



Effects of Straw Return and Soil Amendments on Methane Emissions During Early Anaerobic Incubation of Paddy Soil

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Received: August 31, 2026

Revised: September 9, 2026

Accepted: September 10,
2026

Online: October 7, 2026

Abstract

Rice paddies are one of the largest global sources of anthropogenic methane (CH₄), and straw return can intensify emissions by supplying decomposable carbon and promoting reducing conditions. However, amendment-specific responses to rice straw-induced CH₄ emissions under anaerobic conditions remain insufficiently understood. A 15-day laboratory incubation experiment was conducted using 6 treatments: control (CK), straw only (S), straw combined with biochar (S+Bio), zeolite (S+Zo), CuSO₄ (S+Cu), and FeCl₃ (S+Fe), with three replicates. Flooded paddy soil was incubated at 25°C, and CH₄ flux, soil pH, electrical conductivity (EC), oxidation-reduction potential (ORP), dissolved H₂, and temperature were measured on days 0, 7, and 15. Results showed that straw return increased CH₄ emissions compared to CK, whereas amendment effects changed markedly over time. On day 7, S+Fe reduced CH₄ flux by 91.9% relative to S, the strongest early-stage suppression, but reversed by day 15 to the highest CH₄ flux among amended treatments. S+Bio and S+Zeo instead showed greater suppression at day 15 than at day 7, while S+Cu achieved the highest average suppression (45.2%) across the 15-day incubation. CH₄ was negatively correlated with ORP ($r=-0.63$, $p<0.001$) and positively correlated with dissolved H₂ ($r=0.69$, $p<0.001$), confirming the central role of soil redox status in CH₄ production. These findings demonstrate that amendment effectiveness during post-harvest straw decomposition is strongly time-dependent, highlighting the need to evaluate mitigation performance across the full decomposition period rather than at a single time point.

Keywords *Methane Emissions, Paddy Soil, Straw Return, Soil Amendments, Greenhouse Gas Mitigation*

INTRODUCTION

Rice paddies are one of the largest anthropogenic sources of atmospheric methane (CH₄), contributing an estimated 6–11% of global anthropogenic CH₄ emissions, equivalent to roughly 25–37 Tg CH₄ yr⁻¹ (Saunois et al., 2020). This contribution is closely linked to the global scale of rice cultivation, with more than 160 million hectares cultivated worldwide, with approximately 75% of the area cultivated under flooded conditions (FAOSTAT, 2025; Nawaz et al., 2022). Recent assessments indicate that net CH₄ emissions from rice paddies have increased substantially due to the combined effects of expanding rice cultivation area and intensification of management practices such as fertilizer use, continuous flooding, and straw incorporation (Ocko et al., 2021; Saunois et al., 2020; Tan et al., 2025; Wen et al., 2025). Prolonged flooding creates anaerobic conditions that favor methanogenesis, making rice paddies a persistent CH₄ source (Nwokolo & Enebe, 2025; Sapkota et al., 2020). Indonesia is particularly relevant in this context, with 11.3 million hectares of harvested paddy area in 2025, highlighting the importance of rice management for both production and CH₄ mitigation.

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Returning rice straw can improve nutrient cycling and soil organic matter status, but its decomposition under flooded conditions also supplies readily available carbon substrates for anaerobic microbial processes, with its effects influenced by straw characteristics, climate, soil conditions, and management practices (Cui et al., 2025; He et al., 2024; Paramitha, 2023; Tan et al., 2025). To offset this trade-off, soil amendments such as biochar, zeolite, and silicate have been proposed as management options to reduce CH₄ emissions associated with straw return by modifying carbon availability, adsorption, nutrient retention, and soil redox processes (Paramitha, 2023; Sofyan et al., 2025; Wen et al., 2025). Nevertheless, their effectiveness varies considerably with amendment properties and soil conditions. This variability may also vary throughout straw decomposition as substrate availability and redox conditions evolve.

