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Straw incorporation mitigates methane emissions by facilitating the conversion of particulate organic carbon to mineral-associated organic carbon

Jiayu Qin [a,b], Xinlu Long [a,b], Yuxi Zhou [a,b], Ligeng Jiang [a,b], Pengli Yuan [a,b,*]

[a] Key Laboratory of Crop Cultivation and Physiology, Guangxi University, Education Department of Guangxi Zhuang Autonomous Region, Nanning 530004, China

[b] Guangxi Key Laboratory of Agro-environment and Agric-products Safety, Guangxi University, Nanning 530004, China

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ABSTRACT

Incorporating straw return into tillage systems is a potential strategy for sustaining rice production, while achieving multiple environmental benefits. The effect of different tillage management on methane emissions has been well documented; however, the combined effects with straw return management require further exploration. To investigate this, an experiment was initiated in 2008 using five management practices: conventional tillage, no tillage, conventional tillage with straw mulching, conventional tillage with straw incorporation, and no tillage with straw mulching. Changes in soil carbon pool properties, hydrolytic and oxidative enzyme activities, phospholipid fatty acids, and the abundance of methanogenic and methanotrophic genes were measured during the early and late rice-growing seasons in 2022 and 2023. The results indicated the following: (1) Straw return significantly increased cumulative methane emissions by 75.5 % compared with straw removal. However, conventional tillage with straw incorporation reduced cumulative methane emissions by 37.2 % and 20.3 % compared to conventional tillage with straw mulching and no tillage with straw mulching, respectively. (2) Conventional tillage with straw incorporation enhanced β-acetylglucosaminidase and cellobiohydrolase activities and increased mineral-associated organic carbon content compared to conventional tillage with straw mulching and no tillage with straw mulching. (3) Under conventional tillage with straw incorporation, the content of phospholipid fatty acids in bacteria, fungi, and actinomycetes increased by 7.6 %–19.3 %, 7.2 %–18.3 %, and 6.0 %–19.8 % compared with conventional tillage, no tillage, and conventional tillage with straw mulching, respectively. (4) Conventional tillage with straw incorporation reduced methanogens/methanotrophs by 14.1 % and 4.0 % compared with conventional tillage with straw mulching and no tillage with straw mulching, respectively. Structural equation modeling revealed that tillage and straw management promoted the conversion of particulate organic carbon to mineral-associated organic carbon by increasing the soil microbial populations and β-acetylglucosaminidase and cellobiohydrolase activities, which regulated methane production by methane-related functional communities. Thus, regulating the conversion of activated carbon to inert carbon through a rational combination of tillage and straw return methods can effectively reduce methane emissions from double rice paddies.

1. Introduction

Methane (CH_4) is the second most significant anthropogenic greenhouse gas contributing to climate warming, with 34 times the warming potential of carbon dioxide over a 100-year period (Deng et al., 2022). Furthermore, CH_4 emission accounts for 9 % of the anthropogenic greenhouse effect from soil (Kopittke et al., 2024). Paddy fields represent a major anthropogenic source of CH_4 emissions, contributing approximately 48 % of the total CH_4 emissions from agricultural lands (Carlson et al., 2017). Rice production demand is projected to increase by 28 % by 2050, which is accompanied by a large amount of CH_4 emissions (Zhu et al., 2018). Although straw return is a common practice for enhancing soil fertility and crop yields, it also stimulates CH_4 emission from paddy fields (Zhang et al., 2022a). Consequently, there is an

* Corresponding author at: Key Laboratory of Crop Cultivation and Physiology, Guangxi University, Education Department of Guangxi Zhuang Autonomous Region, Nanning 530004, China.

E-mail address: pengliyuan@gxu.edu.cn (P. Yuan).

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urgent need to investigate CH_4 emission mechanisms under sustainable paddy field management and develop emission reduction technologies to achieve both increased production and reduced emissions.

CH_4 emissions in paddy fields result from the combined activity of methanogens and methanotrophs (Malyan et al., 2016). Methanotrophs can oxidize CH_4 under aerobic conditions using oxygen as an electron acceptor, a process that oxidizes more than 50 % of the CH_4 produced in paddy soils (Thauer, 2010). However, NH_4^+-N in the soil competes with CH_4 for the active site of the enzyme, and NO_2^-, NO_3^-, and NO_3^- bound cations produced during NH_4^+-N oxidation are directly toxic to methanotrophs, thereby inhibiting CH_4 oxidation (Hu et al., 2011). on the other hand, soil microorganisms affect CH_4 emissions by regulating the decomposition and fixation of soil organic carbon (SOC) (Conrad, 2007), which can be categorized as active organic carbon (particulate organic carbon, POC) and inert organic carbon (mineral-associated organic carbon, MAOC) based on the rate of decomposition and turnover (Zhou et al., 2022). In addition, CH_4 production involves hydrolytic enzymes that can decompose complex organic matter into monosaccharides, which are then converted into the substrates required by methanogens. Overall, a variety of factors (e.g., soil organic carbon pools and their components, soil enzyme activities, and microorganisms) influence CH_4 emissions from rice fields. However, all of these factors are affected by field management practices (e.g., tillage, straw return), which directly or indirectly affect CH_4 emissions from rice paddies.

No-tillage (NT) is a sustainable farmland practice that can reduce soil disturbance and enhance soil fertility (Huang et al., 2020; Kan et al., 2021). However, studies have yielded inconsistent results regarding the impact of NT on CH_4 emissions from paddy fields, with some reports indicating significant increases (Zhang et al., 2016; Sainju, 2016), others exhibiting decreases (Tellez-Rio et al., 2015) and some have found no effect (Bayer et al., 2015). These variations might stem from interactions with other field management practices or environmental factors. Straw return, which is known for its benefits to soil fertility and crop production (Yang et al., 2021), can increase CH_4 emissions by promoting methanogen growth owing to higher exogenous organic carbon inputs (Wang et al., 2018). Nonetheless, elevated methane emissions also stimulate methanotrophic growth, which may counteract the negative effects of straw return. Additionally, returning straw from rice and other crops to the field can help reduce CH_4 emissions (Zhou et al., 2020). Different methods of straw return to the field can affect CH_4 emissions differently, with ditch-buried straw return reducing CH_4 emissions by 10.8 % compared with straw incorporation (Hu et al., 2016). The non-rice season straw return promotes the microbial decomposition of straw, reducing CH_4 emissions owing to the lack of favorable conditions for CH_4 production (Zhang et al., 2015). Therefore, the relationship between different tillage and straw return methods and methane emissions from rice fields remains unclear. In particular, tillage or straw return may significantly alter the anaerobic decomposition environment of organic matter, which in turn affects the substrate supply of methanogens and the activity of methanotrophs. Moreover, combination management may amplify or diminish this effect (Zhang et al., 2022b), thus, a sustainable combination management system is needed to enhance the ability to accurately regulate CH_4 emissions from paddy fields.

Paddy fields in China contribute to 29.9 % of global annual CH_4 emissions (Chen et al., 2021). As these fields are essential for national food security and are a major source of anthropogenic CH_4 emissions, the growing conflict between increasing food production and reducing greenhouse gas emissions can become more pronounced with population growth. Therefore, we conducted a field experiment to assess the effects of various tillage and straw management practices on CH_4 emissions from paddy fields. Our objectives were to (1) characterize the CH_4 emission patterns under different tillage and straw practices and (2) examine the relationship between soil physicochemical properties, microbial composition, and the abundance of CH_4 functional bacteria with CH_4 emissions. We hypothesized that although straw return could increase CH_4 emissions, straw incorporation may reduce emissions by improving soil-straw contact, increasing inert carbon content, and modulating CH_4-related microbial communities, compared to straw mulching.

2. Materials and methods

2.1. Site description and experimental design

A long-term positioning experiment initiated in 2008 at Guangxi University's farm in Nanning City, Guangxi Province (Fig. S1) (22°50′N, 108°17′E) was conducted using a double-cropped rice system. The region has an average annual temperature of 21.9°C. January is the coldest month, with an average month temperature of 12.0°C. July and August are the hottest months, with an average month temperature of 28.1°C. The annual rainfall at the site was 1065 mm (Fig. S2). According to the IUSS Working Group WRB classification (IUSS Working Group WRB, 2015), the soil is a Haplic Acrisol, derived from quaternary red clay.

The experiment employed a randomized block design with five management practices: conventional tillage (CT), no tillage (NT), conventional tillage with straw mulching (CT-SMR), conventional tillage with straw incorporation (CT-SR), and no tillage with straw mulching (NT-SMR), each replicated thrice. The area of each experimental plot is 36 m^2 (6 m × 6 m). Ridges, 30 cm high and 20 cm wide, were constructed between plots and covered with a film to prevent fertilizer cascade seepage.

In CT, all rice straw was removed after harvesting, and the soil was rotary-plowed to a depth of 20 cm. In NT, all rice straw was removed, and no plowing was performed. For CT-SMR, after harvesting, the soil was rotary plowed to 20 cm, and straw was mulched. In CT-SR, straw was mulched on the surface after harvesting and then incorporated into the soil by rotary plowing. In NT-SMR, only straw mulching was performed after harvesting. The first rice season was from March to July and the second from August to November. Fertilizer applications per season for each plot included N, P_2O_5, and K_2O at 232, 98, and 180 kg ha^{-1}, respectively. P_2O_5 was applied entirely at sowing, whereas N and K_2O were applied at sowing, tillering, and heading stages in a 5:3:2 ratio. Other management measures (including irrigation, weed and pest control) follow local farming management.

