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Role of nitrate-dependent anaerobic methanotrophs in reducing methane emissions under delayed flooding in straw-amended paddy soil

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Abstract

Straw incorporation is commonly adopted to improve soil fertility in rice paddies, yet it leads to substantial increases in methane (CH_4) emissions. Although water management is recognized as an effective mitigation strategy, the impacts of delayed flooding on CH_4 emissions and the underlying microbial mechanisms under straw incorporation remain poorly understood. To fill this knowledge gap, a laboratory mesocosm study was conducted involving three straw treatments (no straw, maize leaf, and maize stem) and two water regimes (direct and delayed flooding). Results showed that straw addition significantly increased CH_4 emissions, with greater stimulation observed in leaf-amended soil. Relative to direct flooding, delayed flooding reduced CH_4 emissions by 83.3% and 85.6% in leaf- and stem- amended soils, respectively. Delayed flooding significantly decreased methanogenic *mcrA* and aerobic methanotrophic *pmoA* gene abundances but increased *M. nitroreducens*-like archaeal *mcrA* abundance, with no significant effect on *M. oxyfera*-like bacterial *pmoA*. CH_4 emissions were positively associated with methanogenic *mcrA* abundance and negatively associated with *M. nitroreducens*-like archaeal *mcrA* abundance. Random forest identified methanogenic *mcrA* abundance as the strongest predictor of CH_4 emissions. SHAP analysis linked higher methanogenic *mcrA* abundance to higher predicted emissions, whereas higher NO_3^--N content and greater *M. nitroreducens*-like archaeal *mcrA* gene abundance were generally associated with lower predicted emissions. PLS-PM analysis further revealed that the reduced CH_4 emissions caused by delayed flooding under straw addition was primarily mediated by increasing NO_3^--N content, which subsequently stimulated *M. nitroreducens*-like archaea. Collectively, our findings demonstrate that delayed flooding serves as an effective strategy to mitigate CH_4 emissions in straw-incorporated paddy ecosystems.

Keywords: Delayed flooding; Straw addition; CH_4 emissions; methanogen, methanotroph

1. *Introduction*

As the second most prominent anthropogenic greenhouse gas, methane (CH_4) exhibits a 100-year global warming potential 27 times that of carbon dioxide (CO_2), which increases to 82 times over a 20-year horizon (IPCC, 2021). Rice agriculture is vital for global food security, yet it represents a major source of agricultural CH_4 emissions, accounting for nearly 22% of the global agricultural total (Qian et al., 2023). Because these emissions will persist without intervention, mitigating paddy CH_4 emissions is critical for achieving global climate targets (Zhang et al., 2026).

Soil CH_4 emissions are regulated by the dynamic balance between microbial CH_4 production and oxidation, mediated by methanogens and methanotrophs, respectively (Conrad, 2007). Under anaerobic conditions, methanogens produce CH_4 using organic substrates, whereas methanotrophs oxidize CH_4 to CO_2 under both aerobic and anaerobic environments (Murrell and Jetten, 2009). Specifically, aerobic CH_4 oxidation is performed by conventional aerobic methanotroph belonging to *Proteobacteria* phylum (Kolb et al., 2003). In contrast, anaerobic methane oxidation (AOM) is driven by specialized groups, including "*Candidatus Methylomirabilis oxyfera (M. oxyfera)*-like bacteria" of NC10 phylum and "*Candidatus Methanoperedens nitroreducens* (*M. nitroreducens*) -like archaea" of *ANME*-2d (Ettwig et al., 2010; Haroon et al., 2013), which drive nitrite- and nitrate-dependent AMO process, respectively. Therefore, linking CH_4 emissions to these microbial communities is pivotal for elucidating the mechanisms governing paddy CH_4 cycling.

Although straw incorporation effectively improves soil organic matter and crop yield (Liu et al., 2023; Tang et al., 2025), it supplies abundant carbon substrates for methanogenesis, thereby triggering substantial CH_4 emissions during the rice growing season (Liu et al., 2014). A recent meta-analysis showed that straw incorporation can increase CH_4 emissions from rice paddies by 153% (Jia et al., 2024). Consequently, mitigating CH_4 emissions while preserving soil fertility under straw amendment remains a major challenge. Optimized water management, including alternate wetting and drying and mid-season drainage, serves as an effective strategy for curbing CH_4 emissions (Tariq et al., 2017, 2018; Zhou et al., 2025). However, frequent drainage

throughout the rice growing season often poses practical challenges and elevates the risk of nutrient leaching (Huang et al., 2024). In addition, prolonged dry conditions resulting from repeated drainage can restrict rice growth, ultimately leading to yield reductions (Anbumozhi et al., 1998). As a practical alternative, early-season delayed flooding before transplanting is particularly suitable for regions lacking advanced irrigation systems (Tariq et al., 2017). Recent study revealed that delayed flooding accelerates the transformation of plant-derived carbon into microbial biomass carbon and stable fungal necromass carbon (Ma et al., 2026). Zhou et al. (2025) confirmed that a 10-day delayed flooding substantially reduces the carbon footprint without compromising rice yields. Given that paddy CH_4 fluxes concentrate within 7–40 days post-transplanting, typically reaching a peak during the second week (Zhou et al., 2025; Jiang et al., 2022, 2025), early-stage interventions are essential for mitigating seasonal CH_4 emissions. Delayed flooding creates an initial aerobic phase that accelerates the decomposition of straw-derived carbon, thereby limiting substrate availability for methanogens during early rice growth (Song et al., 2021). Studies have confirmed that short-term aerobic pre-decomposition of organic materials (e.g., rice straw or green manure) promotes the rapid consumption of labile carbon (Lee et al., 2020; Liu et al., 2026). This early depletion suppresses the post-flooding DOC pool while maintaining a higher soil redox potential (Eh), ultimately achieving a 60%–98% reduction in CH_4 emissions (Liu et al., 2025; Lee et al., 2020). In line with these findings, Ma et al. (2024) reported that delaying flooding by 5–15 days in a green manure–rice system suppresses CH_4 emissions by 40.9%–60.8%, an effect driven by diminished surface-water DOC concentrations and reduced methanogenic *mcrA* gene abundance.

