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Rotary Tillage Plus Mechanical Transplanting Practices Increased Rice Yields with Lower CH₄ Emission in a Single Cropping Rice System

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Abstract: As the main contributor to greenhouse gas (GHG) in paddy soil, information on methane (CH₄) emission characteristics under different tillage and cultivation practices are limited. A five-year field trial was conducted from 2019 in a single-cropping rice system in Taihu Lake region, east of China. The experiment had a completely randomized block design, and the treatments included rotary tillage plus rice dry direct seeding (RD), rotary tillage plus rice mechanical transplanting (RT), and plowing tillage plus rice mechanical transplanting (PT). We determined the rice yield, GHG emission, soil traits, and methanogens and methanotrophs in 2022 and 2023. The results revealed that PT and RT significantly increased rice yield compared to RD, whereas PT simultaneously increased CH₄ emissions. The year-averaged cumulative CH₄ emissions in PT were increased by 38.5% and 61.4% higher than RT and RD, respectively. Meanwhile, yield-scaled global warming potentials (GWPs) in RT and RD were lower than those in PT. Tillage and cultivation practices shifted *mcrA* and *pmoA* abundances, and PT significantly decreased *pmoA* abundance. The community structure and diversity of the methanogens and methanotrophs were not significantly affected. Structural equation model analyses illustrated that CH₄ emissions were regulated by *mcrA* and *pmoA* directly, which in turn, regulated by soil carbon and nitrogen. Overall, rotary tillage plus mechanism transplanting was a feasible agronomic technology in a single-cropping rice system in Taihu Lake region, exhibiting higher and more stable rice productivity, accompanied with lower CH₄ emissions and yield-scaled GWP.



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1. Introduction

Rice (*Oryza sativa* L.) is an important calorie source for human beings, which feeds more than 50% of the global population on earth [1], especially in East and South Asia [2]. With the rapid development of society and economy, modern rice production must consider agronomic and environmental benefits, such as yield, quality, and greenhouse gas (GHG) emissions [3]. Furthermore, rice fields are the largest artificial wetland systems that play important roles in GHG emissions, and methane (CH₄) is the main contributor to global warming in paddy soils [4]. Thus, mitigating CH₄ emissions from rice fields is a core issue in rice production.

Tillage and cultivation practices are key procedures in crop production and generally strongly depend on local conditions. Recent studies clearly suggested that no-tillage

significantly decreases final CH₄ emissions in rice fields [5–7] but did not change rice yields significantly [8]. Deep tillage, for example, plowing (usually 20–30 cm), was considered by a meta-analysis to be a good choice for increasing rice yield but with higher CH₄ emissions [9]. To reconcile this dichotomy, conventional tillage, such as rotary tillage (10–15 cm), was adopted to balance rice yield and CH₄ emissions. Zheng et al. [10] observed that rotary tillage results in higher rice yield and lower CH₄ emissions compared to no tillage in Guangxi, China. Kim et al. [11] also showed unexpectedly lower CH₄ emissions under rotary tillage in mono-rice paddy soils in South Korea. In contrast, other studies have suggested that rotary tillage increases both rice yield and CH₄ emissions [12,13]. For example, rotary tillage increased CH₄ emissions without any significant yield penalty across rice varieties in India [14]. Thus, it remains uncertain whether rotary tillage strikes an optimal balance between rice yield and CH₄ emissions. Meanwhile, rice planting strategies, such as direct seeding or mechanical transplanting, also play important roles in rice productivity and CH₄ emissions. Pathak et al. [15] reported that rice direct seeding mitigated CH₄ emissions by 3–4 times in water irrigation in different districts of Punjab, India. Hang et al. [16] found that dry direct seeding resulted in higher rice yield and lower CH₄ emissions than wet direct seeding and mechanical transplanting in China. However, although rice direct seeding is easier to be accepted by farmers because of its lower labor and machinery costs, rice direct seeding more frequently suffers from yield reduction due to the lodging of rice in East China, where typhoons often occur during the harvest period.

Methane emissions are mainly governed by complex and diverse microbes, such as hydrolytic, fermenting, syntrophic, methanogenic, and methanotrophic microorganisms. The observation of CH₄ emissions is the net balance of methane production and oxidation [17]. CH₄ production in paddy soil depends on carbon conversion by methanogens in the water-logged and anaerobic environment, in which carbons are derived from plants, organic fertilizers, and root exudates [3]. There are two pathways for CH₄ production: (1) methanogens use H₂ or organic molecules as H donors to reduce CO₂ to form CH₄ or (2) methanogens demethylate acetic acid to produce CH₄ [18]. Conversely, CH₄ oxidation is the process by which methanotrophs convert CH₄ to CO₂ and H₂O. Methanotrophs are widely found in *Proteobacteria* and *Verrucomicrobia*, and the former can be broadly divided into type I (*γ-proteobacteria*) and type II (*α-proteobacteria*) by differential pathways [19]. Methyl coenzyme M reductase (MCR) is responsible for the microbial formation of methane, and the *mcrA* gene encoding the α -subunit is highly conserved as a signature gene in phylogenetic studies of methanogenic archaea [20]. The particulate methane monooxygenase (*pMMO*) is the first enzyme in the C1 metabolic pathway in methanotrophic bacteria, and the *pmoA* gene serves as a biomarker of the methane-oxidizing microorganisms [21]. In recent years, numerous studies have investigated the effects of tillage and cultivation practices on methanogens and methanotrophs using *mcrA* and *pmoA* genes as biomarkers in paddy soil. Kan et al. [22] showed that the timing of tillage regulates CH₄ through changes in *mcrA* gene abundance. Plowing significantly decreased *mcrA* gene abundance compared to rotary tillage in double rice systems [23], whereas conventional plowing simultaneously promoted the abundance of *mcrA* and *pmoA* genes [24].

Taihu Lake region is in the middle-lower Yangtze plains, east of China, which is the most developed area within the region. The region also covers the traditional rice–wheat rotation area. Furthermore, local governments and farmers seek harmonious coexistence between human beings and nature, that is, a balance between high rice productivity and low greenhouse gas emissions is pursued. To address the above issue, eco-friendly agronomic practices, such as feasible tillage strategies and rice cultivation methods, are urgently needed. Hence, according to the effects of tillage and cultivation patterns on grain productivity and global warming potential (GWP) in the previous studies [13,25], we proposed the hypothesis that the combination of rotary tillage and rice mechanical transplanting could maintain sustainable and high rice yields with lower GWP by regulating CH₄ emissions. To accomplish this goal, we conducted a 5-year field experiment to aim at achieving (1) the stability of rice yield under different tillage and cultivation practices,

(2) the combined effects of rice yield and GHG under different tillage and cultivation practices, and (3) the main factors regulating CH_4 emissions under different tillage and cultivation practices.