2.2. Gas sampling and measurements

The CH_4 emission flux from paddy fields was measured by static closed-box technique after rice transplanting (Haque et al., 2015). The chambers constructed from PVC and surrounded by a foam board to maintain a constant temperature were equipped with a small 12 V fan and a temperature sensor to ensure adequate gas mixing and record the temperature changes during sampling. The base of each sampling chamber was inserted into the soil surface of each plot before rice transplantation. Samples were taken once every 7 days during rice growth and once every 10–15 days after rice harvest, with additional gas sampling after days 1, 3, 5 and 7 of fertilizer application.

CH_4 emission flux was calculated using Eq. (1) (Chen et al., 2021).

$$F = \rho \times \frac{V}{S} \times (dc/dt) \times \frac{273}{273 + t} \tag{1}$$

where F is the CH_4 emission flux (mg m^{-2} h^{-1}), ρ is the gas density at the standard temperature (mg m^{-3}), t is the mean temperature (°C), V is the effective volume of the static chamber (m^3), S is the area of the bottom seat (m^2), and dc/dt is the slope of the gas concentration over time.

Cumulative CH_4 emissions (Te) for each season were calculated using Eq. (2) (Haque et al., 2015).

$$Te = \sum_i^n \left(\frac{F_i + F_{i+1}}{2} \times \Delta d \times 24 / 100 \right) \tag{2}$$

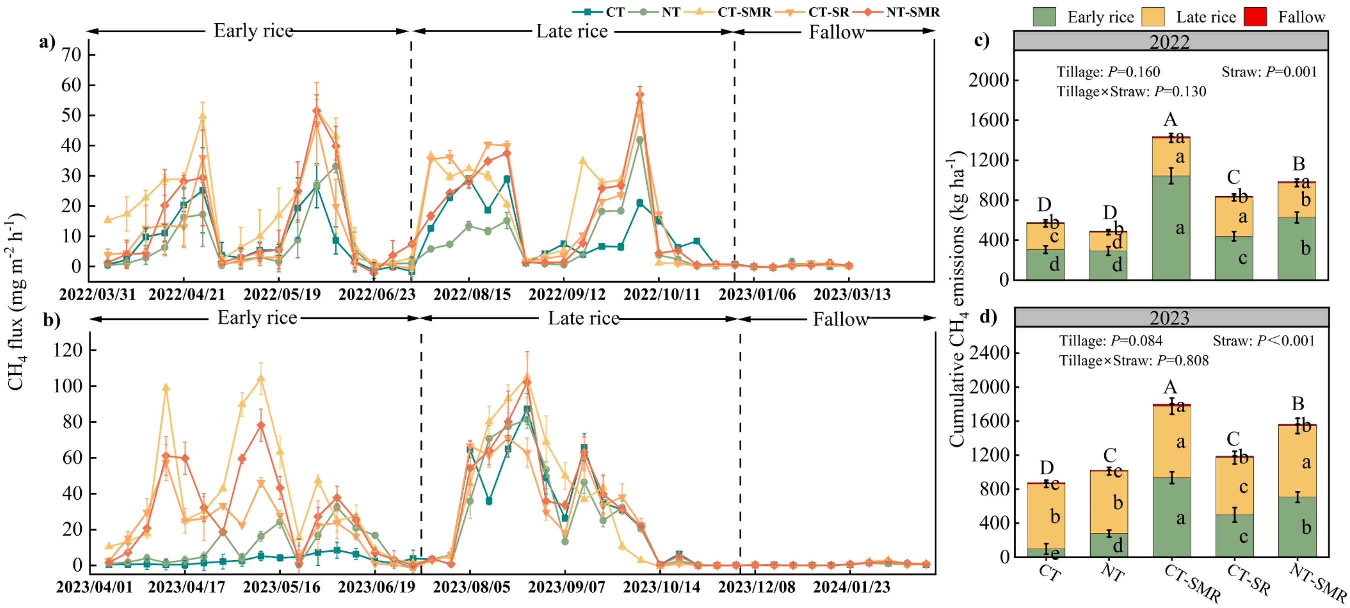

Fig. 1. Effects of different tillage and straw return practices on CH_4 flux and cumulative CH_4 emissions in 2022 (a and c) and 2023 (b and d). CT, conventional tillage; NT, no tillage; CT-SMR, conventional tillage with straw mulching; CT-SR, conventional tillage with straw incorporation; NT-SMR, no tillage with straw mulching. Different letters indicate statistical differences among treatments at $P < 0.05$.

where Te is the cumulative CH_4 emission (kg ha^{-1}), F_i is the CH_4 emission flux on the ith sampling date (mg m^{-2} h^{-1}), F_{i+1} is the CH_4 emission flux on the $i+1$ sampling date (mg m^{-2} h^{-1}), and Δd is the number of days between sampling.

2.3. Soil sampling and measurements

Soil samples were collected at the sowing, tillering, heading, and maturity stages of the early and late rice seasons in 2022 and 2023. Samples were taken from a depth of 0–20 cm, with five samples collected from each block using a soil auger along the S-line. The samples were transported to the laboratory, where they were cleared of rock and plant debris and divided into two subsamples. One subsample was air-dried, passed through a 2 mm sieve, and analyzed for soil chemical properties, while the other was stored in a refrigerator at −80℃ for microbiological analysis.

Soil NH_4^+-N and NO_3^--N were measured using UV spectrophotometry. SOC content was analyzed by external heating with potassium dichromate (Walkley and Black, 1934), and POC was quantified using the wet sieve method (Yeomans and Bremner, 1988). To determine POC, 20 g of air-dried soil, passed through a 2 mm sieve, was mixed with 50 ml of 5 g L^{-1} sodium hexametaphosphate solution and shaken horizontally for 18 h. The suspension was filtered through a 53 μm sieve, and the remaining organic matter dried at 50℃ and weighed was identified as POC, whereas the sieved portion was classified as MAOC. The activities of soil β-glucosidase, cellobiohydrolase, β-xylosidase, β-acetylglucosaminidase, peroxidase, and phenol oxidase were measured using enzyme-linked immunosorbent assay (ELISA). Briefly, 50 μL of standard solution was added to the plate, followed by 40 μL of sample dilution and 10 μL of sample in each well, resulting in a final 5-fold dilution. The plate was incubated at 37°C for 30 min, after which the liquid was removed, and the plate was shaken dry. The wash solution was added to the wells and allowed to stand for 30 s before removal, which was repeated five times. Then, 50 μL of the enzyme reagent was added to each well, excluding the blank well, and the incubation and washing steps were repeated. Subsequently, 50 μL of Color Developer A and 50 μL of Color Developer B were added to each well. The plate was shaken, and color development occurred at 37°C for 10 min in the dark. The reaction was stopped by adding 50 μL of the stopping solution, turning the color from blue to yellow. The absorbance (OD) of each well was measured at 450 nm.

2.4. Phospholipid fatty acid analysis

Phospholipid fatty acids (PLFAs) are crucial components of microbial cell membranes. Different microbial communities synthesize distinct PLFAs through various biochemical pathways, and certain PLFA structures are specific to particular microorganisms. Therefore, specific PLFAs can be selected as biomarkers to indicate changes in microbial community structure. As described by Bossio et al. (1998), PLFA analysis was performed to examine the composition of the main microflora during the tillering, heading, and mature stages of the early and late rice seasons in 2022 and 2023.

The PLFA biomarkers for different microbiological groups were analyzed as follow (Fan et al., 2020): fungi, represented by C18:2ω6c, C18:2ω9c, and C18:1ω9c; bacteria, indicated by iC14:0, iC15:0, aC15:0, iC16:0, iC17:0, aC17:0, C16:1ω7 t, C16:1ω7c, cy17:0, C17:1ω8c, 18:1ω7, and cy19:0; aerobes, identified by 16ω1ω7R1ω7 t, 18:1ω7 t, and 18:1ω7 t; anaerobes, marked by cy17:0 and cy19:0; actinomycetes, characterized by 10MeC18:0 and 10MeC19:0; Gram-positive bacteria, represented by iC14:0, iC15:0, aC15:0, iC16:0, iC17:0, and aC17:0; and Gram-negative bacteria, indicated by C16:1ω7 t, C16:1ω7c, cy17:0, C17:1ω8c, 18:1ω7, and cy19:0. universal, represented by C14:0, C15:0, C165:0, C17:0, C18:0.