In agricultural systems, stems and leaves differ significantly in chemical composition, such as C/N ratios and recalcitrant carbon content. These differences lead to contrasting decomposition dynamics during the initial aerobic phase and subsequently alter substrate availability after flooding (Redin et al., 2014; Ma et al., 2026). However, how delayed flooding combined with specific residue fractions regulates CH_4 emissions and alters the abundances of methanogens and methanotrophs, especially anaerobic methane oxidizers, remains poorly understood. To address these

knowledge gaps, we conducted a microcosm incubation experiment integrating two water regimes (direct vs. delayed flooding) with two maize residue fractions (stem vs. leaf). By quantifying the abundances of both methanogens and methanotrophs (aerobic and anaerobic), this study aims to elucidate the microbial mechanisms by which delayed flooding influences CH_4 emissions under crop residue incorporation.

2. Materials and methods

2.1. Soil and crop residue preparation

Surface soil samples (0–20 cm) were collected from a paddy field at Taihu Experimental Station of Yangtze University (30°21′ N, 112°04′ E), Jingzhou, China. This region experiences a typical subtropical monsoon climate, recording an average annual precipitation of 1200 mm and a mean temperature of 16.2 °C. Five subsamples were taken from each of four replicate plots using a soil auger and composited into a single sample. After removing visible stones and organic residues, the fresh soil was kept at 4 °C prior to incubation, with a subset air-dried for baseline physicochemical analysis. The soil contained 22.3 g kg^{-1} organic matter, 1.3 g kg^{-1} total N, 9.5 g kg^{-1} total K, 0.5 g kg^{-1} total P, with a pH of 6.3. Maize residues were obtained from the same station were oven-dried at 60 °C to a constant weight, separated into stems and leaves, and cut into 2-5 mm pieces. The TC and TN contents were 420 and 7.0 g kg^{-1} for stems, and 394 and 11 g kg^{-1} for leaves, respectively.

2.2 Experiment design

A two-factor microcosm incubation experiment was conducted using three straw treatments (unamended control, maize stem, and maize leaf) and two water regimes (direct flooding and delayed flooding). All treatments were performed in triplicate. Portions of fresh soil containing 40 g of dry mass were transferred into 500-mL containers, with soil moisture set at 60% of water-holding capacity (WHC), and maintained the samples at 25 °C in darkness for a 7-day pre-incubation. Following pre-incubation, the respective maize residues were thoroughly homogenized with the soil at a rate of 5 g kg^{-1} dry soil (equivalent to a field application of 12 t ha^{-1}), while the

control received no amendment. For the direct flooding treatment, distilled water was added on day 0 to establish a 1:2 soil-to-water ratio (2–3 cm floodwater layer). For delayed flooding, samples were kept at 60% WHC for the first 8 days before receiving the same flooding treatment (1:2 ratio, 2–3 cm water layer). All jars were subsequently sealed with rubber stoppers and incubated under dark conditions at 25 °C for 79 days.

2.3 Gas and soil property analysis

Headspace gas sampling was conducted on days 1, 3, 5, 7, 9, 11, 15, 19, 24, 29, 34, 39, 44, 49, 54, 59, 64, 69, 74, and 79 to monitor CH_4 fluxes. Prior to sampling, flasks were ventilated for 10 min to ensure gas equilibrium. A 20-mL headspace gas sample was extracted at 0, 1, and 2 h post-sealing using a gas-tight syringe and transferred into pre-evacuated 12-mL vials. The samples were then analyzed for CH_4 concentrations utilizing gas chromatograph (Agilent 7890A, California, USA). CH_4 fluxes were derived from the linear regression slope of the concentration changes over the enclosure period, and were subsequently expressed on a soil dry-weight basis. Cumulative CH_4 emissions were calculated by linear interpolation of fluxes between successive sampling points.

Soil samples were collected at the end of the incubation. One portion was stored at −80 °C for microbial analyses, while the remainder was utilized to determine soil physicochemical properties following Bao (2000). Soil NH_4^+-N and NO_3^--N were extracted with 2 mol L^{-1} KCl (shaken at 150 r min^{-1} for 1 h) and measured using the indophenol blue colorimetric method. Soil DOC was quantified using the potassium dichromate oxidation method, in which samples were digested in a boiling oil bath and subsequently titrated with a standard ferrous sulfate solution.

2.4 DNA extraction and PCR amplification

Soil (0.25 to 0.5 g) genomic DNA was extracted using a FastDNA SPIN kit according to standard protocols, followed by purification and quantification with a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). The PCR was

conducted to assay the *pmoA* gene abundances of aerobic methanotrophs and *M. oxyfera*-like bacteria, *mcrA* gene abundances of methanogens and *M. nitroreducens*-like archaea, and *amoA* gene abundances of ammonia-oxidizing archaea (AOA) and bacteria (AOB). The specific PCR primers and thermal cycling protocols for amplifying the selected functional genes are provided in Table S1. Standard curves were constructed using 10-fold serial dilutions of target-containing plasmids. All quantitative assays were performed in triplicate alongside negative controls, yielding strong linear correlations between threshold cycles and log-transformed copy numbers ($r^2 > 0.99$) with amplification efficiencies ranging from 90% to 110%. Furthermore, amplicon specificity was verified via melting curve analysis, and gene copy numbers were expressed on a soil dry-weight basis.