Nitrous oxide (N_2O) emission and corresponding GWP in paddy fields is generally much smaller compared with those from upland systems [26], and N_2O emission in paddy soil is strongly affected by drainage and nitrogen availability [27]. Thus, we focused on the effects of tillage and cultivation methods on CH_4 emission in this study.

2. Materials and Methods

2.1. Site Description

The field trial was initiated in 2019 at the National Soil Quality Observation Experiment Station, Xiangcheng, Suzhou City, China ($31^\circ 32' 45''$ N, $120^\circ 41' 57''$ E). The soil is classified as Hydric Anthrosol according to the World Reference Base for Soil Resources of FAO [28], with hydromica and smectite as the dominant clay minerals. The initial soil contained 1.95% total soil organic carbon (SOC), 0.19% total-N (TN), 12.56 mg kg^{-1} Olsen-P (0.5 M NaHCO_3), and $112.50 \text{ mg kg}^{-1}$ available-K (1.0 M $\text{CH}_3\text{COONH}_4$), with a pH of 6.8 (ratio of soil and CO_2 -free water, weight/volume, 1:2.5) and a bulk density (0–20 cm) of 1.41 g cm^{-3} .

Meteorological conditions in 2022 and 2023 are generally similar, with no extreme meteorological events, and average daily temperatures in 2022 were slightly higher than those in 2023, while rainfall in 2023 was slightly higher than that in 2022 (Figure 1).

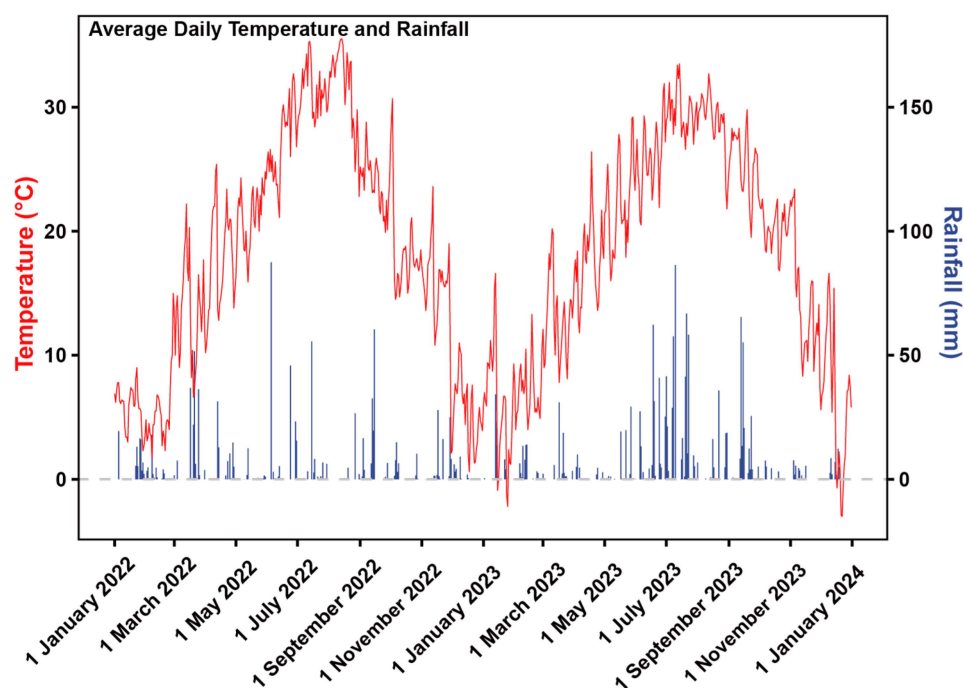


Figure 1. Basic climatic conditions in 2022 and 2023; the data were acquired from Suzhou Weather Bureau, including variations in daily average temperature and precipitation.

2.2. Experiment Design and Implementation

A completely randomized block design was used in this field trial. The treatments included rotary tillage plus rice dry direct seeding (RD), rotary tillage plus rice mechanical transplanting (RT), and plowing tillage plus rice mechanical transplanting (PT). Each treatment was replicated thrice, and each plot area was approximately 150 m^2 . The plots were separated by soil ridges with a width of $\sim 0.30 \text{ m}$ and height of $\sim 15 \text{ cm}$.

A six-position integrated machine (Taicang Xiang's Agricultural Machinery Co., Ltd., Taicang, China) was used for the RD treatment, with a rotary depth of 12–15 cm and a seeding rate of 45 kg ha^{-1} . Kubota M954-K (Kubota Agricultural Machinery Co., Ltd.,

Osaka, Japan) was used for rotary and plowing tillage for RT and PT, with a rotary depth of 12–15 cm in RT and a plowing depth of 20–30 cm in PT. Rice seedlings for mechanical transplanting were raised using the dry seedling method for 20 days. Subsequently, mechanical transplanting of rice was performed using 2ZGQ-6K (Kubota Agricultural Machinery Co., Ltd.). The plant-row spacing in RT and PT was 30 × 16 cm. Notably, wheat straw was completely incorporated into the soil during the rice-growing season.

SXG100, a widely used japonica rice cultivar in Taihu Lake region, was selected for this study. Table 1 indicates the timing of the major agricultural activities in 2022 and 2023. In terms of the local fertilization recommendation, 270 kg N ha⁻¹ and 67.5 kg K₂O ha⁻¹ were dosed throughout the whole rice season. For nitrogen fertilizer, the basal and panicle fertilizer comprised 30% N, respectively, and the remaining 40% N was used at the tillering stage. For potassium fertilizer, the basal and panicle fertilizer was accounted for 50% K, respectively. No phosphorus fertilizer was used during the rice season.

Table 1. Dates of rice direct seeding, transplanting, and harvesting in 2022 and 2023.

Year	Treat	Seeding Date	Transplanting Date	Peak Tillering Date	Harvesting Date
2022	RD	13 June	-	22 July	7 November
	RT	-	23 June	24 July	7 November
	PT	-	23 June	24 July	7 November
2023	RD	12 June	-	17 July	4 November
	RT	-	17 June	19 July	4 November
	PT	-	17 June	19 July	4 November

Water management in paddy fields is closely related to CH₄ emission. Thus, a standardized water management strategy was implemented among the treatments. We kept shallow water (1–3 cm) from transplanting to active tillering, drained and baked the field at the critical period of effective tiller, and performed alternate wetting and drying (AWD) from flowering to harvest. Notably, for rice dry direct seeding, we maintained the saturated water-holding capacity for the first 15 days for the RD treatment and then referred to transplanting practices for the following water management process. Other field management practices, such as pest and weed control, were based on the recommendation of a standardized rice cultivation method in the Taihu Lake region.

2.3. Rice Yield Determination

The grain yields were determined in each plot from all plants in a 1 m² area, and the grain moisture content was adjusted to 13.5% fresh weight.