2.5. Functional genes

The abundances of methanogenic and methanotrophic microorganisms in the soil were assessed by measuring *mcrA* and *pmoA* using absolute real-time quantitative PCR (RT-qPCR). Soil microbial DNA was extracted using the Fast DNA SPIN kit (MP Biomedicals, Santa Ana, CA, USA), and its quality was assessed using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, USA), ensuring an A260/A280 ratio between 1.8 and 2.2. Primer specificity was confirmed by PCR electrophoresis. The PCR amplification was conducted in a 25 μL system, including 1 μL of total soil microbial DNA (15–20 ng/μL), 2.5 μL of 10 × buffer, 0.5 μL of dNTP, 0.5 μL each of left and right primers, 0.25 μL of Taq enzyme, and water to make up the total volume. The

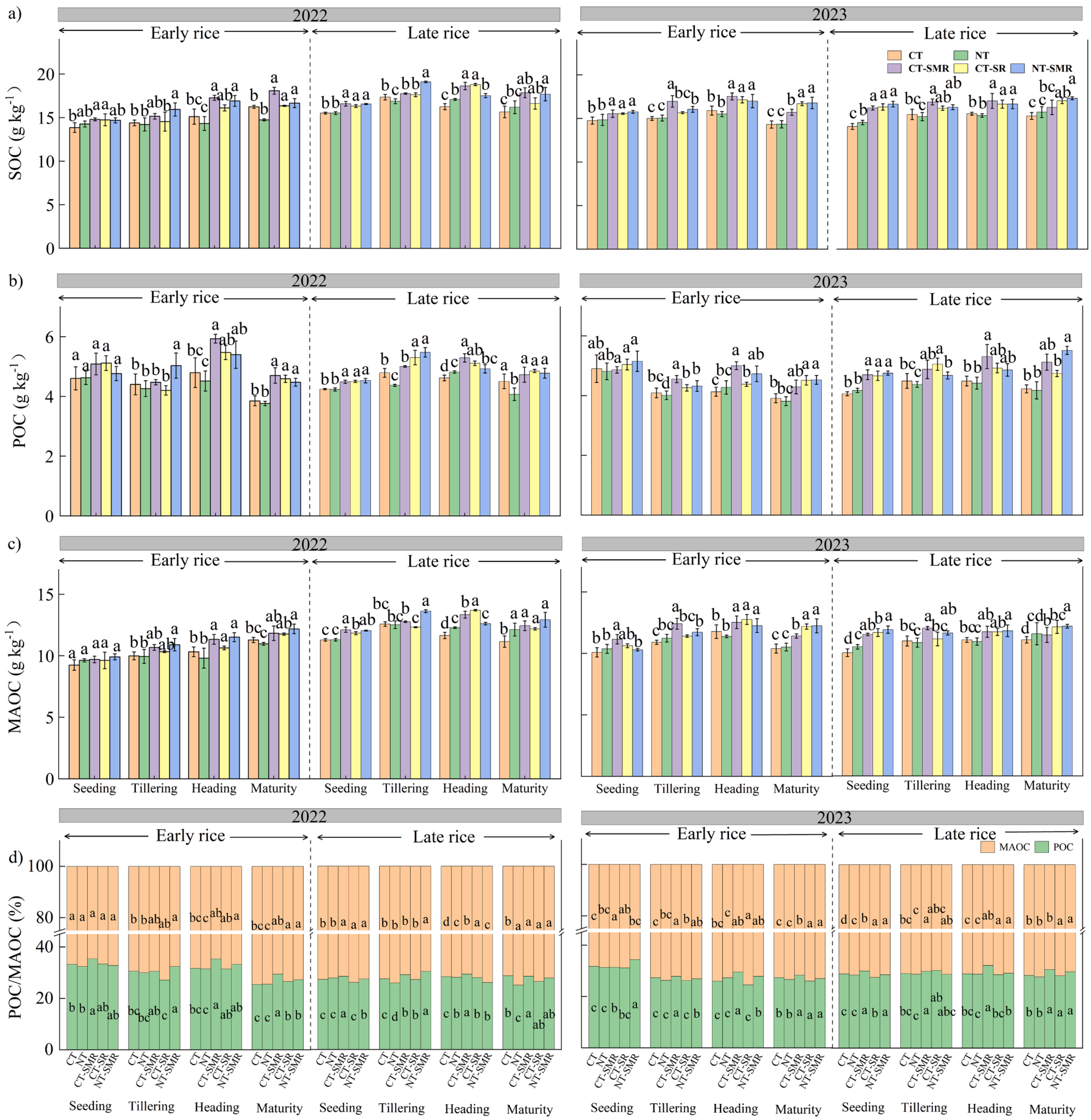

Fig. 2. Soil organic carbon pools under different tillage and straw return practices at early and late rice season seedling, tillering, heading, and maturity stages in 2022 and 2023. (a) SOC; (Soil organic carbon; (b) POC, particulate organic carbon; (c) MAOC, mineral-associated organic carbon; (d) POC/MAOC, the ratio of particulate organic carbon to mineral-associated organic carbon. CT, conventional tillage; NT, no tillage; CT-SMR, conventional tillage with straw mulching; CT-SR, conventional tillage with straw incorporation; NT-SMR, no tillage with straw mulching. Different lowercase letters indicate statistical differences among treatments at $P < 0.05$.

primers used for amplification were MLAS/MCRA-rev (Angel et al., 2012) for *mcrA* and A189f/Mb661r (Mao et al., 2015) for *pmoA*.

2.6. Statistical analysis

The data collected at various fertility periods were analyzed by two-way (straw and tillage) repeated measure analysis of variance (ANOVA) and Tukey's honest significance test ($P < 0.05$) using SPSS (SPSS Inc., Chicago, Illinois, USA; version 22.0). The figures were created using Origin 2024 (Origin Lab, Inc., USA, version 2023). Pearson correlation analysis showing the relationships between soil properties, enzyme activities, microorganisms, gene abundances, and CH_4 fluxes. The structural equation modeling was conducted with the "piecewiseSEM" packages in R 4.3.2, to explore the mechanisms of straw and tillage effects on CH_4.

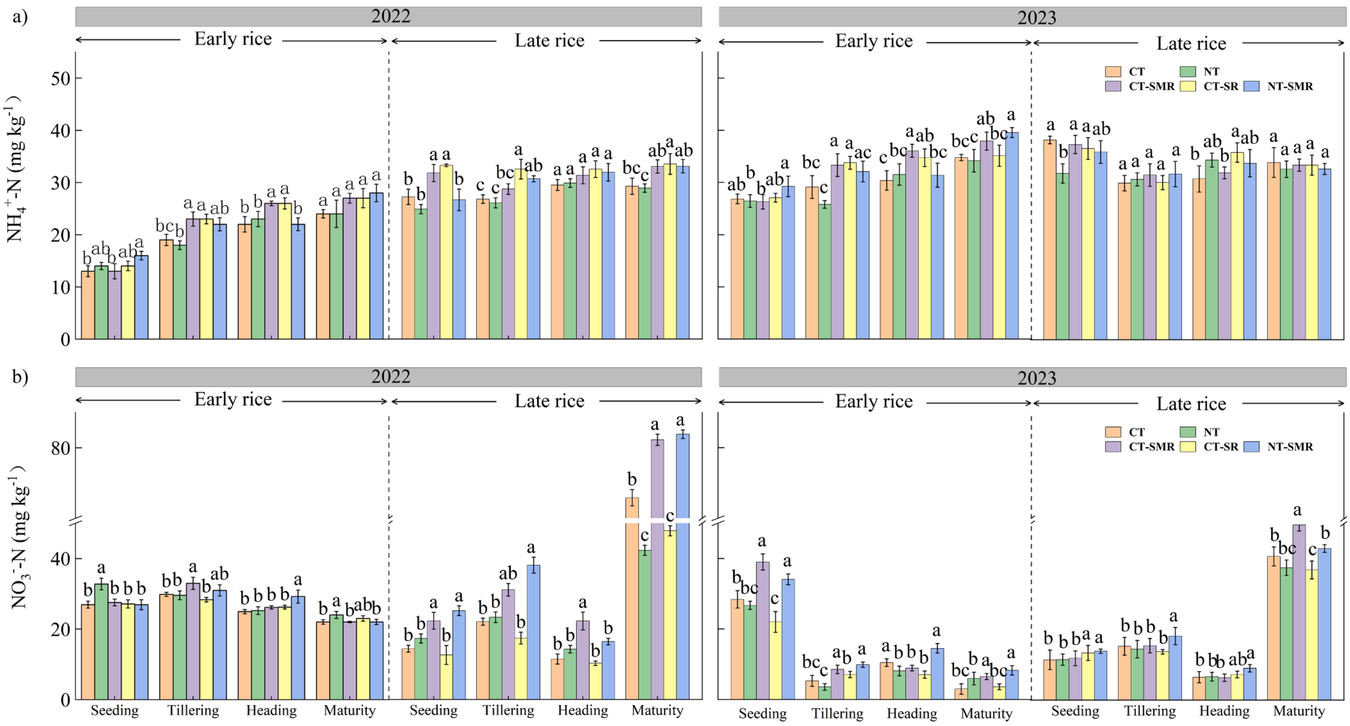

Fig. 3. Soil NH_4^+-N (a) and NO_3^--N (b) concentrations under different tillage and straw return practices at early and late rice season seedling, tillering, heading, and maturity stages in 2022 and 2023. CT, conventional tillage; NT, no tillage; CT-SMR, conventional tillage with straw mulching; CT-SR, conventional tillage with straw incorporation; NT-SMR, no tillage with straw mulching. Different lowercase letters indicate statistical differences among treatments at $P < 0.05$.