Most previous studies have examined individual amendments, optimized application rates, or field systems, where plant and rhizosphere processes may confound amendment effects. Direct comparisons among different amendments under standardized anaerobic conditions are therefore limited, particularly across different stages of straw decomposition. Although CH₄ production and the conditions governing methanogenesis change as decomposition progresses (Conrad, 2020; Sapkota et al., 2020), whether amendment effectiveness changes accordingly remains poorly understood. Therefore, this study aimed to compare the effects of biochar, zeolite, CuSO₄, and FeCl₃ applied with rice straw on CH₄ flux during a 15-day anaerobic incubation and to identify the soil physicochemical indicators associated with CH₄ variation. By evaluating CH₄ flux at two incubation stages, this study examines temporal variation in amendment effectiveness during post-harvest straw decomposition.

LITERATURE REVIEW

Methane emissions in flooded soils result from the interaction between organic carbon availability and anaerobic redox conditions. After flooding, oxygen availability declines rapidly, and microbes progressively consume alternative electron acceptors, shifting metabolism toward increasingly reducing conditions that favor methanogenesis (Conrad, 2007; Sapkota et al., 2020). Irrigation practices, organic matter inputs, soil biogeochemistry, and climate conditions modulate this process (Qian et al., 2023; Sapkota et al., 2020; Yusong et al., 2026). Soil organic carbon is a key substrate for methanogenesis and directly influences CH₄ production and oxidation dynamics (Luo et al., 2022; Xiao et al., 2025).

Rice straw return can intensify this carbon-redox coupling after incorporation into flooded soil. Its decomposition supplies readily available organic substrates and stimulates microbial activity, increasing electron-acceptor demand and promoting reducing conditions that favor methanogenesis (Kan et al., 2023; Tan et al., 2025). Emission intensity depends on straw quality and quantity, soil properties, and environmental conditions (Haque et al., 2021; He et al., 2024). Accordingly, applying straw as an amendment may influence CH₄ emissions by altering carbon accessibility and transformations or by modifying the development of reducing conditions.

The potential effect of amendments depends on their physicochemical characteristics. Previous studies have found that biochar addition reduced CH₄ emission by modifying carbon accessibility, nitrogen availability, pH, and microbial habitat through its porous structure and reactive surface, although its effect on CH₄ emissions varies with feedstock, production conditions, application rate, and soil environment (Liu et al., 2019; Pertiwi & Abdila, 2026; Wen

et al., 2025). Zeolite amendments have been shown to reduce CH₄ emissions by improving soil structure, increasing cation exchange capacity, and enhancing methane oxidation, while silicate has been associated with changes in soil structure and redox processes (Chen et al., 2025; Kan et al., 2023). Metal-containing amendments may affect anaerobic processes through different pathways. Iron (Fe) can function as an alternative electron acceptor during organic matter decomposition, potentially competing with methanogens for electron donors and suppressing CH₄ production (Galgo et al., 2024; Qiu et al., 2026). In contrast, copper (Cu) is a redox-active trace element that may enhance expression of methane-oxidation genes, although its effectiveness depends on concentration and soil conditions (Fan et al., 2025; Hu et al., 2026).

Taken together, these differences among amendments suggest that their effectiveness may change during straw decomposition. Therefore, compare amendment performance under the same experimental conditions rather than infer it from studies using different soils, straw characteristics, or application practices. The post-harvest period is particularly relevant because incorporated straw decomposes under flooded conditions, increasing carbon availability and promoting reducing conditions that favor CH₄ production. A controlled anaerobic incubation can stimulate these conditions while minimizing the influence of plants and field-management factors, providing a consistent system to evaluate whether amendment effectiveness changes across stages of straw decomposition.

RESEARCH METHOD

Sandy loam soil and rice straw samples were collected from paddy fields in Sleman Regency, Special Region of Yogyakarta. A laboratory incubation experiment was conducted from May to July 2026 at room temperature, using a completely randomized design with six treatments and three replications: control without straw (CK), straw only (S), straw combined with biochar (S+Bio), zeolite (S+Zeo), CuSO₄ (S+Cu), and FeCl₃ (S+Fe). Each incubation unit contained 100 g of air-dried soil and 3 g of straw. Biochar and zeolite were added at 3.8% and 0.77% (w/w), respectively, while CuSO₄ · 5H₂O and FeCl₃ were added at 5 mg Cu kg⁻¹ and 0.6 g FeCl₃ kg⁻¹ soil, and all treatments received urea at 200 kg N ha⁻¹. CH₄ fluxes were determined at days 0, 7, and 15 using the closed-chamber method coupled with gas chromatography. After gas sampling, we measured soil oxidation-reduction potential (ORP), dissolved hydrogen (H₂), pH, and electrical conductivity (EC). Treatment effects were assessed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test ($p < 0.05$). Pearson correlation analysis was performed to evaluate relationships between CH₄ fluxes and redox-related soil properties.