2.4. Gas Sampling and Determination

Gas samplings were carried out using a static closed-chamber technique, which has been widely utilized and reported [29]. Chambers with dimensions of 50 × 50 × 50 cm and 50 × 50 × 100 cm were deployed to collect gas before and after the panicle initiation stage, respectively. Gas sampling was performed weekly between 8:00 and 10:00 a.m. During the gas sampling, the chamber was carefully placed above the vegetation, fitting its rim above the waterfilled collar groove. To homogenously mix the gas, small fans were installed at the top and in the middle of the chambers. An automatic program-controlled sampler was connected to the chamber by a silicone hose, and each time, 50 mL of gas was sampled into four independent pre-evacuated gas bags at 10 min intervals.

The concentration of CH₄ gas was simultaneously determined for CH₄ and N₂O using a gas chromatograph (Agilent 7890, Waldbronn, Germany). A flame ionization detector and ⁶³Ni electron capture detector were used for CH₄ and N₂O, respectively. The oven and two detectors were operated at 60 °C and 300 °C. The carrier gases for CH₄ and N₂O determination by gas chromatography were nitrogen and argon–CH₄ (95:5) mixtures,

respectively. The CH₄ and N₂O fluxes were calculated from the linear increases in the CH₄ and N₂O concentrations over time ($r^2 > 0.90$, $n = 4$) [25].

The gas emission flux is calculated according to Equation (1):

$$F = \rho h \frac{dc}{dt} \times \frac{273}{273 + T} \quad (1)$$

where F is the gas emission flux [$\text{mg (m}^{-2} \text{ h}^{-1})$], ρ is the gas density under standard conditions (kg m^{-3}), h is the net height of the sampling chamber (m), dc/dt is the change rate of the gas concentration in the sampling chamber per unit time, 273 is the gas equation constant, and T is the average temperature ($^{\circ}\text{C}$) in the sampling chamber during gas sampling.

The cumulative emissions of CH₄ and N₂O were calculated using Equation (2):

$$CF = \sum_{i=1}^n F_i D_n \quad (2)$$

where CF ($\text{kg CH}_4/\text{N}_2\text{O ha}^{-1}$) represents the cumulative emission of CH₄ or N₂O in the rice field, F_i represents the average emission flux of gas, and D_n represents the number of days during the sampling period [30].

GWP ($\text{kg CO}_2\text{-eq ha}^{-1}$) in the rice season was calculated based on a 100-year time horizon using a radiative forcing potential of 28 for CH₄ and 265 for N₂O [31], as shown in Equation (3):

$$\text{GWP} = CF_{\text{CH}_4} \times 28 + CF_{\text{N}_2\text{O}} \times 265 \quad (3)$$

Yield-scaled GWP is a trade-off index between GWP and crop production, which is calculated as the ratio of GWP to yield, as shown in Equation (4):

$$\text{Yield-scaled GWP} = \text{GWP} / \text{Yield} \quad (4)$$

2.5. Soil Sampling and Determination

Considering that the rice tillering stage is the peak period of CH₄ emissions according to previous studies in the Taihu Lake region [13,25,30], fresh soil of 0–20 cm was collected at the tillering stage on 24 July 2023, based on a five-point sampling method. Plant residues were carefully removed and the soil from each sample was completely mixed and made homogeneous in the laboratory. Fresh soil of 10 g was immediately stored at -80°C after passing a 2 mm soil sieve for soil microbial DNA extraction. Then, the left soil was air-dried, ground, mixed, and passed through 4 mm and 0.85 mm sieves sequentially. Soil total nitrogen (TN) and total organic carbon (TOC) were measured using an element analyzer (Elementar, Shanghai, China). Soil total phosphorus and Olsen-P (NaHCO_3 extract) were determined using a spectrophotometer [32].

2.6. Methanogen and Methanotroph

Soil microbial genomic DNA was extracted using a FastDNA[®] Spin Kit for Soil (MP Bio-medicals, Santa Ana, CA, USA) according to the manufacturer's instructions. The extracted DNA was dissolved in 100 μL of TE buffer, quantified using a microspectrophotometer (Thermo Scientific, Waltham, MA, USA), and stored at -20°C until further analysis. Real-time quantitative PCR (qPCR) was performed to quantify *mcrA* and *pmoA* gene copies using the primer sets *mals/mcrA*-rev and *A189F/Mb661R*, respectively [30]. The 20 μL PCR reaction consisted of a 2 μL DNA template, 1.6 μL primer, and 16.5 μL ChamQ SYBR Color qPCR Master Mix. The qPCR conditions for the *mcrA* and *pmoA* genes were as follows: 95°C for 5 min, followed by 40 cycles of 95°C for 5 s, 55°C for 30 s, and 72°C for 40 s.

PCR amplicons of the *mcrA* and *pmoA* genes were sequenced to investigate methanogenic and methanotrophic communities. The 50 μL PCR reactions consisted of 25 μL of $2 \times \text{Taq Plus Master Mix}$, 4.0 μL of primer, 1 μL (~ 50 ng) of DNA template, and 20 μL ddH₂O. PCR conditions for both the *mcrA* and *pmoA* genes were as follows: 95°C for 3 min, followed by 36 cycles of 95°C for 20 s, 57°C for 40 s, and 72°C for 60 s. The PCR products of each DNA sample were checked by 1% agarose gel electrophoresis. The targeted band was

purified using the E.Z.N.A Gel Extraction Kit (Omega Bio-Tek, Norcross, GA, USA) and quantified by QuantiFluor™-ST (Promega, Madison, WI, USA). The library was constructed using the ALFA-SEQ DNA Library Prep Kit (Ark Biosafety Technology Co., Ltd., Shanghai, China). The purified amplicons were pooled in equimolar ratios and sequenced on an Illumina Nova 6000 Platform by MAGIGENE (<https://www.magigene.com/>, accessed on 14 January 2024). The data reported in this paper have been deposited in the GenBase in National Genomics Data Center [33], Beijing Institute of Genomics, Chinese Academy of Sciences/China National Center for Bioinformation, under accession number PRJCA026297, which is publicly accessible at <https://ngdc.cnbc.ac.cn/genbase> (accessed on 22 May 2024).

The raw sequence reads were demultiplexed in the Qiime2 environment (<https://qiime2.org/>). The paired-end sequences with an average quality of less than 20 were then filtered using fastp [34]. Barcode and PCR primers were removed using Cutadapt [35]. The paired-end clean reads were then merged using “fastq_mergepairs” command in USERCH10 [36], and OTU-table was produced from the sequence reads using the Unoise3 algorithm. After the reads were corrected against the FunGene database [37] by the FrameBot software (version 1.2) [38], the taxonomic classification of OTUs was performed using “syntax” algorithm in USERACH against NCBI database with a confidence threshold of 0.8 [39].