3. Results

3.1. CH_4 emissions

CH_4 emission fluxes from paddy soils exhibited significant seasonal variations in 2022 and 2023, with peak emissions occurring during the tillering and heading stages. The emission trends were consistent across both years, whereas the CH_4 emission fluxes were higher in 2023 than in 2022 (Fig. 1a and b). The fluxes ranged from −2.1–105.2 mg m^2 h^{-1}, with a mean of 51.6 mg m^2 h^{-1} for both years (Fig. 1a and b). The highest mean CH_4 emission fluxes were observed in CT-SMR, with the values of 16.1 and 29.3 mg m^2 h^{-1}, respectively. Although the differences in CH_4 emission fluxes among the various tillage practices were not significant, straw return increased emissions (Fig. 1a and b). Overall, the trends in early rice, late rice, and total cumulative CH_4 emissions were consistent: CT-SMR > NT-SMR > CT-SR > NT > CT. Repeated ANOVA showed that methane emissions were significantly affected by straw; while no significant effect of tillage and the interaction of tillage with straw (Fig. 1c and d). Compared with CT, NT, CT-SMR, NT-SMR, and CT-SR increased cumulative CH_4 emissions by 4.2 %, 122.4 %, 68.3 % and 39.7 %, respectively. Compared to CT-SMR and NT-SMR, CT-SR reduces cumulative CH_4 emissions by 37.2 % and 20.3 % (Fig. 1c and d).

3.2. Soil chemical properties

3.2.1. Organic carbon and its components

Overall, the SOC content increased with the rice growth stage. SOC concentration was the highest in NT-SMR and the lowest in CT at most rice growth stages (Fig. 2a). Repeated ANOVA showed that SOC was significantly affected by straw management (Fig. S3a and b), with straw return higher than straw removal by 8.8 % on average. However, the mean soil SOC content did not differ significantly among NT-SMR, CT-SMR, and CT-SR (Fig. 2a).

The POC content exhibited a dynamic pattern, decreasing, then increasing, and decreasing again during the early rice season, and increasing and then decreasing during the late rice season (Fig. 2b). CT-SMR had the highest average POC content, followed by NT-SMR, CT-SR, CT, and NT (Fig. 2b). Repeated ANOVA showed that POC was significantly affected by straw management (Fig. S3c and d), with straw return higher than straw removal by 11.8 % on average. Repeated ANONA showed no significant effects of tillage on POC concentration (Fig. S3c and d). CT-SR had 8.1 % and 4.6 % lower POC contents than CT-SMR and NT-SMR, respectively (Fig. 2b). The MAOC content trend over the two years was similar to that of POC, with the average MAOC content ranking as CT-SR > NT-SMR > CT-SMR > NT > CT (Fig. 2c). Repeated ANOVA analyses showed no significant effect of tillage and the interaction of tillage and straw on MAOC concentrations (Fig. S3e and f). Straw return increased MAOC content by 7.6 % compared to straw removal. CT-SR increased the MAOC content by 2.6 % and 1.5 % compared with CT-SMR and NT-SMR, respectively. The POC/MAOC ratio in SOC decreased with the rice growth stage, and straw return increased this ratio compared to straw removal, with the lowest POC/MAOC ratio observed in CT-SR under straw return conditions (Fig. 2d).

3.2.2. Soil ammonium and nitrate nitrogen content

Soil ammonium nitrogen (NH_4^+-N) concentration in the early rice season increased gradually with the rice growth stage, late rice season remained relatively stable (Fig. 3a). Compared to conventional tillage, no tillage decreased NH_4^+-N concentration by 3.3 %; compared to straw removal, straw return increased NH_4^+-N concentration by 9.5 %. Repeated ANONA showed no significant effects of tillage, straw, and the interaction on NH_4^+-N concentration (Fig. S4). The nitrate nitrogen (NO_3^--N) concentration in early- and late-season rice exhibited a consistent trend across both years. In the early season, the soil NO_3^--N concentration decreased progressively with rice growth stages, whereas in the late season, the NO_3^--N concentration increased, except during the tasseling period (Fig. 3b). Compared to CT, NT decreased NO_3^--N concentration 4.5 %; Compared to CT-SMR and NT-SMR, CT-SR reduces NO_3^--N concentration by 26.4 % and 28.2 % (Fig. 3b). Repeated ANONA

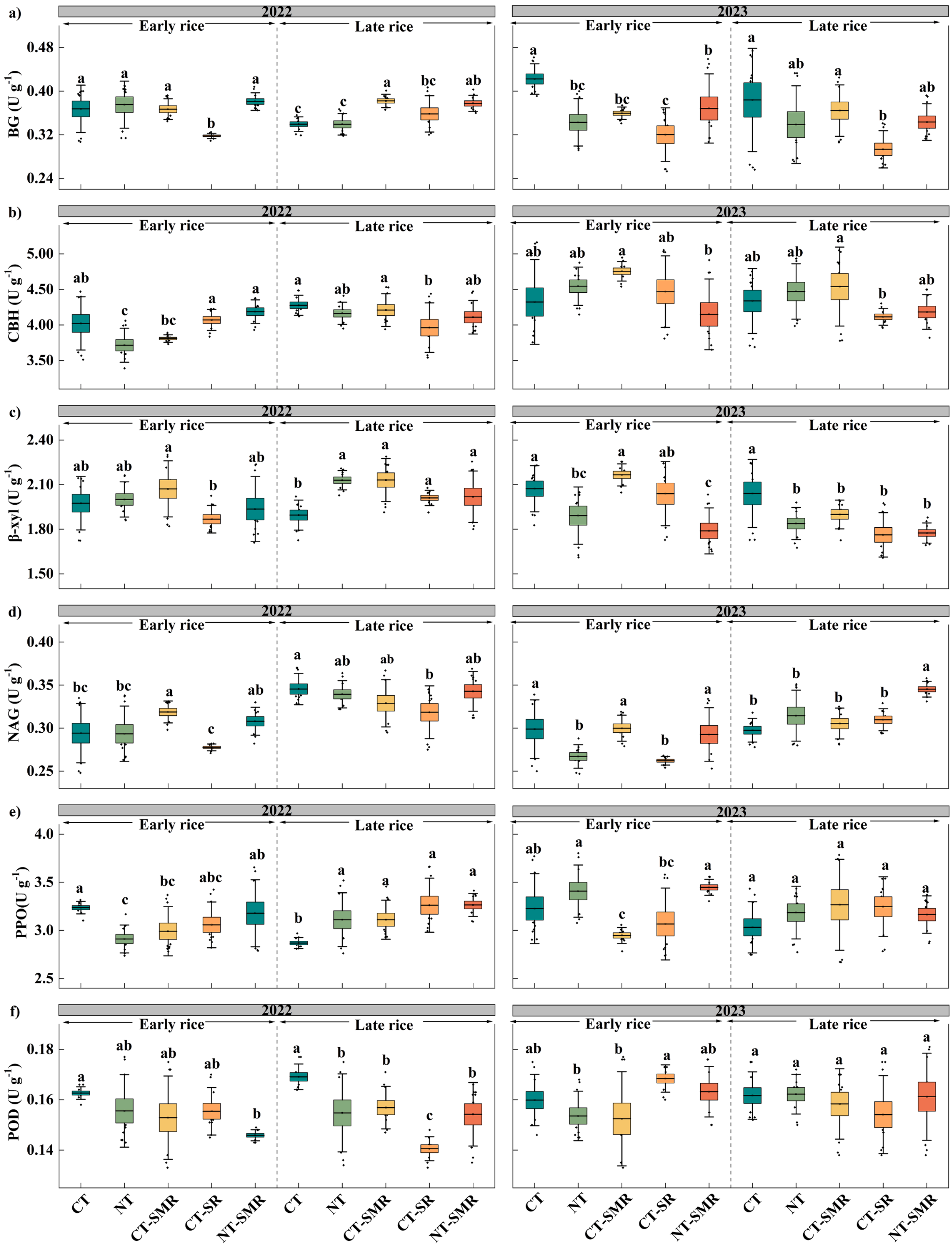

Fig. 4. Activities of soil hydrolases [(a), β-1,4-glucosidase; (b), cellobiohydrolase; (c), β-xylosidase; and (d), β-acetylglucosaminidase] and oxidases [(e), phenol oxidase; and (f), peroxidase] under different tillage and straw return practices in the early and late rice seasons in 2022 and 2023. CT, conventional tillage; NT, no tillage; CT-SMR, conventional tillage with straw mulching; CT-SR, conventional tillage with straw incorporation; NT-SMR, no tillage with straw mulching. Different lowercase letters indicate statistical differences among treatments at $P < 0.05$.

