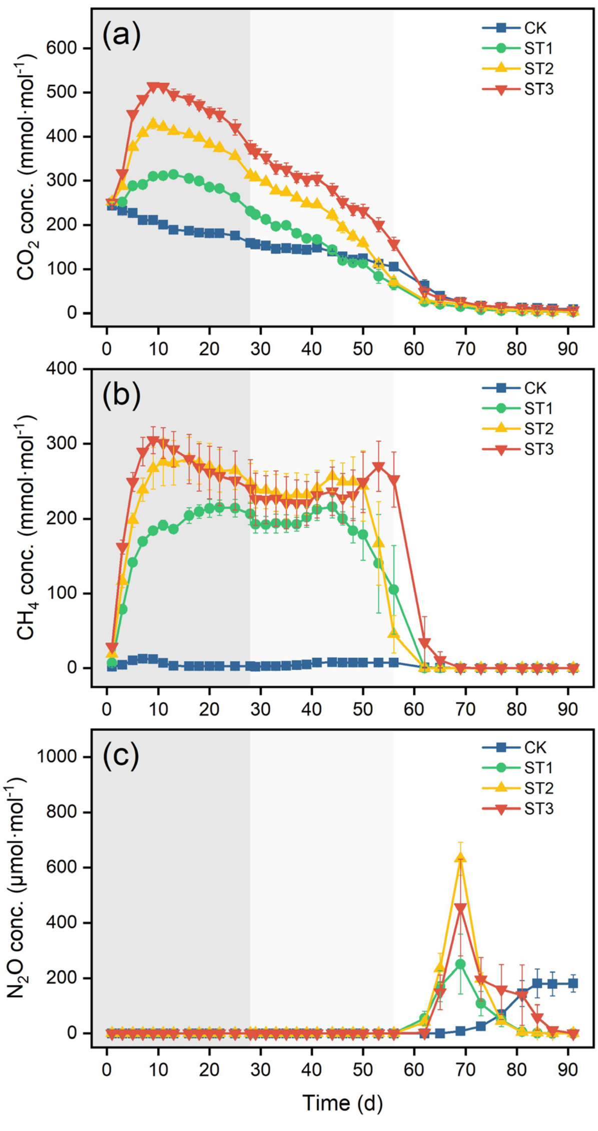

Figure 2: Dynamics of GHG concentrations in soils during the incubation. (a) CO_2; (b) CH_4; (c) N_2O. The abbreviation *conc.* means concentration. Data are given in average ± standard error (n = 3). Grey, lighter grey, and white areas indicate flooding, the early drained period, and the late drained period, respectively.

3.4 Variations of microbial abundance

3.4.1 The abundance of bacteria and fungi

In general, the abundance of both bacterial 16S rRNA and fungal 18S rRNA followed the order of ST3 > ST2 > ST1 > CK (Fig.3). The average copy numbers of 16S rRNA were 3.92E+12, 4.50E+12, 4.76E+12, and 5.71E+12 per ~~gram~~ g dry soil for CK, ST1, ST2, and ST3, respectively. The average abundances of 18S rRNA were 2.04E+9, 2.78E+9, 3.03E+9, and 3.63E+9 per ~~gram~~ g dry soil, respectively.