FINDINGS AND DISCUSSION

CH₄ Flux Response to Straw Return and Amendments

Methane flux exhibited clear treatment-dependent responses during the 15-day anaerobic incubation (Figure 1). On day 7, all straw-amended treatments showed higher CH₄ fluxes than CK, with the straw-only treatment (S) showing the highest emissions. The elevated CH₄ following straw incorporation is consistent with the increased availability of readily decomposable carbon substrates that support methanogenesis under flooded conditions (Paramitha, 2023; Tan et al., 2025). However, amendment addition substantially altered this response.

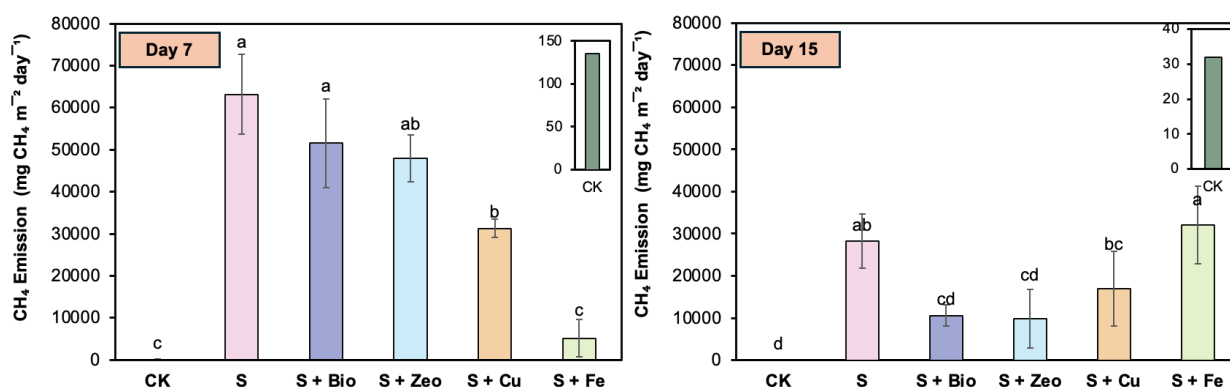


Figure 1. Effects of straw return and amendment application on CH₄ emissions during the early stages of flooded soil incubation

Relative to S, CH₄ flux was reduced by 91.9% in S+Fe, 50.5% in S+Cu, 24.1% in S+Zeo, and 18.4% in S+Bio. Thus, FeCl₃ produced the strongest early-stage suppression, whereas biochar addition showed the smallest reduction among the tested amendments. The limited suppression under S+Bio contrasts with the CH₄ mitigation reported for biochar in other flood systems (Liu et al., 2019; Pertiwi & Abdila, 2026; Wen et al., 2025). Previous studies have likewise shown that biochar effects on CH₄ emission can vary substantially with pyrolysis temperature and the resulting composition of labile and reactive carbon fractions (Wen et al., 2025). These differences suggest that amendment-specific properties may influence the short-term response in CH₄ production.

By day 15, the relative effects of the amendment had changed markedly, underscoring the reason a single measurement day would have given an incomplete picture. The suppressive effects of S+Bio and S+Zeo became more pronounced, with CH₄ fluxes 62.5% and 65% lower than S, respectively. S+Cu also maintained lower CH₄ flux than S, although its relative reduction was smaller than at day 7. In contrast, S+Fe showed a complete reversal, shifting from the strongest suppression to a 13.6% higher CH₄ flux than S, accompanied by a 5.24-fold increase from its day-7 value. This temporal reversal suggests that iron's initial suppressive effect did not persist during anaerobic decomposition. One plausible explanation is that Fe(III) reduction initially competed with methanogenesis for available electron donors, and this competing effect diminished as reducible Fe was progressively consumed (Liu et al., 2019; Wen et al., 2025). Despite these temporal differences, S+Cu showed the highest average CH₄ suppression over the 15-day incubation, indicating the strongest overall short-term mitigation among the tested amendments.