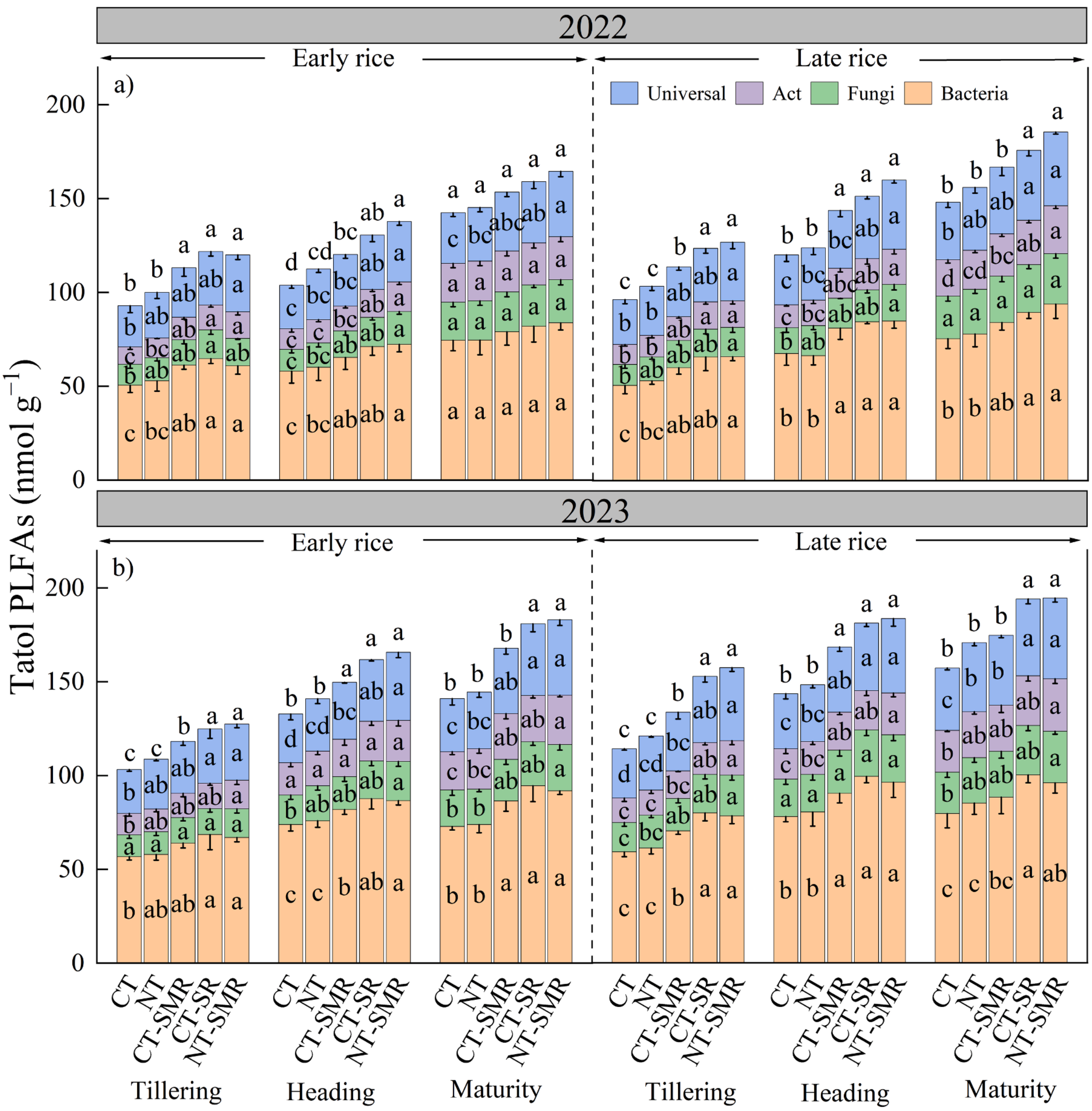

Fig. 5. PLFA content of different microbial populations under different tillage and straw return practices at early and late rice season tillering, heading, and maturity stages in 2022 and 2023. CT, conventional tillage; NT, no tillage; CT-SMR, conventional tillage with straw mulching; CT-SR, conventional tillage with straw incorporation; NT-SMR, no tillage with straw mulching. Different lowercase letters indicate statistical differences among treatments at $P < 0.05$.

showed no significant effects of tillage, straw, and the interaction on NO_3-N concentration (Fig. S4).

3.3. Soil enzyme activity

Compared to no-tillage, conventional tillage increased the activities of β-acetylglucosaminidase, peroxidase, cellobiohydrolase, and β-xylosidase, while decreasing the activities of β-1,4-glucosidase and phenol oxidase, when no straw return was considered. Compared to CT, NT reduced β-1,4-glucosidase, β-xylosidase, cellobiohydrolase, β-acetylglucosaminidase, and peroxidase activities by 7.6 %, 1.7 %, 1.0 %, 1.7 %, and 4.4 %, respectively, while enhancing phenol oxidase activity by 2.0 % (Fig. 4). Straw return increased peroxidase and β-acetylglucosaminidase activity but decreased β-xylosidase, cellobiohydrolase, β-1,4-glucosidase, and peroxidase activities compared with straw removal. However, compared to CT-SMR and NT-SMR, CT-SR increased cellobiohydrolase and β-acetylglucosaminidase activities by 4.2 % and 2.8 %, and 4.1 % and 10.4 %, respectively (Fig. 4). Repeated ANONA showed no significant effect of tillage, straw and the interaction on soil hydrolases and oxidases (Tables S1 and S2).

3.4. Soil microbiological composition

The bacterial, fungal and actinomycete phospholipid fatty acids (PLFAs) increased in the five treatments with the growth period of the rice (Fig. 5). Significant difference of bacteria and total PLFAs were observed in the different treatments. Compared to CT, NT decreased bacterial and total PLFAs 2.2 % and 5.3 %; Compared to CT-SMR, CT-SR reduces bacterial and total PLFAs by 6.2 % and19.1 % (Fig. 5). Repeated ANOVA showed that bacterial and total PLFAs was significantly affected by straw management (Table. S3), with straw return higher than straw removal by 18.7 % on average. fungal and actinomycetes PLFAs gradually increased with the rice growth stage in early and late season rice. Compared to CT, NT increased fungal and actinomycetes PLFAs by 8.9 % and 8.1 %, respectively; compared to straw removal, straw return increased fungal and actinomycetes PLFAs by 17.2 % and 20.2 %, respectively (Fig. 5). Repeated ANONA showed no significant effects of tillage, straw, and the interaction on fungal and actinomycetes PLFAs

Table 1
Ratios of microbial phospholipid fatty acids in tillering, heading and maturity stages under different tillage and straw management practices in 2022.

	Period	Treatment	2022		
			Fungal/ Bacteria	Aerobic/ Anaerobic	Gram-positive/ Gram-negative
Early	Tillering	NT-SMR	0.242 a	1.392 a	1.034 a
		CT-SR	0.237 a	1.589 a	0.950 a
		CT-SMR	0.220 a	1.571 a	1.021 a
		NT	0.233 a	1.405 a	1.109 a
		CT	0.220 a	1.767 a	1.087 a
	Heading	NT-SMR	0.242 a	1.611 b	0.966 a
		CT-SR	0.219 a	1.748 b	0.820 b
		CT-SMR	0.215 a	2.059 ab	0.831 b
		NT	0.217 a	1.712 a	0.924 ab
		CT	0.203 a	2.268 b	0.864 ab
	Maturity	NT-SMR	0.274 a	2.521 b	0.733 a
		CT-SR	0.268 a	3.410 a	0.629 b
		CT-SMR	0.269 a	3.438 a	0.616 b
		NT	0.280 a	2.505 b	0.623 b
		CT	0.272 a	3.643 a	0.572 b
Late	Tillering	NT-SMR	0.238 a	1.530 a	0.944 a
		CT-SR	0.224 a	1.615 a	0.751 b
		CT-SMR	0.240 a	1.909 a	0.806 ab
		NT	0.240 a	1.595 a	0.894 ab
		CT	0.219 a	1.933 a	0.797 ab
	Heading	NT-SMR	0.230 a	1.597 a	0.854 a
		CT-SR	0.201 a	1.792 a	0.791 a
		CT-SMR	0.198 a	1.889 a	0.789 a
		NT	0.239 a	1.713 a	0.852 a
		CT	0.207 a	1.909 a	0.832 a
	Maturity	NT-SMR	0.286 a	1.545 b	0.881 a
		CT-SR	0.285 a	2.131 a	0.714 b
		CT-SMR	0.293 a	2.174 a	0.718 b
		NT	0.305 a	1.574 b	0.686 b
		CT	0.302 a	2.277 a	0.655 b

Fungal/Bacteria ratio of fungal to bacterial PLFAs, Aerobic/Anaerobic ratio of aerobic bacterial to anaerobic bacterial PLFAs, Gram-positive/Gram-negative ratio of Gram-positive bacteria to Gram-negative bacteria PLFAs. CT, conventional tillage; NT, no tillage; CT-SMR, conventional tillage with straw mulching; CT-SR, conventional tillage with straw incorporation; NT-SMR, no tillage with straw mulching. Different letters in the same column indicate significant differences at the level of 0.05.

Table 2
Ratios of microbial phospholipid fatty acids in tillering, heading and maturity stages under different tillage and straw management practices in 2023.