2.5. Statistical analyses

Initial statistical evaluations involved the Shapiro-Wilk and Levene's tests to verify normality and homoscedasticity across all datasets. Subsequent two-way ANOVAs were performed to evaluate the responses of CH_4 emissions and soil biochemical indicators to fooding conditions and straw addition. Pearson correlation analysis was utilized to investigate the relationships linking measured soil variables with CH_4 emissions. Additionally, a random forest (RF) modeling was deployed to rank the essential abiotic and biotic drivers governing CH_4 emissions, and SHapley Additive exPlanations (SHAP) analysis was performed using the treeshap package to quantify their contributions to RF predictions. Furthermore, a partial least squares path modeling (PLS-PM) was used to disentangle the direct and indirect factors driving CH_4 emissions. All analyses were done in R 4.5.3, with statistical significance defined as $p < 0.05$.

3. Results

3.1 CH_4 emission

In straw-unamended control, CH_4 fluxes remained consistently low throughout the incubation period (Figs. 1a and b). Straw addition markedly stimulated CH_4 fluxes during the early stage, with maize leaves exerting a stronger stimulating effect than

stems. Under direct flooding, straw-amended treatments exhibited two distinct flux peaks on days 15 and 30, followed by a gradual decrease. Under delayed flooding, straw addition induced only a single emission peak on day 30 before gradually decreasing.

Flooding time, straw addition, and their interaction significantly regulated cumulative CH_4 emissions ($p < 0.05$, Fig. 1c). Specifically, adding crop straw substantially enhanced cumulative CH_4 emissions, with higher values observed in leaf-amended soil. Notably, compared with direct flooding, delayed flooding suppressed cumulative CH_4 emissions by 85.6% and 83.3% in maize stem and leaf treatments, respectively.

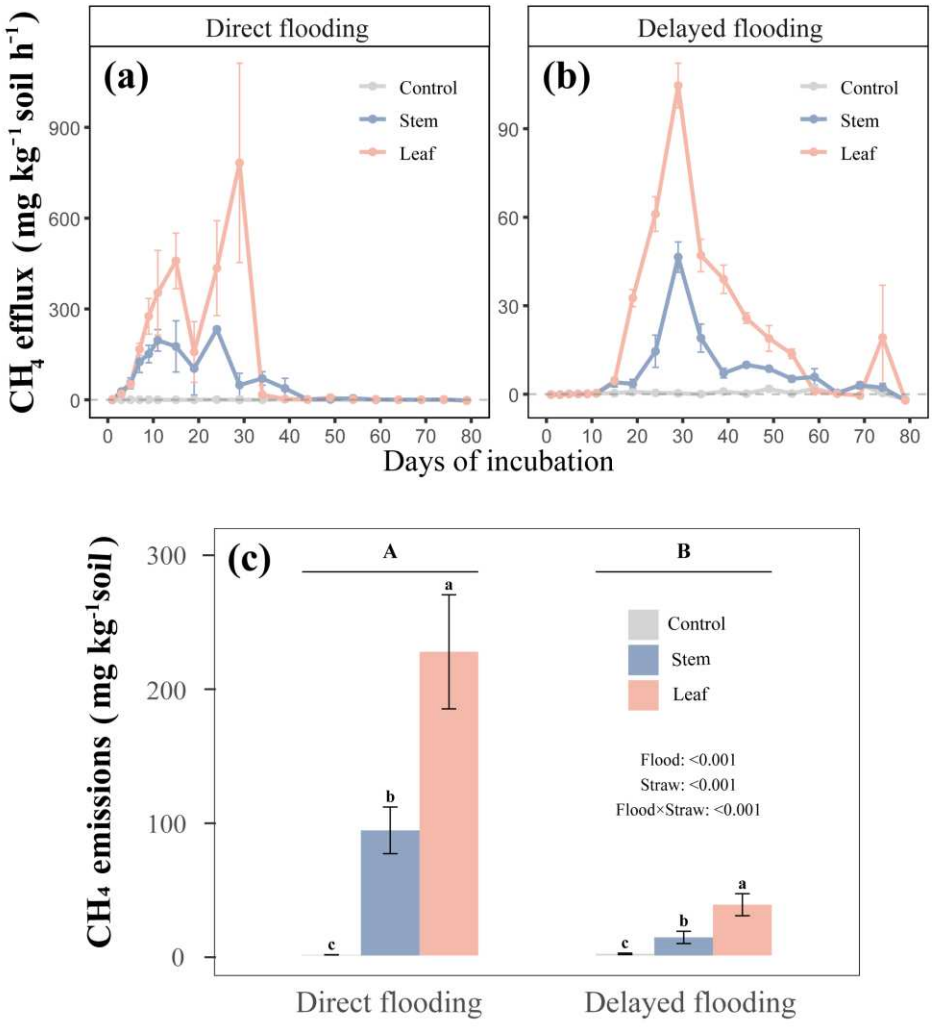


Fig. 1 Temporal effluxes and cumulative emissions of CH_4 under different treatments over a 79-day incubation. Error bars indicate standard errors (n = 4). Different uppercases denote significant differences between direct and delayed flooding

treatments, different lowercase letters denote significant differences among straw addition treatments ($p < 0.05$).