3. Statistical Analysis

Statistical analysis and plotting were performed using the R software environment [40]. The α -diversity indices of methanogens and methanotrophs were calculated by ‘vegan’ package. To avoid collinearity, only the Chao1 and Shannon indices were left for subsequent analysis. CH₄ and N₂O emission fluxes were expressed as the average and standard error of four replicates per observation. Two-way analysis of variance (Two-way ANOVA) was adopted to test the H₀ hypothesis that there were no significant differences between years and treatments, that is, rice yields, cumulative CH₄ and N₂O emissions, GWP, and yield-scaled GWP. One-way ANOVA was used to test the differences of function gene copies, diversity indices, and soil nutrient indices between treatments. Tukey’s honest significant difference (HSD) test was used for multiple comparisons. The Kruskal–Wallis test was used to detect significant differences in methanogen and methanotroph abundance between treatments. The structural equation model (SEM) was constructed by the ‘lavvan’ package [41]. The relationships between CH₄ emission flux and methanogens and methanotrophs were fitted by a linear regression model.

4. Results

4.1. Rice Yields

Analysis of variance revealed that the rice yields of RT and PT were significantly higher than those of RD in 2022, with an average rice yield of 10,845 kg ha^{−1} for RT and PT, which was 1.6 times that of RD (Figure 2). There were no significant differences in rice yield between the different treatments in 2023, with an average yield of 10,850 kg ha^{−1} (Figure 2).

4.2. CH₄ and N₂O Emissions in Paddy Fields

Tillage and cultivation practices did not have a significant influence on the dynamic characteristics of CH₄ and N₂O emissions during the rice-growing season in 2022 and 2023, with CH₄ emission flux mainly peaking at the tillering stages (Figure 3A,B), while N₂O emission fluxes from heading to maturity stage were higher than those from transplanting or seeding to the tillering stage (Figure 3C,D). It is noteworthy that the maximum CH₄ emission fluxes of PT in the tillering stage were 35.45 mg m^{−2} h^{−1} and 25.20 mg m^{−2} h^{−1} in 2022 and 2023, respectively, which were significantly higher than those of RT and RD ($p < 0.05$). The cumulative CH₄ emissions of PT were also significantly higher than those of RT and RD tested by two-way ANOVA ($p < 0.001$) (Figure 4A). On average, PT increased cumulative CH₄ emissions by 38.5% and 61.4% in 2022 and 2023 compared to RT and RD, respectively. Cumulative N₂O emissions varied significantly across years. PT

showed significantly higher cumulative N₂O emissions than NT in 2022 (two-way ANOVA, $p < 0.05$), while RT had significantly higher cumulative N₂O emissions in 2023 than PT and RD (Figure 4B).

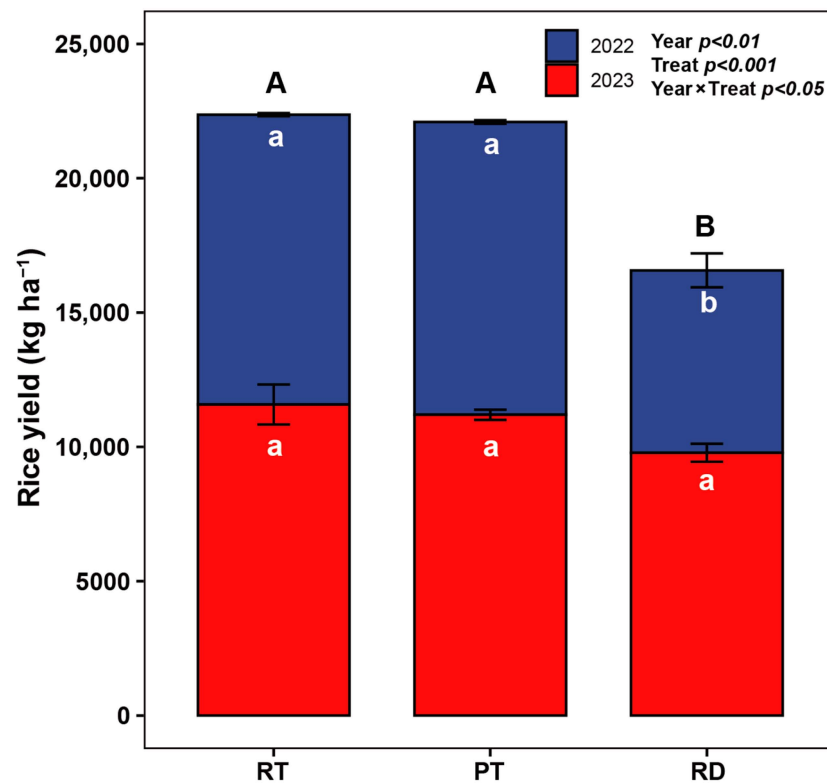


Figure 2. Rice yields in 2022 and 2023. Different lowercase letters in bars represent significant differences of rice yields between treatments at 0.05 levels (Tukey's HSD test) in 2022 and 2023, respectively. Different uppercase letters above bars indicate significant differences of the total rice yields of 2022 and 2023 at 0.05 levels (Tukey's HSD test). Vertical bars indicate standard errors of four replicates.

4.3. Global Warming Potential (GWP) and Yield-Scaled GWP

Tillage and cultivation practices significantly increased GWP. There were significant differences in GWP between treatments, and the trends of change were consistent across years. In 2022 and 2023, the average GWP of PT, RT, and RD were 6167.5 kg CO₂-eq. ha⁻¹, 4589.2 kg CO₂-eq. ha⁻¹, and 3515.0 kg CO₂-eq. ha⁻¹ (Figure 4C). The GWPs of PT were 34.4% and 75.5% higher than those of RT and RD treatments, respectively.

There were significant differences in yield-scaled GWP between the treatments (Figure 4D). The two-year averaged yield-scaled GWPs of PT, RT, and RD were 0.56 kg CO₂-eq kg⁻¹, 0.41 kg CO₂-eq kg⁻¹, and 0.42 kg CO₂-eq kg⁻¹, respectively.

4.4. Abundances, Diversity, and Composition of Methanogens and Methanotrophs

Fluorescence-based quantitative PCR (qPCR) data indicated that although there was no significant difference in the copies of *mcrA* gene between treatments, the copies of the *mcrA* gene of PT were still much higher than those in RT and RD, with increases of 58.5% and 114.5%, respectively. The copies of *pmoA* gene differed significantly between treatments, and RT had 1.85- and 4.05-times copies of *pmoA* gene than RD and PT, respectively (Figure 5A,B).