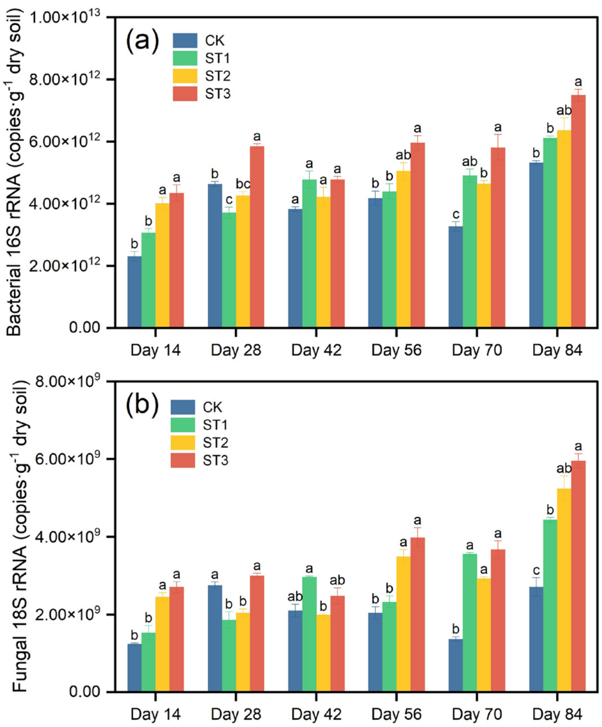

Figure 3: Dynamics of bacterial 16S rRNA abundance (a) and fungal 18S rRNA abundance (b) during the incubation. Different lower-case letters denote significant differences between treatments (α = 0.05). Data are given in average ± standard error (n = 3).

3.4.2 The abundance of methanogens ~~(*mcrA*)~~ and methanotrophs ~~(*pmoA*)~~

The copy number of *mcrA* was ~~obviously~~ markedly affected by straw incorporation and positively related to the straw input rate (Fig.4a, Table 3). The average *mcrA* abundance during the CH_4 fluxing period between day 14 and day 56 was significantly and positively related to the straw input rate. In comparison with CK, the least increase occurred in ST1 with 32%, while the highest happened in ST3 with 129%.

For *pmoA*, although straw incorporation also clearly promoted its copy number, the treatment with the lowest amount of straw (ST1) induced a substantial increase in methanotrophs compared to CK by approximately 81%. Further increasing the straw amount failed to cause significant elevations in comparison with ST1 (Fig.4b).

When concerning *mcrA*/*pmoA*, the ratio of ST1 was quite similar to that of CK, while the ratios of ST2 and ST3 were significantly higher than that of CK (Fig.4c).