3.2 Soil physicochemical properties

Soil DOC content was markedly affected by flooding time, straw addition, and their interaction ($p < 0.05$, Fig. 2a). Under direct flooding, leaf and stem additions significantly increased DOC by 64.0% and 34.7% compared with straw-unamended soil, respectively. Leaf addition still maintained the highest DOC content under delayed flooding. Delayed flooding reduced DOC contents in leaf- and stem-amended soils by 25.0% and 31.1% compared with direct flooding.

Straw addition served as the sole factor influencing soil NH_4^+-N content ($p < 0.05$, Fig. 2b). Under direct flooding, leaf and stem additions significantly elevated NH_4^+-N contents by 40.8% and 31.1% relative to straw-unamended soil, respectively. Under delayed flooding, however, a significant increase in NH_4^+-N was observed only under leaf-amended treatment.

Soil NO_3^--N content was dramatically influenced by flooding time, straw addition, and their interaction ($p < 0.05$, Fig. 2c). Under direct flooding, stem and leaf additions reduced NO_3^--N by 28.1% and 47.9% compared with straw-unamended soil, respectively. Conversely, under delayed flooding, only leaf addition caused a significant reduction. Relative to direct flooding, delayed flooding markedly elevated soil NO_3^--N levels by 21.7%, 77.1% and 65.6% in straw-unamended, leaf and stem addition treatments, respectively.

3.3 Soil biological properties

The gene abundances of methanogen-*mcrA*, aerobic methanotroph-*pmoA* and *M. nitroreducens*-like archaea-*mcrA* was markedly affected by flooding time, straw addition, and their interaction ($p < 0.05$; Figs. 2d and e). AOA-*amoA* and AOB-*amoA* were influenced only by individual main effects ($p < 0.05$; Figs. 2g and i), whereas *M. oxyfera-like* bacteria-*pmoA* showed no significant response to either factor ($p > 0.05$; Fig. 2f).

Straw addition increased the gene abundance of methanogen-*mcrA* by 1.0- to 4.2-fold, with leave exerting a stronger effect than stem (Fig. 2d). Compared with direct flooding, delayed flooding significantly reduced the gene abundance of methanogen-*mcrA* by 50.5%, 76.4%, and 65.0% in straw-unamended, stem, and leaf treatments, respectively. Straw addition increased the gene abundance of aerobic methanotroph-*pmoA* by 0.8- to 4.4-fold (Fig. 2e). Relative to direct flooding, delayed flooding significantly decreased the gene abundance of aerobic methanotroph-*pmoA* by 78.4%, 53.7% and 34.9% in straw-unamended, leaf and stem addition treatments, respectively. Under direct flooding, stem and leaf additions reduced the gene abundance of *M. nitroreducens*-like archaea-*mcrA* by 42.6% and 91.9% respectively, whereas only leaf addition caused a significant reduction (12.7%) under delayed flooding (Fig. 2g). Remarkably, compared to direct flooding, delayed flooding significantly elevated the gene abundance of *M. nitroreducens*-like archaea-*mcrA*, increasing it by 19.7% in straw-unamended soil, and by 1.3- and 11.9-fold in the stem and leaf addition treatments, respectively. Compared to straw-unamended soil, straw addition decreased the abundance of the AOA-*amoA* gene by 42.7%−47.7%, while increasing AOB-*amoA* gene abundance by 1.1−1.2-fold (Fig. 2h, j). Relative to direct flooding, delayed flooding significantly enhanced AOA- *amoA* gene abundance by 68.5%, 125.8%, and 97.8%, and AOB-*amoA* gene abundance by 60.6%, 9.8%, and 31.0% in the straw-unamended, stem, and leaf treatments, respectively.