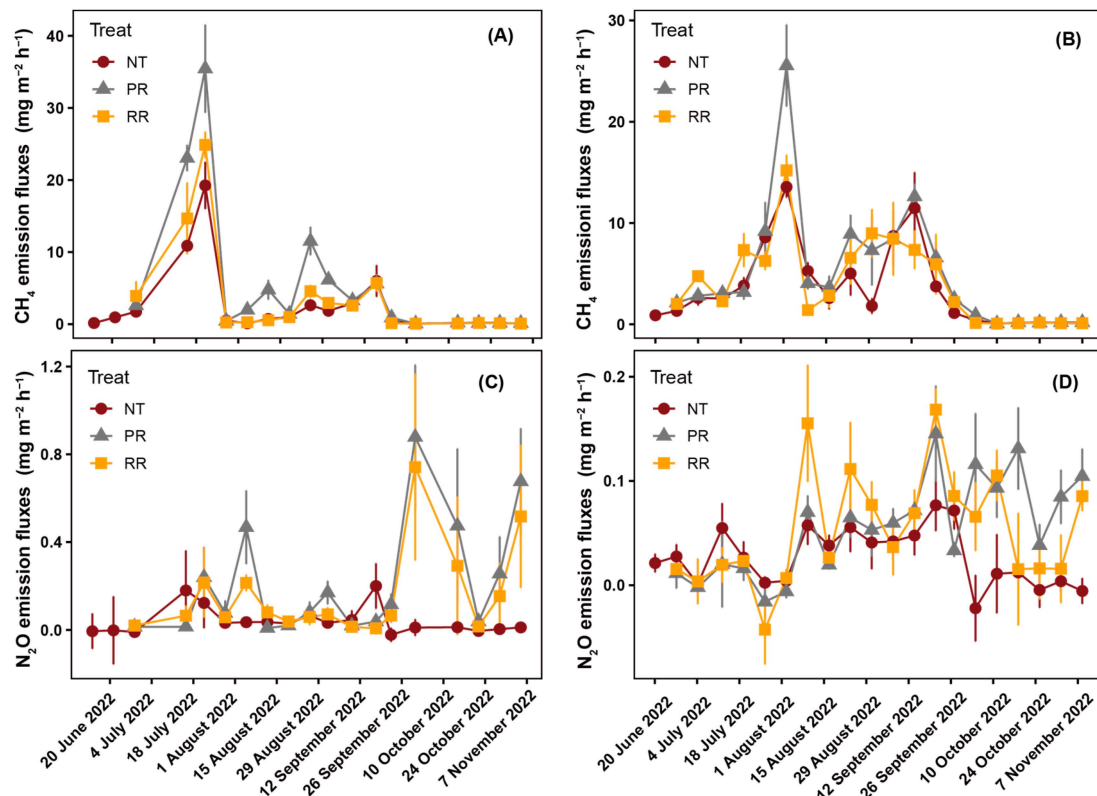


Figure 3. Seasonal dynamics of CH₄ (A,B) and N₂O (C,D) emission fluxes under different soil tillage and cultivation practice in 2022 and 2023. RR and PR represent rotary tillage and plowing with rice transplanting in rice season, respectively. NT represents no tillage and direct seedling of rice. Vertical bars indicate standard errors of four replicates.

Tillage and cultivation practices did not significantly influence the community structure of methanogens and methanotrophs (adonis, $p > 0.05$), but partly shaped the abundance composition of methanogens and methanotrophs (Figure 6A,B). The main methanogens (relative abundance $>1\%$), i.e., *Candidatus methanogranum* (average abundance 2.60%), *Methanocella* (average abundance 27.7%), and *Methanoregula* (average abundance 15.1%), showed significant differences in relative abundance between treatments (Kruskal–Wallis test, $p < 0.05$). In particular, the relative abundance of *Candidatus methanogranum* in RD was 4.12%, which was 2.02 and 2.51 times higher than that in RT and PT, respectively. In the RD treatment, the relative abundance of *Methanocella* was 38.74%, which was 91.3% and 60.1% higher than that in the PT and RT treatments, respectively. The *Methanoregula* abundance of PT was 21.18%, which was 25.7% and 191.3% higher than that of RT and RD treatments, respectively. Among the major methanotrophs ($>1\%$), type I methanotroph *Methyloglobulus* (average abundance 1.43%) showed significant differences between treatments ($p < 0.05$). The relative abundance of RT was 2.65%, i.e., 2.02 and 8.28 times compared to RD and PT, respectively.