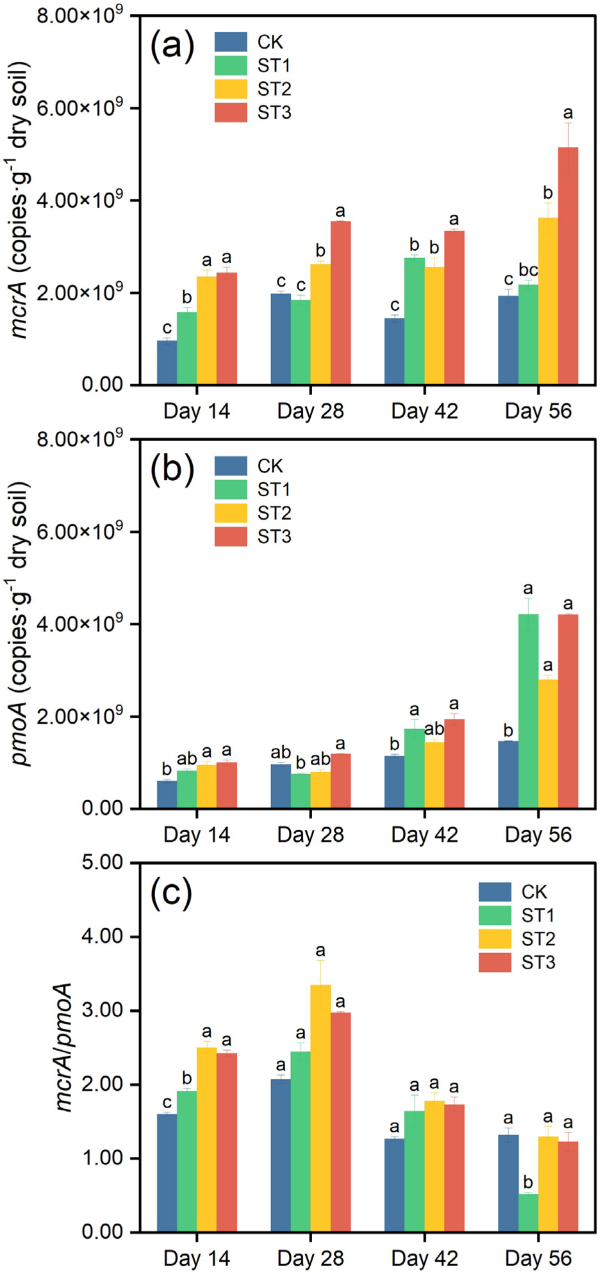

Figure 4: Dynamics of *mcrA* abundance (a), *pmoA* abundance (b), and *mcrA*/*pmoA* ratio (c) during the CH_4 fluxing period. Different lower-case letters denote significant differences between treatments (α = 0.05). Data are given in average ± standard error (n = 3).

3.4.3 The abundance of N_2O-producing ~~(*nirS*)~~ and N_2O-reducing ~~(*nosZII*)~~ communities

The population size of *nirS* was relatively stable, and the variations among ~~the~~ treatments were negligible under flooding (Fig.5a). ~~However~~In contrast, under drying, the copy number of *nirS* increased and was clearly affected by ~~the~~ straw incorporation~~s~~. All the straw–amended treatments outstandingly increased *nirS* abundance compared to CK. Among the treatments, ST3 possessed the highest *nirS* copy numbers ~~of *nirS*~~, while ST2 and ST1 had similarly lower abundances.~~ ~~ By comparison, the abundances of *nirK* declined after drainage and were less affected by straw incorporation than those of *nirS* (except ST3, Fig. S2).

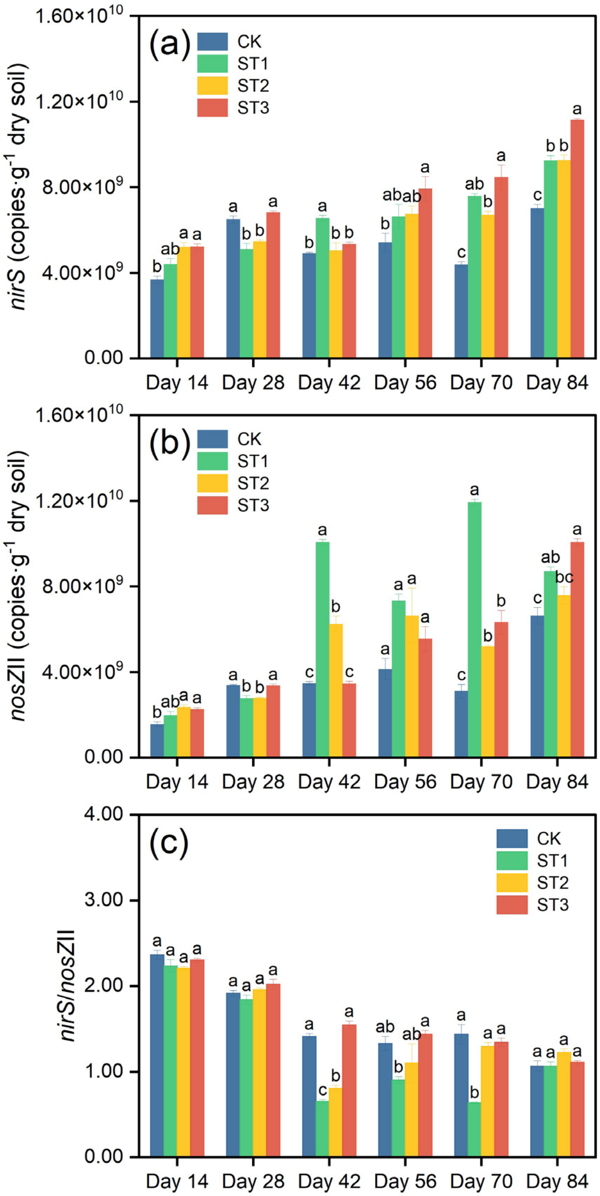

Figure 5: Dynamics of *nirS* abundance (a), *nosZ*II abundance (b), and *nirS*/*nosZ*II ratio (c) during the incubation. Different lower-case letters denote significant differences between treatments (α = 0.05). Data are given in average ± standard error (n = 3).