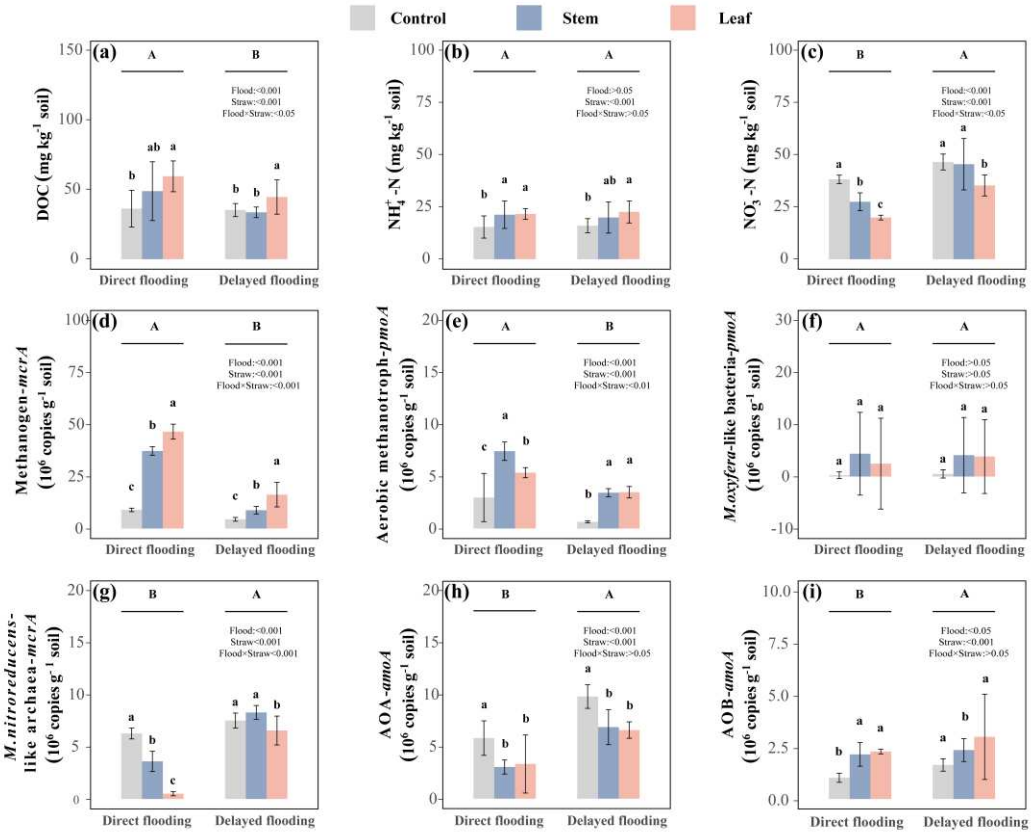


Fig. 2 Soil DOC (a), NH_4^+-N (b), NO_3^--N (c) contents, gene abundances of methanogen-*mcrA* (d), aerobic methanotroph-*pmoA* (e) and *M. oxyfera*-like bacteria-*pmoA* (f), *M. nitroreducens*-like archaea-*mcrA* (g), AOA-*amoA* (h), AOB-*amoA* (i) under different treatments at the end of the incubation. Error bars indicate standard errors (n = 4). Different uppercases denote significant differences between direct and delayed flooding treatments, different lowercase letters denote significant differences among straw addition treatments ($p < 0.05$).

3.4 Factors governing CH_4 emissions

Correlation heatmap and linear regression analyses revealed that CH_4 emissions were strongly regulated by soil chemical and biological properties (Fig. 3 and Fig. 4). Specifically, CH_4 emissions showed significant positive correlations with DOC, NH_4^+-N, and the gene abundances of methanogen-*mcrA* and aerobic methanotroph-*pmoA* ($p < 0.05$). Conversely, CH_4 emissions were negatively correlated with NO_3^--N and *M. nitroreducens*-like archaea-*mcrA* ($p < 0.05$). *M. oxyfera*-like bacteria-*pmoA* exhibited no significant correlation with CH_4 emissions or other environmental factors ($p > 0.05$).

RF analysis indicated that functional genes and soil properties jointly influenced CH_4 emissions (Fig. 5a). Among all variables, the gene abundance of methanogen-*mcrA* was the most dominant predictors ($p < 0.05$), followed by NO_3^--N, *M. nitroreducens*-like archaea-*mcrA*, aerobic methanotroph-*pmoA*, and DOC. In contrast, NH_4^+-N and *M. oxyfera*-like bacteria-*pmoA* showed minor influences that did not achieve significance ($p > 0.05$).

SHAP analysis of the random forest model showed that Methanogen-*mcrA* exhibited the widest range of SHAP values, with high-abundance samples showing large positive contributions, making it the most influential predictor (Fig. 5b). NO_3^--N and *M. nitroreducens*-like archaea-*mcrA* ranked next in overall importance, with higher values predominantly associated with negative SHAP values and substantial negative contributions to predicted CH_4. Higher DOC and aerobic methanotroph-*pmoA* abundance were also generally associated with positive SHAP values. In contrast, the SHAP values of NH_4^+-N and *M. oxyfera*-like bacteria-*pmoA* were concentrated closer to zero, indicating relatively smaller overall contributions.

PLS-PM analysis revealed the pathways through which flooding time and straw addition regulated CH_4 emissions (Fig. 6). Specifically, *M. nitroreducens*-like archaea-*mcrA* exerted a direct negative effect on CH_4 emissions. Flooding time and straw addition significantly impacted soil NO_3^--N content, which subsequently promoted *M. nitroreducens*-like archaea-*mcrA* while suppressing methanogen-*mcrA*. Overall, straw addition increased CH_4 emissions mainly through indirect positive effects (total effect = 0.73), whereas delayed flooding substantially inhibited CH_4 emissions by stimulating NO_3^--N accumulation and stimulating *M. nitroreducens*-like archaea-*mcrA* (total effect = -0.47).