	Period	Treatment	2023		
			Fungal/ Bacteria	Aerobic/ Anaerobic	Gram-positive/ Gram-negative
Early	Tillering	NT-SMR	0.229 a	1.590 b	0.859 a
		CT-SR	0.203 a	1.561 b	0.708 b
		CT-SMR	0.213 a	1.815 ab	0.701 b
		NT	0.208 a	1.545 b	0.801 ab
		CT	0.206 a	1.915 a	0.718 ab
	Heading	NT-SMR	0.242 a	1.798 a	0.716 a
		CT-SR	0.229 a	1.750 a	0.618 a
		CT-SMR	0.215 a	1.869 a	0.627 a
		NT	0.248 a	1.777 a	0.622 a
		CT	0.213 a	1.843 a	0.626 a
	Maturity	NT-SMR	0.267 a	3.584 b	0.848 a
		CT-SR	0.249 a	3.496 b	0.706 a
		CT-SMR	0.257 a	4.249 ab	0.705 a
		NT	0.256 a	4.307 ab	0.809 a
		CT	0.266 a	5.594 a	0.796 a
Late	Tillering	NT-SMR	0.279 a	1.371 a	0.948 a
		CT-SR	0.258 a	1.425 a	0.793 a
		CT-SMR	0.246 a	1.600 a	0.757 a
		NT	0.286 a	1.369 a	0.827 a
		CT	0.266 a	1.599 a	0.786 a
	Heading	NT-SMR	0.264 a	1.546 a	0.764 a
		CT-SR	0.248 a	1.629 a	0.677 a
		CT-SMR	0.256 a	1.727 a	0.652 a
		NT	0.249 a	1.592 a	0.706 a
		CT	0.256 a	1.725 a	0.639 a
	Maturity	NT-SMR	0.287 a	3.166 b	0.942 a
		CT-SR	0.264 a	3.447 b	0.848 a
		CT-SMR	0.276 a	3.931 ab	0.868 a
		NT	0.284 a	3.759 ab	0.969 a
		CT	0.278 a	4.215 a	0.87 5a

Fungal/Bacteria ratio of fungal to bacterial PLFAs, Aerobic/Anaerobic ratio of aerobic bacterial to anaerobic bacterial PLFAs, Gram-positive/Gram-negative ratio of Gram-positive bacteria to Gram-negative bacteria PLFAs. CT, conventional tillage; NT, no tillage; CT-SMR, conventional tillage with straw mulching; CT-SR, conventional tillage with straw incorporation; NT-SMR, no tillage with straw mulching. Different letters in the same column indicate significant differences at the level of 0.05.

(Table S3). Compared to CT, NT increased fungal/bacterial, Gram-positive/Gram-negative by 5.5 % and 11.2 % and decreased aerobic/anaerobic by 15.0 %. Compared to straw removal, straw return decreased aerobic/anaerobic by 6.9 % (Tables 1 and 2). Repeated ANONA showed no significant effects of straw, and the interaction of tillage with straw on fungal/bacterial, aerobic/anaerobic, Gram-positive/Gram-negative (Table S4).

Shannon, Simpson and Pielou relatively stable with rice growth stage (Table 3). Repeated ANOVA showed that Simpson and Pielou was significantly affected by the main effects of tillage and straw (Table S5). Both no-tillage and straw return increased Simpson and Pielou. NT increased the Shannon, Simpson, and Pielou indices by 0.2 %, 0.7 %, and 0.8 %, respectively, compared with CT. Straw return increased Simpson and Pielou by 0.2 % and 1.7 %, respectively, compared to straw removal. Similarly, CT-SR increased the Shannon, Simpson, and Pielou indices by 0.1 %, 0.5 %, and 0.5 %, respectively, compared with CT-SMR (Table 3).

3.5. Functional gene abundance

The trend in *pomA* abundance was consistent between the two years, increased with the progression of the growth stage (Fig. 6a). However, the *mcrA* abundance increased with rice growth stage in 2022, while the opposite was true in 2023 (Fig. 6b). Repeated ANOVA showed that *mcrA* and *pmoA* abundances were significantly affected by straw return (Fig. S5). NT reduced *pomA* abundance by 4.2 % and increased *mcrA* abundance by 1.7 %, compared with CT. CT-SR increased *pomA* abundance by 4.2 % compared with NT-SMR, whereas CT-SR decreased *mcrA* abundance by 12.3 % compared to CT-SMR. The *mcrA/pmoA* ratio generally decreased with the rice growth stage, with significant differences between treatments at the tillering and heading stages (Fig. 6c). Repeated ANOVA showed that *mcrA/pmoA* was significantly affected by straw return (Fig. S5). CT-SR reduced the *mcrA/pmoA* ratio by 14.1 % and 4.0 % compared with CT-SMR and NT-SMR, respectively.

CH_4 fluxes was significantly positively correlated to POC and *mcrA/pmoA*, but significantly negatively correlated to *pomA*, *mcrA*, actinomycetes, fungi, bacteria (Fig. 7). Structural equation modeling revealed that soil chemical and biological properties accounted for 47 % of the variation in CH_4 emission fluxes under different tillage and straw types. The tillage and straw management positively and directly influenced the CH_4 emission fluxes through the soil microbial composition and CH_4-related microbial communities, with direct flux coefficients of 0.40 ($P < 0.001$), 0.50 ($P < 0.001$), and 0.26 ($P < 0.05$), respectively. These practices promoted CH_4 emissions directly or indirectly by regulating POC content and affecting the abundance of functional genes such as *pmoA* and *mcrA*. Additionally, tillage and straw management negatively regulated soil microorganisms and indirectly mitigated CH_4 emissions by promoting the conversion of POC to MAOC by increasing β-acetyl-glucosaminidase and cellobiohydrolase activities. Overall, different tillage and straw management practices directly and indirectly affected CH_4 emissions by influencing biochemical properties (Fig. 8).

Table 3
Species diversity under different tillage and straw management practices at tillering, heading and maturity stages in 2022 and 2023.

	Period	Treatment	2022			2023		
			Shannon	Simpson	Pielou	Shannon	Simpson	Pielou
Early	Tillering	NT-SMR	0.929 a	0.882 a	2.725 a	0.932 a	0.893 a	2.760 a
		CT-SR	0.928 a	0.872 ab	2.696 ab	0.931 ab	0.882 ab	2.727 ab
		CT-SMR	0.927 a	0.868 ab	2.683 ab	0.931 ab	0.883 ab	2.730 ab
		NT	0.924 ab	0.857 bc	2.650 bc	0.929 bc	0.874 b	2.703 b
		CT	0.922 b	0.841c	2.598c	0.928c	0.867 b	2.678 b
	Heading	NT-SMR	0.930 a	0.888 a	2.745 a	0.932 a	0.893 a	2.761 a
		CT-SR	0.929 a	0.875 ab	2.704 ab	0.932 ab	0.885 ab	2.735 ab
		CT-SMR	0.928 a	0.869 ab	2.688 ab	0.930 ab	0.873 b	2.698 b
		NT	0.928 a	0.868 ab	2.683 ab	0.930 b	0.873 b	2.700 b
		CT	0.927 a	0.850 b	2.628 b	0.929 b	0.874 b	2.701 b
	Maturity	NT-SMR	0.931 a	0.882 a	2.725 a	0.932 a	0.893 a	2.759 a
		CT-SR	0.929 b	0.872 b	2.696 b	0.931 ab	0.882 ab	2.728 ab
		CT-SMR	0.929 b	0.869c	2.686c	0.929 b	0.873 b	2.699 b
		NT	0.929 b	0.866 d	2.677 d	0.929 b	0.870 b	2.690 b
		CT	0.927c	0.862 e	2.665 e	0.928 b	0.870 b	2.690 b
Late	Tillering	NT-SMR	0.931 a	0.891 a	2.755 a	0.931 a	0.892 a	2.758 a
		CT-SR	0.930 ab	0.877 ab	2.711 ab ab	0.931 a	0.888 ab	2.743 ab
		CT-SMR	0.931 ab	0.881 ab	2.723 ab	0.931 a	0.882 ab	2.727 ab
		NT	0.927 bc	0.862 b	2.665 b	0.929 a	0.875 b	2.706 b
		CT	0.926c	0.858 b	2.657 b	0.929 a	0.874 b	2.702 b
	Heading	NT-SMR	0.932 a	0.891 a	2.754 a	0.933 a	0.898 a	2.777 a
		CT-SR	0.930 ab	0.88 ab	2.719 ab	0.932 a	0.892 ab	2.757 ab
		CT-SMR	0.929 b	0.875 b	2.705 b	0.931 ab	0.884 abc	2.733 b abc
		NT	0.928 bc	0.868 bc	2.683 bc	0.930 b	0.877 bc	2.712 bc
		CT	0.927c	0.861c	2.662c	0.930 b	0.875c	2.704c
	Maturity	NT-SMR	0.931 a	0.884 a	2.733 a	0.932 a	0.893 a	2.760 a
		CT-SR	0.931 ab	0.886 a	2.738 a	0.932 a	0.892 a	2.757 a
		CT-SMR	0.931 b	0.886 a	2.739 a	0.930 a	0.884 a	2.734 a
		NT	0.931 bc	0.880 b	2.721 b	0.931 ab	0.885 b	2.737 a
		CT	0.930c	0.875c	2.705c	0.929 b	0.872 b	2.697 b

CT, conventional tillage; NT, no tillage; CT-SMR, conventional tillage with straw mulching; CT-SR, conventional tillage with straw incorporation; NT-SMR, no tillage with straw mulching. Different letters in the same column indicate significant differences at the level of 0.05.