In general, the dynamics of *nosZ*II abundance during the incubation were similar to the variations of *nirS* abundance (Fig.5b). Interestingly, the abundance of *nosZ*II in ST1 was the highest, which was significantly higher than the second highest treatment (ST3) during drying, especially during the peaking time of N_2O flux on day 70. In contrast, the abundance differences of *nosZ*I among treatments were far less pronounced than those of *nosZ*II (Fig. S2).

When concerning the abundance ratio of *nirS*/*nosZ*II during drying, ST1 displayed the lowest average ratio of 0.81, while the average ratios of other treatments were higher, ranging from 1.11 to 1.36 (Fig.5c).

4 Discussion

Rice straw is an important organic resource and has been used to improve soil productivity (Huo et al., 2024; Zhou et al., 2024), which was also observed in this study. Since rice straw return may also exacerbate GHG emissions from paddy fields (Shi et al., 2023; He et al., 2024), which could offset the benefits of rice straw returning to paddy systems, it is urgent to investigate the feasibility of straw incorporation in relation to soil fertility improvement and GHG emission control. We hypothesized that there should be a suitable straw incorporation amount, which could not only modify soil fertility, but also generate minimal GHG emissions without causing a significant increase in GWP. Soil fertility is the capability of soil to continuously supply water, nutrients, air, and suitable thermal conditions for plant growth, which can be reflected by soil physical, chemical, and microbial characteristics (Theresa et al., 2026). We found that soil BD was negatively related to the straw incorporation rate, and soil CEC and soil available nutrient concentrations were positively related to the rate. The results strongly suggested that five-year rice straw incorporation improved soil fertility by increasing soil aeration, soil cation holding capacity, and nutrient supply ability (Xing et al., 2025). In this study, the least amount of straw incorporation (ST1) generated the lowest GWP among straw–amended treatments. If this treatment can also lead to the elevation of soil fertility, it would support our hypothesis. The results showed that although the soil physicochemical properties of ST1 were not significantly different from those of CK, the characteristics were systematically improved. For instance, BD decreased by 2.76%, and TC and AvK increased by 4.19% and 38.52%, respectively. It is obvious that after five years of rice straw incorporation under ST1, soil porosity, soil organic matter, and nutrient supply ability all increased, indicating its soil fertility was improved.

We found that ST1 treatment clearly improved soil physicochemical properties. More importantly, it failed to ST1 treatment did not cause a significantly increase in GWP compared to the treatment without straw input (CK)CK. It was observed that the cumulative CO_2 and CH_4 emissions of ST1 were significantly higher than those of CK, but the cumulative N_2O emission was significantly lower. Since the warming potentials over a 100-year horizon of CO_2, CH_4, and N_2O are 1, 27.9, and 273, respectively (Forster et al., 2021), the major contributor to the low GWP under ST1 would be the significantly lower N_2O emission. The interesting point is why this happened.

It was detected that the CO_2 fluxes were positively related to the straw incorporation rate, and the higher input induced the higher CO_2 emission. Among straw–amended treatments, ST1 generated the lowest cumulative CO_2 flux, which was about 1.7 times of CK. This would be linked to the small amount of organic substrate for the soil microbial communities and the limited growth of microbial population (Miller et al., 2008; Li et al., 2022). Although the CO_2 emission of ST1 increased significantly compared to CK, its SOC content rose simultaneously, indicating this rate of straw returning was beneficial for the elevation of SOC.