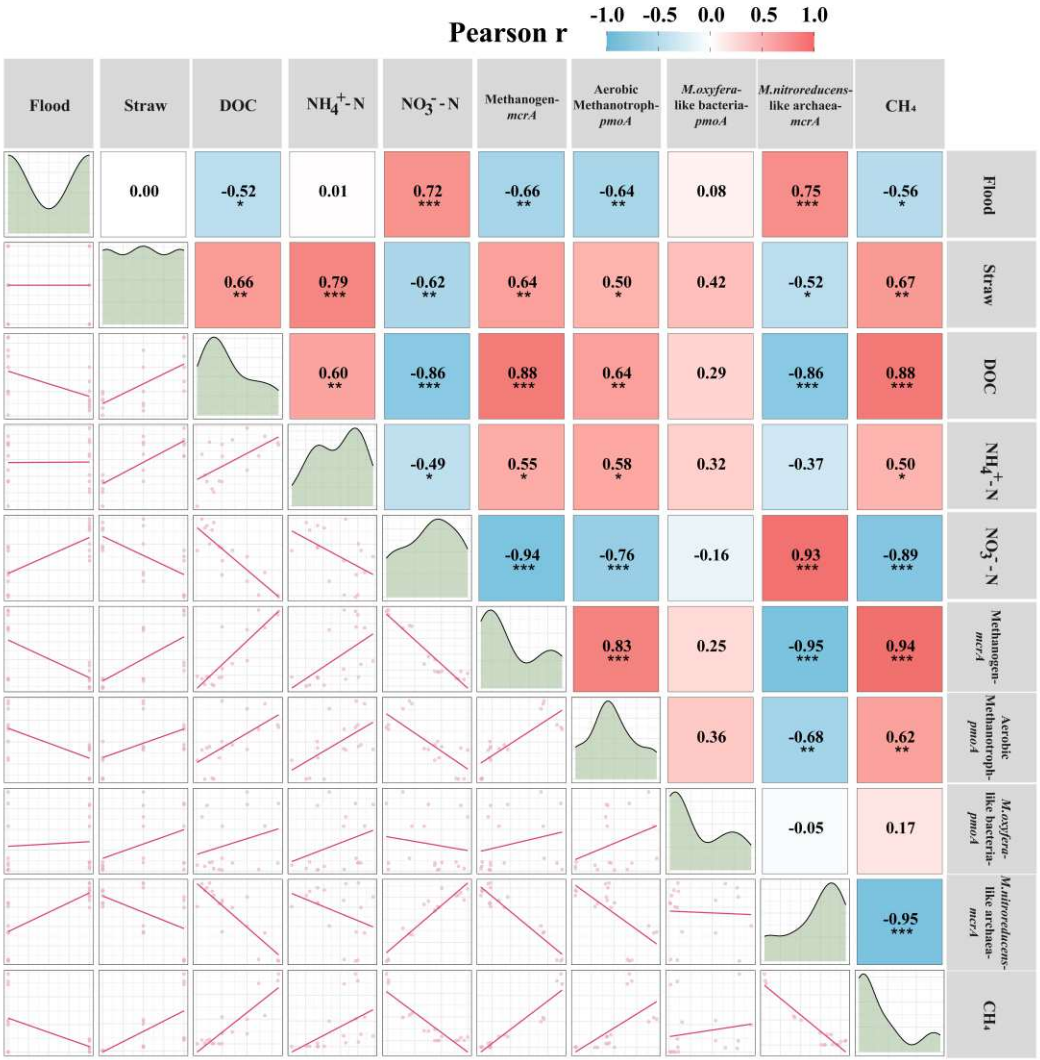


Fig. 3 Relationships between CH_4 emissions and soil properties based on Pearson correlation analysis , Asterisks denote statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

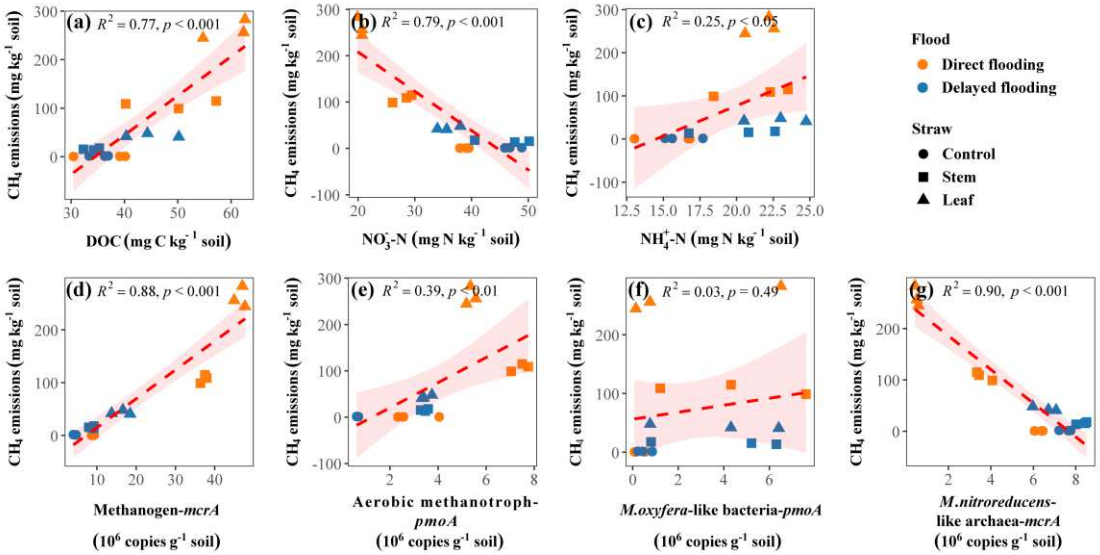


Fig. 4 Relationships between CH_4 emissions and soil properties based on linear regression analysis.