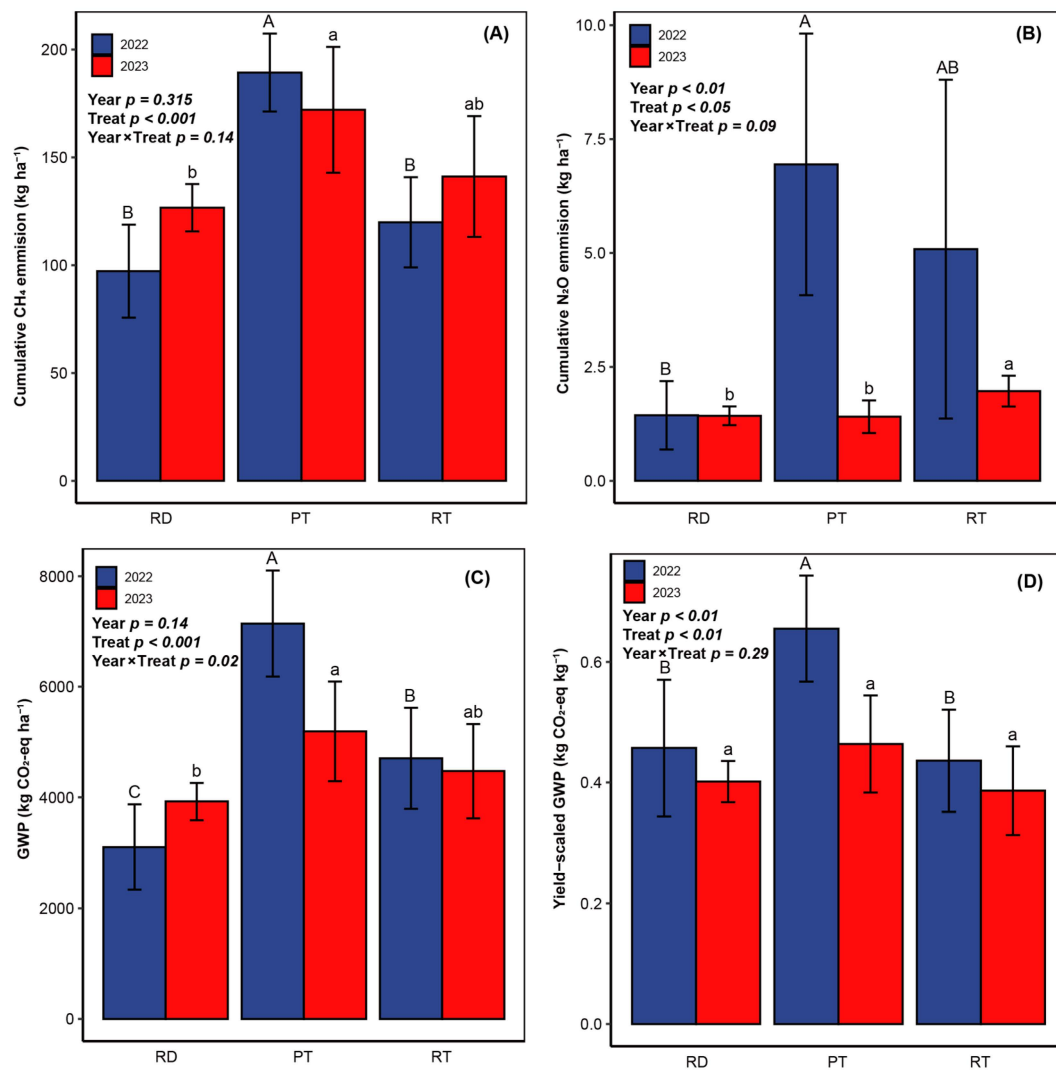


Figure 4. Impacts of soil tillage and cultivation practices on CH₄ (A), N₂O (B) cumulative emissions, global warming potential (GWP) (C) and yield-scaled GWPs (D) for 2022 and 2023. RR and PR represent rotary tillage and plowing with rice transplanting in rice season, respectively. NT represents no tillage with direct seedling of rice. Vertical bars indicate standard errors of four replicates. Different uppercase and lowercase letters on bars represent significant differences between treatments at 0.05 levels (Tukey's HSD test) in 2022 and 2023, respectively.

Although tillage and cultivation practices did not significantly influence the alpha diversity indices of methanogen and methanotroph communities, PT contributed to higher richness (Chao1) and diversity (Shannon-H) of methanogens, whereas its impact on the alpha diversity of methanotrophs was relatively minor (Table 2).

Table 2. Alpha diversity indices of methanogen and methanotroph communities at tillering stage.

Treat	Methanogens		Methanotrophs	
	Chao1	Shannon	Chao1	Shannon
RD	496.37 a	6.86 a	437.29 a	6.21 a
PT	674.26 a	7.06 a	412.39 a	6.12 a
RT	625.52 a	6.88 a	412.03 a	6.23 a

Note: The same small letters in columns indicate no significant differences at 0.05 levels (Tukey's HSD test), $n = 3$.

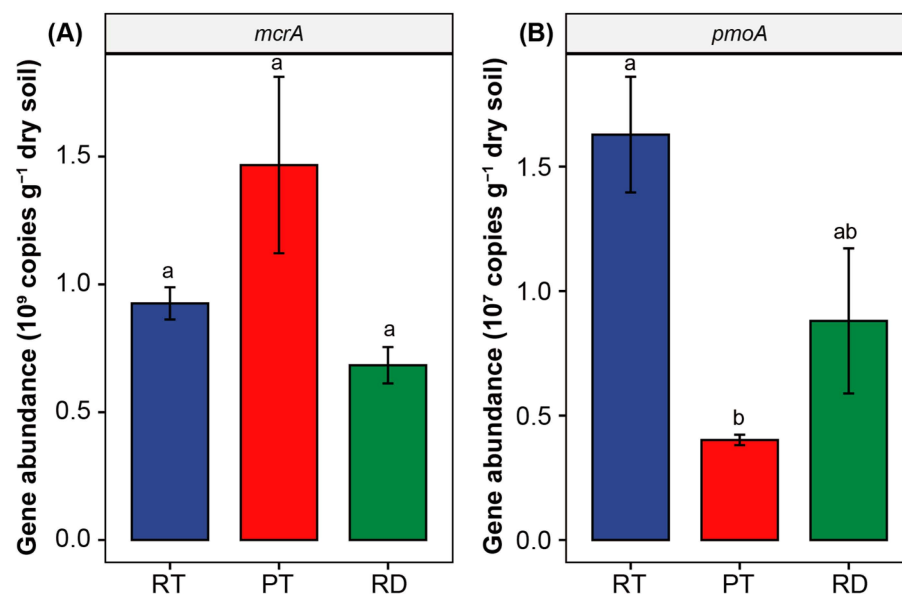


Figure 5. Copies of methanogens gene (*mcrA*) (A) and methanotrophs gene (*pmoA*) (B) in 0–20 cm topsoil at tillering stage in 2023. Different lowercase letters in bars represent significant differences between treatments at 0.05 levels (Tukey's HSD test) in 2023. Vertical bars indicate standard errors of three replicates.

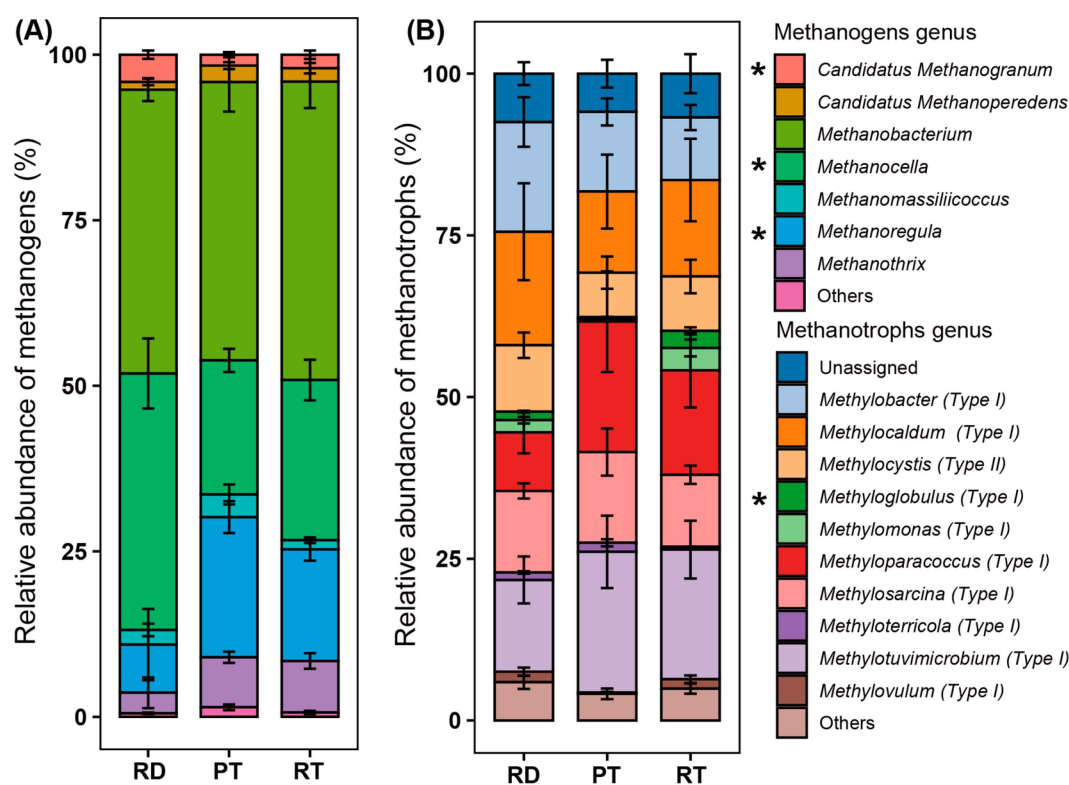


Figure 6. Community compositions of methanogenic (A) and methanotrophic (B) microbes of genus levels in 0–20 cm topsoil at tillering stage in 2023. Groups with <1% relative abundance were merged into the “Others” taxa. Asterisks represent significant differences of relative abundance among treatments according to Kruskal–Wallis test, with a significance level of 0.05. Vertical bars indicate standard errors of three replicates.