Among CO_2, CH_4, and N_2O, N_2O has the highest warming potential over a 100-year horizon, followed by CH_4 and CO_2 (Forster et al., 2021). It was detected that there were two peaks of N_2O fluxesWe detected two N_2O flux peaks during the incubation: one occurred after N fertilization under flooding and the other appeared within drying, which were consistent with other studies (Zhou et al., 2020b; Senbayram et al., 2022; Li et al., 2025b): one was under flooding just after nitrogen fertilization and the other occurred during drying. We found that only CK emitted a large amount ofconsiderable N_2O while the emissions from straw–amended treatments were negligible markedly lower at the first peakduring the flooding phase. This would be partially due to the competition of soil microorganisms for soil available nitrogenN. Generally, organic materials incorporated into paddy soil will stimulate mass propagation of soil microorganisms, and these fast reproducing microbial communities will compete for soil free nitrogen N as their nutrient (Cucu et al., 2014; L'espérance et al., 2024; Zhang et al., 2025). On the contrary, the nitrogen N fertilizer in CK could be an available nitrogen N source for nitrification and denitrification. As a consequence, this treatment emitted a lot of, leading to a substanial N_2O emission. However, during drying, oxygen continuously penetrated the soil profile, and abundant available nitrogen N was released mainly through the mineralization of soil organic matter (Chiriac et al., 2025). Organic matter also contributed more electron donors to denitrification when the soil moisture declined (Yi et al., 2022). These means if a soil contains higher SOC, it would produce more mineral nitrogen N as the substrate and provide more electron donors for N_2O production and reduction (Yi et al., 2022; Chiriac et al., 2025). Although ST1 contained slightly higher SOC than CK, there was no clear difference in N_2O fluxes between them during the drying process. The reasons for this phenomenon would be as follows. N_2O emission largely relies on N_2O production ability and consumption activity, which are driven by the related functional microorganisms (Hu et al., 2015; He et al., 2025). Among these microorganisms, the denitrifiers of *nirS*-type and *nosZ*II-type communities were indicated as the more active microbial groups in paddy soils (Jones et al., 2013; Wei et al., 2015; Jin et al., 2020; Chen et al., 2024; Yang et al., 2024). Among all treatments, It was detected that ST1 treatment contained a relatively low abundance of *nirS* and the highest *nosZ*II copy number among the treatments, as well as the lowest *nirS*/*nosZ*II ratio. These results strongly suggested that ST1 would possess relatively low N_2O production but and high N_2O transformation activitiescapabilities. As a result, the N_2O emission from ST1 was significantly lower than that from CK.

Applying organic materials to paddy fields often intensified CH_4 flux from soils (Han et al., 2023; Song et al., 2024; Qin et al., 2025). Similar results were also observed in this study, and the CH_4 emission was significantly and positively related to the straw input rate. Interestingly, the cumulative CH_4 emission from ST1 treatment was quiteremained low with less than 5 $g \cdot m^{-2}$. , whereas Further increasing the straw incorporation rateST2 and ST3 sharply elevated theCH_4 emissions, such as the cumulative CH_4 emissions of ST2 and ST3 were to about 5 and 9 times those that of ST1, respectively. The question is why ST1 treatment maintained such a low level of CH_4 flux. CH_4 emission depends on CH_4 production and consumption in soil, which are driven by methanogens and methanotrophs (Conrad, 2020; Nwokolo and Enebe, 2025). The population size of *mcrA* in ST1 was clearly higher than that in CK but significantly lower than ST2 and ST3, indicating that the CH_4 production capacity of ST1 would be higher than that of CK but lower than the treatments receiving higher amounts of straw. Another important aspect in determining CH_4 flux is the soil CH_4 consumption capability (Conrad, 2020; Nwokolo and Enebe, 2025).