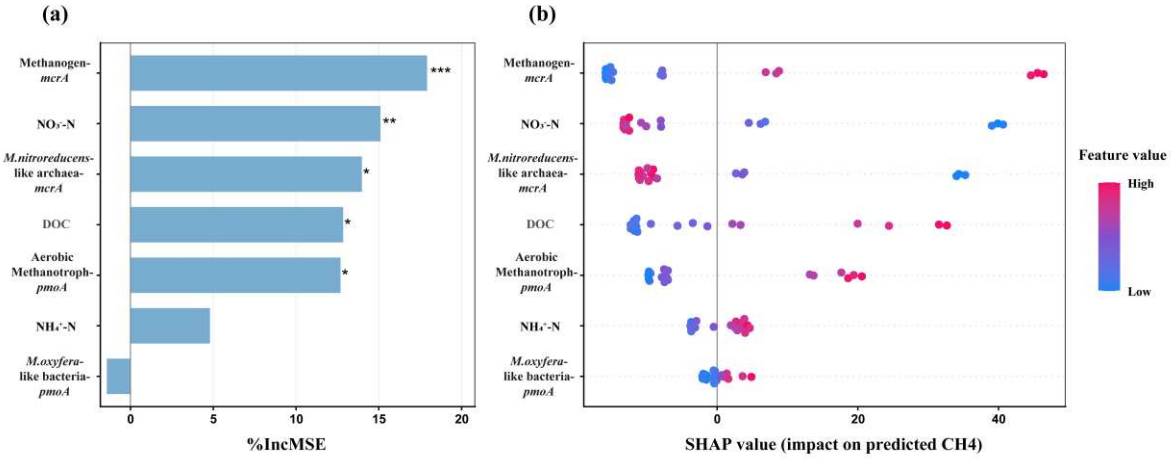


Fig. 5 Predictor variable importance for CH_4 emissions based on the random forest model (a). Asterisks indicate significance of variable importance assessed using permutation tests implemented in rfPermute: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. TreeSHAP beeswarm plot showing each variable's contribution to individual predictions (b). Each point represents one sample, with blue and red indicating low and high feature values, respectively. Positive and negative SHAP values indicate increases and decreases, respectively, in predicted cumulative CH_4 emissions relative to the model baseline.

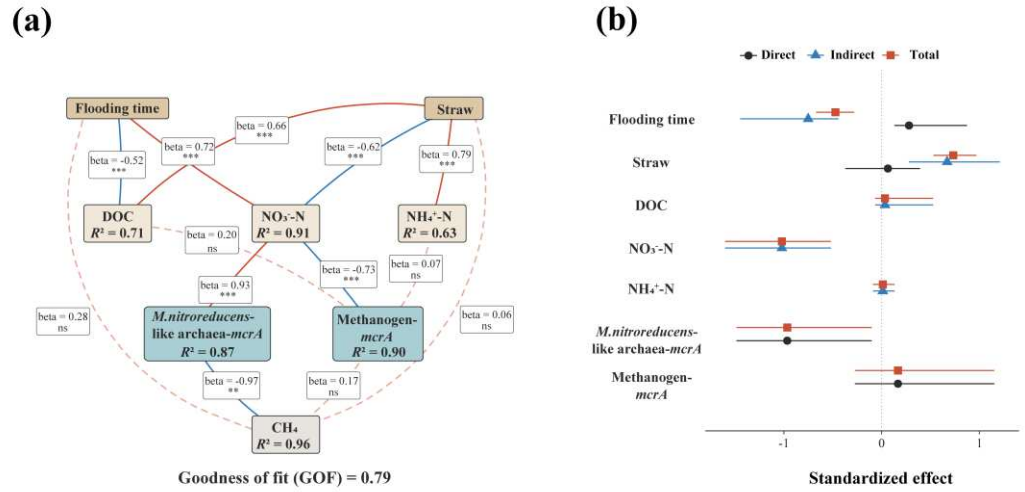


Fig. 6 PLS-PM analysis illustrating the pathways through which water regimes and straw addition affect CH_4 emissions (a), along with the corresponding standardized total, direct, and indirect effects (b). Solid blue and red lines denote significant negative and positive effects, respectively; dashed lines represent non-significant paths. Numbers along the arrows correspond to standardized path coefficients, and R^2 highlights the proportion of explained variance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

Our findings demonstrated that straw amendment significantly increased soil CH_4 emissions (Fig. 1). As the final stage of anaerobic carbon decomposition, methanogenesis strongly depends on the availability of substrate and highly reducing conditions (Conrad, 2007). Straw degradation directly releases labile organic carbon, fueling the growth of methanogens (Shen et al., 2025), as evidenced by the marked increase in the gene abundance of methanogen-*mcrA* in straw-amended soils (Fig. 2d). Notably, the stimulating effect differed markedly between tissue types, with leaf inducing substantially higher CH_4 emissions than stems (Fig. 1). This discrepancy could be attributed to their distinct physicochemical properties (Li et al., 2012). In contrast to stems, which are rich in recalcitrant components such as lignin and cellulose, maize leaves possess a higher proportion of labile organic carbon as well as a more porous physical structure (Xu and Li, 2019). Consequently, leaf incorporation promoted rapid

DOC accumulation (Fig. 2a), thereby providing abundant precursors for methanogenesis (Shen et al., 2025).

Water regime critically governs soil redox states, carbon turnover, and greenhouse gas emissions in paddy ecosystems (Xu et al., 2003; Liu et al., 2019). In this study, delayed flooding markedly reduced CH_4 emissions by 85.6% and 83.3% in stem- and leaf-amended soils, respectively (Fig. 1c), demonstrating its strong mitigation efficacy following straw incorporation. This aligns with Zhou et al. (2025), who observed a 37.7%–76.1% reduction in CH_4 emissions under 5-to-15-day delayed flooding during Chinese milk vetch incorporation. During the initial aerobic phase following straw incorporation, aerobic microbes rapidly decomposed a large portion of labile carbon (De Troyer et al., 2011). This pre-decomposition process depleted soil DOC and NH_4^+-N prior to reflooding (Fig. 2a and b), thereby imposing strict substrate limitations on methanogens (Shen et al., 2025). Furthermore, the diminished gene abundance of methanogen-*mcrA* demonstrated that delayed flooding suppressed methanogens, directly lowering soil's capacity for methane production.