4. Discussion

4.1. Effects of management measures on CH_4 emissions and associated microbial communities

Previous studies have explored the effects of tillage and straw management on CH_4 emissions from rice fields mainly from the perspectives of soil carbon and methane-functional bacteria, or have focused on the regulatory role of fertilizer and irrigation management (Fan et al., 2020; Liang et al., 2022; Ma et al., 2022; Kan et al., 2023). However, this paper comprehensively investigated all the factors related to CH_4 emission, such as soil carbon, nitrogen, enzyme activity, PLFA value and functional genes, and then clarified the mechanism of the effect of different tillage and straw return practices on CH_4 emission from paddy fields. Our study found that tillage management had a negligible impact on CH_4 emissions, which straw return significantly increased emissions (Fig. 1). This finding is inconsistent with previous study by Kan et al. (2023), which reported that cumulative CH_4 emissions were significantly correlated with tillage. This discrepancy may be attributed to differences in experimental conditions, particularly the interaction between tillage and water regimes in their study. Specifically, our results indicated that CT-SR had lower cumulative CH_4 emissions compared to CT-SMR and NT-SMR (Fig. 1). The lower emissions in CT-SR could be explained by two factors. On the one hand, this may be due to the higher temperature and moisture obtained straw mulch conditions promoting POC decomposition, reducing substrate availability for methanogens. On the other hand, the CT-SR treatment obtained lower mcrA/pmoA ratio, indicating a shift in microbial community structure that may favor methanotrophs over methanogens. Additionally, the CH_4 emission fluxes in 2023 were significantly higher than those in 2022, likely due to increased rainfall in 2023 (Fig. S2). Soil microbial activity plays a crucial role in CH_4 emissions, as simple metabolites produced by microorganisms serve as substrates for methanogens (Wang et al., 2018). Our study found that no-tillage and straw return practices increased microbial biomass, which was accompanied by higher CH_4 emissions. These findings are consistent with a split-plot experiment by Kan et al. (2023), which reported that straw management significantly affected cumulative CH4 emissions during the rice-growing season, with straw return increasing emissions by 58.8 %. CT-SR improved the contact area between the soil and straw, facilitating better decomposition and utilization of straw during the winter fallow season. This resulted in reduced substrate availability for methanogens during the rice-growing season, and consequently, lowered CH_4 emissions (Fig. 1). In contrast, the lower emissions from CT compared to NT were attributed to a higher abundance of methane-oxidizing bacteria, likely because of the effect of tillage on soil permeability, which benefited CH_4 oxidation. However, carbon from straw return can be stabilized by chemical resistance or interaction with mineral surfaces, thus mitigating the positive impact of straw on CH_4 emissions (Liu et al., 2022).

Conventional tillage has been associated with lower *mcrA* copy numbers and *mcrA/pmoA* copy number ratios (Deng et al., 2022), which is in contrast to the findings of this study. Both conventional tillage and straw addition increased the abundance of CH_4 functional bacterial genes (Fig. 6). This increase could be attributed to carbon exposure due to tillage, as well as to straw providing nutrients for the growth of methanogenic (Conrad et al., 2012). On the other hand, increased methane production can stimulate oxidation by methanotrophs, thereby mitigating the increase in methane emissions. The return of straw to the field enhances substrate availability and accelerates carbon turnover in the presence of microorganisms (Sainju, 2016). At the same time straw can be used as a fresh source of carbon that promotes the mineralization of natural organic carbon in the soil (Guenet et al., 2018). Effective field management practices play a crucial role in enhancing soil carbon sequestration and nutrient retention by improving soil structure, thereby reducing the adverse effects of priming effects. This shows that proper field management can mitigate the significant methane emissions

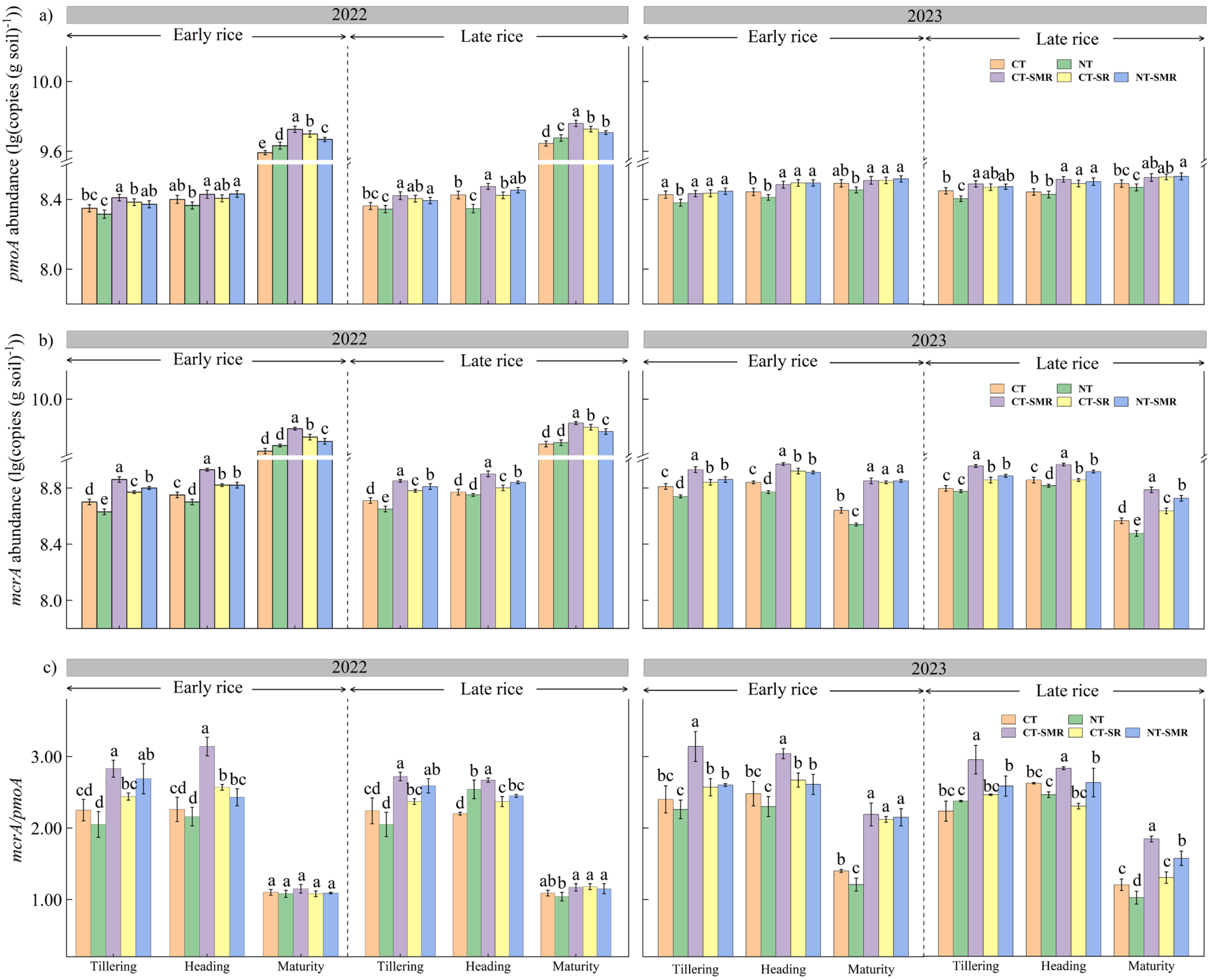

Fig. 6. Effects of different tillage and straw return practices on the dynamics (a, b, and c) of *pmoA*, *mcrA* abundance, and *mcrA/pmoA* ratio in 2022 and 2023. CT, conventional tillage; NT, no tillage; CT-SMR, conventional tillage with straw mulching; CT-SR, conventional tillage with straw incorporation; NT-SMR, no tillage with straw mulching. Different lowercase letters indicate statistical differences among treatments at $P < 0.05$.

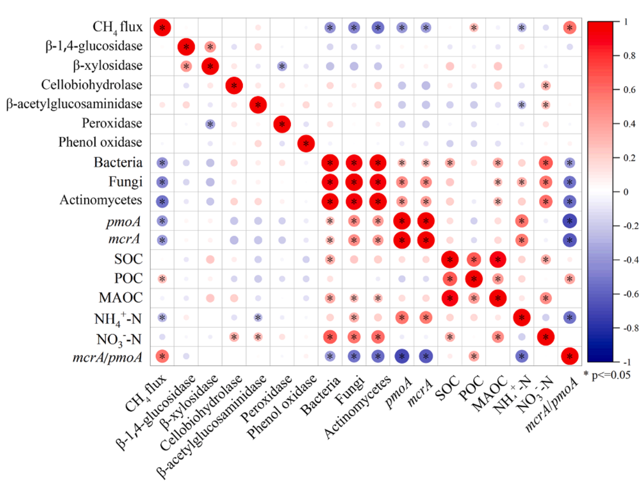

Fig. 7. Pearson correlation analysis showing the relationships between soil properties, enzyme activities, microorganisms, gene abundances, and CH_4 fluxes. *mcrA,* methanogenic; *pmoA*, methanotroph; *mcrA/pmoA*, methanogenic/ methanotroph. "* " indicated $P < 0.05$.

that are accompanied by straw returning. In our study, the lowest methane emissions were observed in the CT-SR treatment under straw returning conditions, potentially because of the lower *mcrA/pmoA* and POC/MAOC ratios under CT-SR (Figs. 2 and 6). Therefore, conventional tillage combined with straw incorporation effectively reduced CH_4 emissions by promoting inert carbon and enhancing CH_4 oxidation.