4.5. Soil Nutrient Traits

Tillage and cultivation practices significantly altered the contents of surface soil (0–20 cm) TOC, TN, TP, and Olsen-P. Soil TOC and TN contents of PT were significantly higher than those in RT and RD ($p < 0.05$). The soil TP and Olsen-P contents of RD were significantly higher than those of RT and PT ($p < 0.05$).

4.6. Structural Equation Modeling Analysis

Structural equation modeling (SEM) was employed to explain the hypothesis that CH₄ emission flux is driven by a combination of biological and environmental factors due to tillage and cultivation practices (Figure 7A). By model comparison, only two bio-factors (*mcrA* and *pmoA*) and two abio-factors (TOC and TN) were left for the SEM model. The final model fitted well, with a chi-square of 0.723, a high goodness-of-fit index (GFI = 0.999), and a low root mean square error of approximation (RMSEA = 0.000). It was clearly found that the CH₄ emission flux was strongly influenced by biological factors ($p < 0.01$), that is, *pmoA* and *mcrA* gene copies, followed by soil TOC and TN content ($p < 0.05$). Notably, *pmoA* gene copies showed negative and stronger influence than *mcrA*. TOC and TN also directly affected CH₄ emission flux negatively. TOC and TN negatively regulated *pmoA* gene copies ($p < 0.05$), but positively regulated *mcrA* gene copies ($p > 0.05$). We also used the linear regression model to fit the relationships between CH₄ emission flux and methanogens (Figure 7B) and methanotrophs (Figure 7C), respectively. Likewise, *mcrA* copies positively contributed to CH₄ emission flux while *pmoA* copies negatively related to CH₄ emission flux.

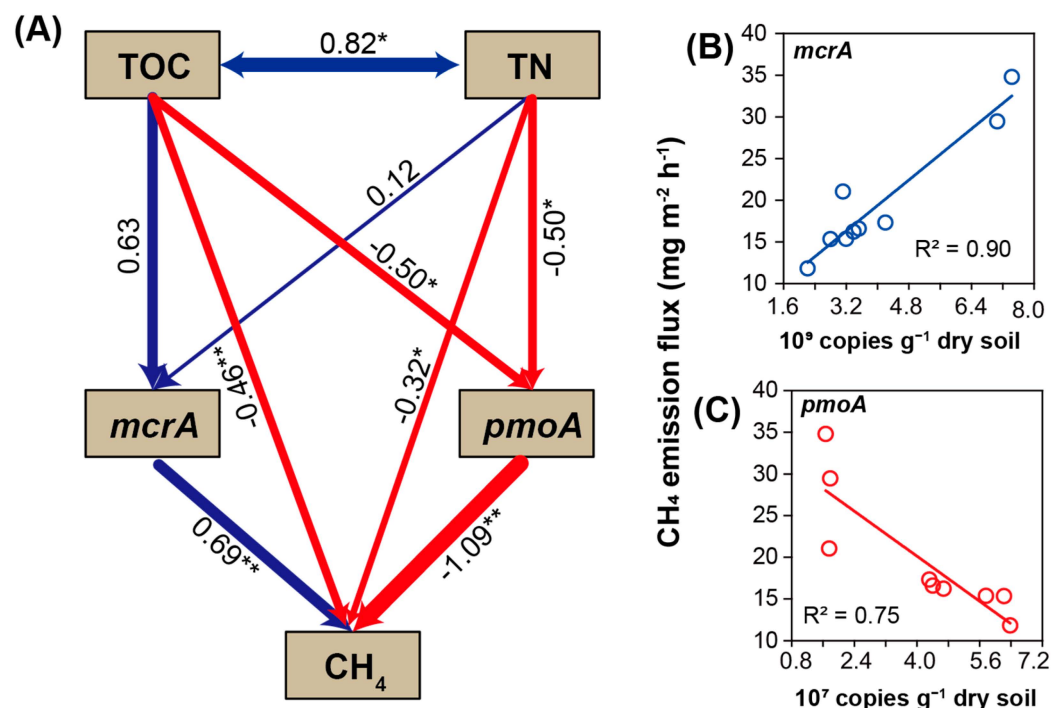


Figure 7. The structural equation models (SEM) based on the relationships and interactions among soil total organic carbon (TOC), soil total nitrogen (TN), methanogen abundance (*mcrA*), methanotroph abundance (*pmoA*), and CH₄ emission fluxes at tillering stage in 2023 (A). The numbers listed on the arrows are standardized parameter estimates (* $p < 0.05$, ** $p < 0.01$). The size of each path coefficient is represented by the thickness of the arrow, where red represents a negative effect and blue represents a positive effect. The assessment parameters of SEM are listed as follows: $\chi^2 = 0.723$, $df = 1.000$, p value = 0.395, GFI = 0.999, CFI = 1.000, and RMSEA = 0.000. The linear model represents the relationships between CH₄ emission flux and methanogens (B) and methanotrophs (C), respectively.

5. Discussion

5.1. Rice Productivity and Global Warming Potential

This study partly supported our hypotheses that RT maintained sustainable and high rice yields as PT (Figure 2), but RT did not show the lowest CH₄ emission and GWP between treatments. Our results are consistent with those of Zheng et al. [10] that rotary tillage kept a high and stable yield of rice in South China. Although Zhang and Hu [42] reported that the adoption of direct seeding increased rice yield in the Yangtze River Basin in China, dry direct seeding of rice significantly decreased rice yields in both 2022 and 2023 in our study, indicating that direct seeding was still at high risk in Taihu Lake region. These adverse effects of yield reduction were probably due to nutrient competition from weeds and rice lodging in direct-seeding treatments [43]. Meanwhile, rotary tillage is preferred because it reduces the fuel energy of machinery, that is, the total carbon footprint could be lower when using rotary tillage comparing to conventional plowing method [44].

Subsequently, we found significantly higher GHG emissions and GWP in PT, followed by RT and RD (Figure 4A,B), suggesting that deeper tilling promoted GHG emissions and GWP. Soil tillage can improve soil aeration, increase contact between crop residues and soil, and expose aggregate-protected SOM to microbial attack [45]. Thus, deeper soil tilling would accelerate soil carbon transformation and GHG emissions [46]. Furthermore, yield-scaled GWP, a trade-off index between rice yield and GWP, is widely used for agronomic innovation and policy making. Our results indicated that RT and RD achieved lower yield-scaled GWP than that of PT (Figure 4D). Similarly, Feng et al. [47] reported no tillage tended to decrease yield-scaled GWP down by 20% compared with conventional tillage. Interestingly, RT and RD contributed to yield-scaled GWP differently. For RT treatment, lower GHG emissions played an important role in yield-scaled GWP. Otherwise, for RD treatment, lower grain yields were the major driver of corresponding yield-scaled GWP. The above results suggest that lower GWP or yield-scaled GWP should be based on an acceptable yield, not just low emissions.