Net CH_4 emissions are determined by the balance between methanogenesis and methane oxidation (Conrad, 2007, Jiang et al., 2022), with the latter occurring via both aerobic and anaerobic pathways (Knief, 2019). Compared with direct flooding, delayed flooding significantly reduced the gene abundance of aerobic methanotroph-*pmoA* (Fig. 2e). Although aerobic conditions typically stimulate the growth of aerobic methanotrophs (Wang et al., 2024), the unexpected decrease observed in this study might be attributed to substrate limitation. During the initial aerobic phase under delayed flooding, rapid carbon decomposition substantially reduced labile substrate availability (Ma et al., 2026), thereby possibly suppressing CH_4 production upon flooding. The growth of methanotrophs was ultimately restricted by the scarcity of CH_4, which serves as their essential growth substrate (Ahmadi and Lackner, 2024).

M. oxyfera-like bacteria play a pivotal role in mediating the AOM process (Ettwig et al., 2010), their abundance exhibited no significant variation under straw amendment or flooding regimes (Fig. 2f), indicating their insensitivity to exogenous carbon input and water management in our study. Conversely, straw amendment decreased the gene

abundance of *M. nitroreducens*-like archaea-*mcrA* (Fig. 2d). However, delayed flooding effectively alleviated this inhibition, markedly boosting their abundance. This response could be attributed to the following reasons: (i) the initial aerobic environment established by delayed flooding could increase soil SO_4^{2-} and Fe^{3+} concentrations, supplying critical electron acceptors for sulfate- and iron-reducing bacteria (Ratering et al., 2024). Upon subsequent flooding, these microorganisms not only outcompete methanogens for electron donors (e.g., acetate and H_2), but also drive iron and sulfate - coupled AOM (Conrad, 2009, 2020), thereby reducing CH_4 emissions. (ii) Given that AOA and AOB drive soil ammonia oxidation and subsequent nitrification (Huang et al., 2021; Shen et al., 2024), the increased gene abundances of AOA-*amoA* and AOB-*amoA* (Fig. 2h and i) confirmed that delayed flooding promoted nitrification, thereby accelerating the conversion of NH_4^+-N to NO_3^--N (Fig. 7), as supported by the higher soil NO_3^--N under delayed flooding (Fig. 2c). Because NO_3^--N is an essential electron acceptor for denitrifying anaerobic methane oxidation (DAMO) (Fujii et al., 2010; Haroon et al., 2013), its accumulation might relieve electron acceptor limitations and increase DAMO rate driven by *M. nitroreducens*-like archaea. PLS-PM analysis further confirmed that delayed flooding reduced CH_4 emissions by increasing the gene abundance of *M. nitroreducens*-like archaea-*mcrA* through NO_3^--N accumulation.

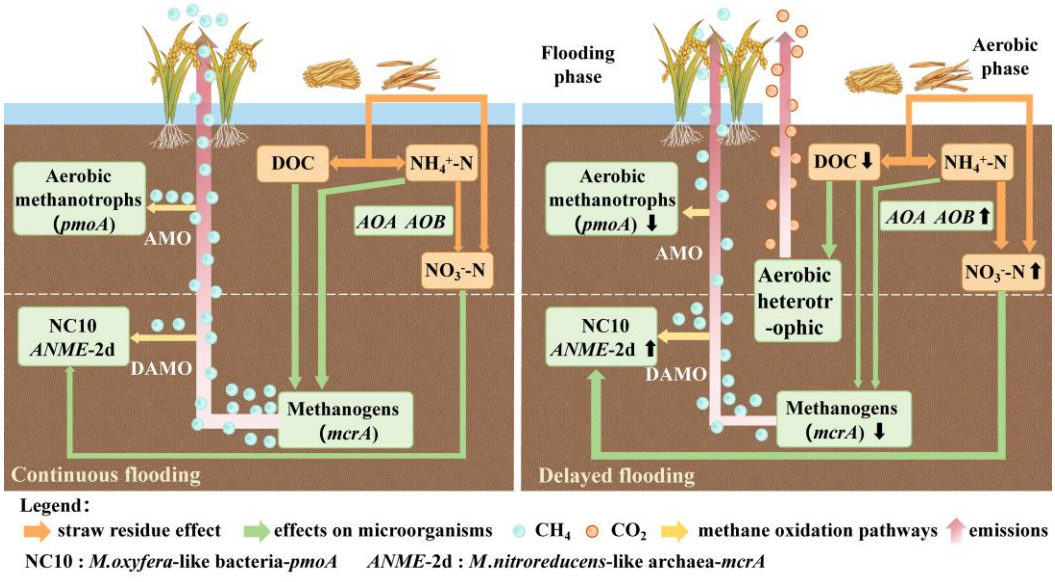


Fig. 7 Schematic diagram illustrating the mechanisms by which delayed flooding decreases CH_4 emissions under straw amendment. Arrow colors signify distinct

processes: orange for straw decomposition products, green and yellow for microbial substrate utilization and the methane oxidation pathway, respectively, and pink for gaseous emissions. Line thickness reflects the relative intensity of each process.

Conclusion

Both maize leaf and stem amendments significantly stimulated CH_4 emissions, with leaves exerting a markedly stronger effect than stems. In straw-unamended soil, delayed flooding had no significant impact on CH_4 emissions; however, it effectively suppressed CH_4 emissions when straw was amended. This mitigation effect was mainly attributed to the enhancement of soil NO_3^--N content, which subsequently increased the gene abundance of *M. nitroreducens*-like archaea-*mcrA*. In contrast, *M. oxyfera*-like bacteria-*pmoA* exhibited no significant response to delayed flooding, whereas the gene abundances of methanogen-*mcrA* and aerobic methanotroph-*pmoA* were markedly reduced. Overall, these findings provide a theoretical basis for optimizing water management to mitigate CH_4 emissions in straw-amended paddy fields.

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