4.2. Effect of management practices on soil carbon pool

The chemical characteristics of paddy soils are closely related to organic matter and tillage management practices. This study observed that NT and straw return increased SOC and MAOC fractions, with straw incorporation being more effective than straw mulching in enhancing these fractions (Fig. 2), This finding support the point made by Gattinger et al. (2012), which reported higher SOC concentrations and stocks in the tillage layer under organic farming systems, which reported higher SOC concentrations and stocks in the tillage layer under organic farming systems. These differences could arise from variations in crop residue inputs and soil disturbance levels. Specifically, straw return directly enhances soil organic carbon accumulation by increasing exogenous organic carbon inputs, whereas tillage practices can lead to soil disturbance, thereby accelerating microbial decomposition of organic carbon.

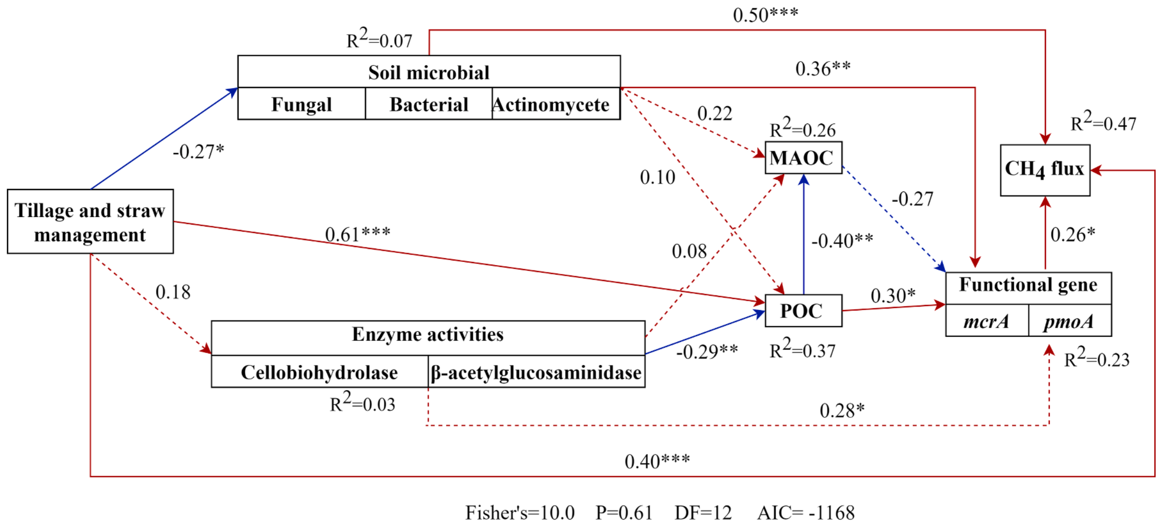

Fig. 8. Structural equation modeling (SEM) showing the relationships and interactions between tillage and straw return, enzyme activity, POC, MAOC, soil microorganisms, methanogenic and methanotroph (represented by *mcrA* and *pmoA*, respectively), and CH_4 emissions. The numbers listed next to the arrows are standardized path coefficients (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). The red and blue lines represent positive and negative effects, respectively. The solid and dashed lines indicate significant and non-significant effects, respectively.

POC is a crucial energy source for soil microorganisms and plays a significant role in the carbon cycle (Wang et al., 2015). The primary sources of POC include straw, residues, root secretions, and microbial residues (Li et al., 2016). The addition of exogenous organic carbon through straw return to the field directly promoted organic carbon accumulation, leading to a significant increase in POC content (Fig. 2b). According to an 8-year field experiment conducted by Zhao et al. (2019), while the priming effect associated with straw return caused certain carbon losses, net soil organic carbon sequestration occurred when the carbon derived from newly added straw exceeded the balance between gains and losses. Straw addition also influenced MAOC, a form of inert carbon that resists decomposition and persists in soil for extended periods. The formation of MAOC primarily occurs through two pathways: plant residues and microbial processes (Zhou et al., 2022). This highlights the importance of straw return in enhancing long-term carbon sequestration in soils. Compared to straw mulching, straw incorporation resulted in higher MAOC content (Fig. 2c). Due to straw incorporation into the soil there is more contact between straw and soil, which favours the formation of soil mineral associated organic carbon (Gosling et al., 2013). Our findings align with previous studies, which have shown that burying straw residues deeper in the soil profile is more effective for SOC and MAOC sequestration than surface mulching (Xue et al., 2018; Hao et al., 2022). On the other hand, straw mulching to obtain higher temperature and moisture will promote POC decomposition (Shakoor et al., 2021). A portion of straw carbon may be utilized by microbial communities, leading to the accumulation of microbial biomass and necromass. Microbial necromass can form MAOC through combination with minerals, which enhances the conversion of POC to MAOC. Our study obtained the same results, in that the CT-SR obtained a lower POC/-MAOC, compared to the CT-SMR and NT-SMR(Fig. 2d). Thus, straw return is recommended as an effective practice to increase the soil fertility and carbon sequestration in paddy fields. Combining straw return with tillage can further enhance soil carbon sequestration.

4.3. *Effect of management practices on soil microorganisms, hydrolytic and oxidative enzyme activities*

Previous research has highlighted the crucial role of soil enzymes in nutrient cycling in agroecosystems (Ai et al., 2012). Hydrolytic enzymes such as cellobiohydrolase, β-xylosidase, and β-1,4-glucosidase play a key role in decomposing cellulose and hemicellulose from straw into glucose, which is subsequently involved in soil nutrient cycling (Ling et al., 2014). Our findings reveal that the implementation of NT reduced the activities of both soil hydrolases and oxidases, while straw return increased the activities of β-1,4-glucosidase and β-acetylglucosaminidase but decreased β-xylosidase, cellobiohydrolase, and oxidases (Fig. 4). These observations can likely be attributed to the fact that both no-tillage and straw return enhance soil organic matter content, which in turn stimulates microbial activity and improves soil nutrient availability. Notably, straw incorporation was found to increase cellobiohydrolase and β-acetylglucosaminidase activities compared to straw mulching (Fig. 4). This difference may be explained by the fact that incorporated straw allows for more favorable microbial contact, thereby promoting microbial growth. In agreement with our findings, Liu et al. (2023) reported that soil enzyme activities are often physically limited by their exposure to organic matter, leading to uneven distribution of enzyme activities within the soil structure.

Soil microorganisms are central to nutrient cycling processes (Martinez-Garcia et al., 2018) and play a key role in driving various biochemical transformations. Straw return has been shown to enhance soil microbial diversity by decomposing organic matter and supplying essential nutrients for crop growth (Yan et al., 2019). This improvement in soil nutrient status has a positive impact on microbial activity (Rabbi et al., 2016). In this study, CT-SMR, NT-SMR, and CT-SR treatments increased the abundance and diversity of bacteria and fungi compared to CT and NT (Fig. 5 and Table 3). This can be attributed to the stimulation of C-related enzyme activity by straw addition, which accelerates the breakdown of plant residues. A 14-year study further supports this, showing that long-term straw incorporation enhances enzyme activity and improves the utilization of recalcitrant carbon sources (Liu et al., 2022). This increase was attributed to variations in soil nutrients, which not only accelerated the decomposition of soil organic matter but also facilitated the efficient utilization of soil nutrients. Straw can increase the carbon source available to microbial communities, thereby boosting the microbial biomass. Conservation tillage with reduced soil disturbance promotes microbial activity (Yang et al., 2020). Our data showed that the fungal and bacterial biomass increased under NT compared to that under CT, and straw return further enhanced both fungal and bacterial biomass, with a greater increase observed with straw incorporation. Among the five treatments, NT-SMR had the highest levels of fungi, bacteria, actinomycetes, and total microbial biomass (Fig. 5). Liu et al. (2022) also showed that the addition of straw significantly increased the abundance and diversity of soil microorganisms in a rice-wheat rotation system. Additionally, straw incorporation can enhance the contact surface between soil and straw, facilitating microbial decomposition and the application of straw, thus promoting microbial growth. Meanwhile, combining straw return with tillage can improve soil fertility, stimulate microbial community activity, and increase rice yields.

5. Conclusion

This study examined the effects and mechanisms of tillage and straw management on CH_4 emissions from paddy fields. Tillage and straw management influenced the methanogenic and methanotrophic communities to mitigate CH_4 emissions by regulating the microbial biomass and increasing the β-acetylglucosaminidase and cellobiohydrolase activities, which promoted the conversion of particulate organic carbon to mineral-associated organic carbon. Conventional tillage with straw incorporation exhibited higher bacterial, fungal, and actinomycete biomass, increased mineral-associated organic carbon content, and lower methanogens/methanotrophs, leading to mitigation of increased methane emissions under straw return conditions. Compared with straw mulching, straw incorporation significantly reduced the cumulative CH_4 emissions by 20.3 %–37.2 %. These results indicated that straw incorporation promoting soil carbon sequestration and mitigating CH_4 emissions under straw mulching conditions.

CRediT authorship contribution statement

Xinlu Long: Methodology, Investigation. **Qin Jiayu:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Yuxi Zhou:** Investigation, Formal analysis, Conceptualization. **Pengli Yuan:** Writing – review & editing, Conceptualization. **Ligeng Jiang:** Writing – original draft, Conceptualization.

Declaration of Competing Interest

We declare that we have no conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109780.

Data availability

Data will be made available on request.

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