Soil CH_4 consumption ~~in a soil~~ would be strongly linked to ~~its~~ soil CH_4 holding capacity (Ariani et al., 2022)~~, because this gas remaining in the soil would be mostly transformed~~ as the retained CH_4 can be oxidized by methanotrophs. Once CH_4 producing ability is over the soil CH_4 holding capacity, the extra CH_4 would be emitted into the atmosphere. We observed that the soil CH_4 holding capacity was about 190 mmol·mol^{-1} under the anaerobic environment, ~~which was determined from the soil CH_4 concentrations where~~and below this level CH_4 emission~~s~~ ~~were~~ was almost undetectable. It was detected that the CH_4 concentrations of ST2 and ST3 were clearly over the holding capacity, and CH_4 emissions notably occurred. In contrast, the~~We also observed that~~ soil CH_4 concentrations of ST1 fluctuated ~~about~~ around 200 mmol·mol^{-1} under anaerobic conditions, leading to ~~and coincidently,~~ the minimal CH_4 emission among straw–amended treatments.~~of this treatment was minimal. While the CH_4 concentrations in ST2 and ST3 were clearly over 190 mmol·mol^{-1}, CH_4 emissions notably occurred. Therefore, the CH_4 production activity in ST1 treatment was slightly over the soil CH_4 holding capacity, which would be the main mechanism for its minimal CH_4 flux among straw–amended treatments.~~ Moreover, the microbial gene abundances in this study were at the higher end of the ranges reported in the literature (Ma et al., 2012; Zhou et al., 2020b; Wang et al., 2022; Yi et al., 2024; Cai et al., 2025; Zheng et al., 2026). This could be attributed to the differences in soil properties among studies and to straw incorporation that provided substrates for microbial growth.

5 Conclusion

Rice straw could be incorporated into paddy soil at a suitable amount. Compared to no straw input, it could not only improve soil fertility, but also avoid significantly increasing GWP. The major contributors to this consequence were strongly linked to the highest *nosZ*II copy number and the lowest *nirS/nosZ*II ratio, and the CH_4 producing ability fluctuated around the soil CH_4 holding capacity and most of the produced CH_4 was consumed by soil methanotrophs. Because different soils possess various soil properties, their CH_4 holding capacities and nitrogen transformation processes would be different. Therefore, the field tests with different soil types are necessary.~~the feasibilities of straw returning for different soils require further investigations.~~

Data availability

Data will be made available on reasonable request.

Supplement link

The link to the supplement will be included by Copernicus, if applicable.

Author contributions

MZ: Conceptualization, Data Curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - Original Draft, Writing - Review & Editing. **RL**: Data Curation, Formal analysis. **WZ**: Conceptualization, Resources, Supervision, Writing - Review & Editing. **CF**: Resources. **SGC** and **AT**: Writing - Review & Editing. **BZ**: Conceptualization, Resources, Supervision, Writing - Review & Editing. **WW**: Conceptualization, Methodology, Resources, Supervision, Validation, Writing - Original Draft, Writing - Review & Editing. **RS**: Conceptualization, Funding acquisition, Resources, Supervision, Writing - Original Draft, Writing - Review & Editing.

Competing interests

The authors declare that they have no conflict of interest.

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Acknowledgements

We thank Xingan Fu and Xiaohua Yang from Taoyuan Agroecosystem Research Station for their help in soil and gas sampling. We thank Laurent Philippot from INRAE, France for his constructive suggestions in paper writing.

Financial support

This work was supported by the International Partnership Program of Chinese Academy of Sciences (Grant No. 092GJHZ2022057FN), and the Training Program for Outstanding Youth of Changsha (Grant No. kq2306030). Simon Guerrero-Cruz received support from the NXPO Thailand under PMU-B, through the European and SE Asia joint funding scheme (JFS-2021-100: Micro-GRICE).

Review statement

The review statement will be added by Copernicus Publications listing the handling editor as well as all contributing referees according to their status anonymous or identified.

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