5.2. Effects of Microbes, Function Genes, and Soil Nutrients on CH₄ Emissions

Considering that CH₄ contributes more to GWP than N₂O in the rice season [3,48], we focus on the discussion of CH₄ emissions in the following paragraphs. Previous studies have shown that CH₄ emission peaks generally occur in the vegetative stage because of waterflooding [13,25]. Hence, bio- and abiotic factors are vital for affecting CH₄ emissions during this vegetative stage. We used *mcrA* and *pmoA* genes as biomarker of methanogens and methanotrophs that coding methyl coenzyme M reductase and the particulate methane monooxygenase, respectively [20,21]. The plowing treatment (PT) had higher *mcrA* and lower *pmoA* abundances than those of the rotary treatments (RT and RD) at the tillering stage (Figure 5A,B), and these corresponding enzymes were tightly correlated with CH₄ emission fluxes (Figure 7B,C). We inferred that PT promoted rice growth due to its higher yields, coupled with higher root exclusion [49], in which carbon substrates simultaneously promoted the growth of methanogens [50]. Man et al. [51] pointed out that tillage management exerted stronger controls on soil microbial community structure, likely due to the higher decomposition of lignin after a 24-year field trial, and our results (Figure 6, Table 2) were partly in line with theirs. We found changes in the relative abundances of typical methanogens and methanotrophs, for example, *Methanocella*, *Methanoregula*, and *Methyloglobulus*, suggesting that tillage and cultivation practices shifted the ecological niches of methanogens and methanotrophs [52].

The SOC and TN contents in PT were significantly higher than those in RT and RD (Table 3), consistent with previous studies documenting that deep plowing with straw incorporation increased SOC and TN [53,54]. Deep plowing is helpful for the sufficient mixing of straw and soil, and subsequently, amounts of nutrients, that is, C and N, are released into the soil with straw decomposition [55]. Thus, PT could supply more substrate-C for methanogens to release CH₄, and tillage also changed soil physical conditions, such as soil porosity, which accelerated CH₄ emissions [48]. Meanwhile, CH₄ is transported

from the soil to the atmosphere predominantly through rice plants [56]. Large biomasses of rice plants in PT naturally discharge high CH₄ emissions. Furthermore, RD reduced CH₄ emissions more compared to RT, and it is widely known that the significant reduction in CH₄ emissions is mainly attributed to the differential irrigation and drainage management in the seedling stage between direct seeding and transplanting practices [57]. For direct seeding of rice, long-term waterflooding was not suggested to avoid the anaerobic respiration of rice that could damage rice seedlings. In our study, we performed a saturated water-holding capacity in the first 15 days of RD treatment, resulting in higher soil redox potential and lower CH₄ emissions. Meanwhile, considering that we did not determine the CH₄ emissions while raising rice seedlings for transplanting, CH₄ emissions in PT and RT were probably underestimated. A systematic and full monitoring process of GHG emissions is required in future studies.

Table 3. Soil traits (0–20 cm) at tillering stage.

Treat	TOC (%)	TN (%)	TP (mg kg ^{−1})	Olsen-P (mg kg ^{−1})
RD	1.98 c	0.20 c	743.37 a	19.88 a
PT	2.24 a	0.22 a	681.73 ab	13.42 b
RT	2.14 b	0.21 b	637.86 b	14.55 ab

Note: Different small letters represent significant differences at 0.05 levels (Tukey's HSD test), $n = 3$.

5.3. Inherent Regulation Mechanism of CH₄ Emission

Methane emission is generally co-affected by various biotic and abiotic factors under tillage and cultivation management. SEM analysis was used to help understand the patterns of correlation/covariance among a set of variables and explain as much of their variance as possible with the model specified [58]. Although bio- and abio-factors simultaneously affected CH₄ emission, SEM analysis clearly showed that CH₄ emission fluxes were mainly regulated by bio-factors according to the estimates of path effect, i.e., directly regulated by *pmoA* and *mcrA*, and in turn, by abio-factors, i.e., indirectly regulated by TOC and TN (Figure 7A). Notably, SEM analysis supported that methane-oxidizing bacteria played the most important roles in CH₄ emission ($p < 0.01$), and most of the methanotrophs belonged to *γ-proteobacteria* (type I) in our study (Figure 6B), which takes advantage of the RuMP pathway to assimilate formaldehyde in the inner membrane. Type I methanotrophs were widely found in anaerobic environments, such as paddy soils and wetlands [59], suggesting that biological effects on CH₄ emission fluxes largely through regulating soil aeration and redox potential. Considering that TOC and TN were negatively correlated with *pmoA* in SEM, we propose that methanotroph metabolism could be versatile and opportunistic. Zhou et al. [60] reported that *γ-proteobacteria* not only mediates methylated compounds but also utilizes complex organic compounds as substrates. Thus, PT, with significantly higher TOC and TN, unexpectedly inhibited *pmoA* gene abundance.

Methanogens belong to archaea, generally accounting for a small proportion of soil microbial community, and they have crucial roles in mediating soil carbon and nitrogen cycles [61]. Although no significant difference of methanogenic abundance between treatments was found (Figure 5A), TOC and TN positively supported *mcrA* gene abundance by SEM analysis, which was in line with higher CH₄ emissions. Song et al. [62] also found that *mcrA* gene abundance was positively associated with soil TC, TN, and DOC contents in permafrost peatlands. These results provide insights into the methanogens distribution and their relationships with soil properties. However, we constructed the SEM only based on the independent sampling time at tillering stage, and time-series investigations through the rice growing season are required to clarify the impacts of tillage and cultivation methods on CH₄ emission in detail in further studies.

6. Conclusions

In summary, the five-year field trial showed that rice yields of PT and RT were higher and more stable than those of RD. RT and RD exhibited significantly lower CH₄ emissions and yield-scaled GWP than PT. Overall, the agronomic technology of rotary tillage plus mechanical transplanting is preferred for rice production in the Taihu Lake region. The functional gene abundances of *mcrA* and *pmoA* were the most important regulatory factors of CH₄ emissions, rather than the community structure and diversity of methanogens and methanotrophs. Soil TOC and TN directly regulated CH₄ emissions by changing C-substrate concentration and indirectly regulated CH₄ emissions by affecting *mcrA* and *pmoA* expression. In future studies, we suggest focusing on regulating the activities of methanogens and methanotrophs to obtain lower CH₄ emissions and higher rice productivity.

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