Soil Environmental Responses and Their Association with CH₄ Flux

Temporal changes in CH₄ flux were accompanied by progressive changes in the soil chemical and redox environment (Table 1). On day 7, soil pH was generally lower in straw-amended treatments than CK, with S+Fe showing the lowest values, whereas differences in EC and ORP among treatments were negligible ($p > 0.05$). Thus, despite the lower CH₄ fluxes observed in the amended treatments relative to S, differences in the bulk redox environment among amendments were not yet evident on day 7 ($p > 0.05$).

Table 1. Changes in key soil physicochemical indicators during incubation

Treatment	Parameter				
	pH	EC (dS m ⁻¹)	ORP (mV)	H ₂ (ppm)	Temperature (°C)
Day 7					
CK	6.83±0.07 ^a	0.34±0.11 ^a	-219.67±10.97 ^a	0.41±0.03 ^a	26.4±0.00 ^a
S	6.27±0.25 ^b	0.53±0.14 ^a	-213.00±8.00 ^a	0.41±0.05 ^a	25.7±0.61 ^{ab}
S+Bio	6.21±0.14 ^b	0.47±0.12 ^a	-202.67±24.79 ^a	0.36±0.06 ^a	25.83±0.29 ^{ab}
S+Zeo	6.05±0.08 ^b	0.44±0.11 ^a	-185.67±23.12 ^a	0.35±0.04 ^a	25.47±0.45 ^b
S+Cu	6.08±0.21 ^b	0.56±0.04 ^a	-217.00±20.07 ^a	0.41±0.04 ^a	25.53±0.23 ^b
S+Fe	5.21±0.17 ^c	0.28±0.08 ^a	-185.67±28.43 ^a	0.35±0.04 ^a	25.27±0.23 ^b
Day 15					
CK	6.37±0.07 ^a	0.31±0.02 ^a	-144.00±33.29 ^a	0.16±0.1 ^c	28.87±0.14 ^a
S	6.24±0.07 ^{ab}	0.84±0.35 ^b	-225.33±11.24 ^b	0.42±0.02 ^{ab}	28.13±0.08 ^b
S+Bio	6.22±0.05 ^{ab}	0.82±0.04 ^b	-199.33±23.54 ^b	0.36±0.07 ^a	27.95±0.09 ^b
S+Zeo	6.06±0.03 ^b	1.00±0.12 ^b	-212.33±18.50 ^b	0.38±0.06 ^{ab}	27.77±0.14 ^b
S+Cu	6.30±0.07 ^a	0.8±0.09 ^b	-225.67±9.61 ^b	0.42±0.03 ^{ab}	27.87±0.16 ^b
S+Fe	5.28±0.14 ^c	4.12±0.2 ^c	-172.33±12.01 ^{ab}	0.24±0.04 ^b	26.95±0.39 ^c

Values followed by the same letter within an incubation day are not significantly different.

Values are presented as mean ± standard deviation (SD).

By day 15, treatment differences in soil conditions became more pronounced. Soil pH remained lower in the straw-amended treatments than CK, with S+Fe maintaining the lowest value. Soil EC increased in all straw-amended treatments relative to CK, with a particularly marked increase under S+Fe. ORP also became more negative in most straw-amended treatments, with the most reducing conditions observed in S+Cu and S, compared with CK. In contrast, S+Fe showed a distinct soil response characterized by a less negative ORP, lower H₂, markedly higher EC, and the lowest pH. The marked increase in EC under S+Fe may reflect changes in soluble ion concentration during FeCl₃ transformation and organic matter decomposition. Although soil EC did not correlate with CH₄ flux, it was therefore unlikely to be a major predictor of CH₄ variation in this experiment. Temporal variation in CH₄ responses among amendments, especially the reversal under S+Fe, corresponded with gradual changes in these soil parameters between days 7 and 15.

The relationship between soil conditions and CH₄ production was further evaluated using Pearson correlation analysis (Figure 2). CH₄ flux was significantly associated with ORP ($r = -0.63$, $p < 0.001$), dissolved H₂ ($r = 0.69$, $p < 0.001$), and temperature ($r = -0.39$, $p < 0.001$), whereas pH and EC showed no significant correlation ($p > 0.05$). The negative relationship between ORP and CH₄ suggests that more reducing conditions were generally associated with higher CH₄ flux, consistent with the progressive transition toward methanogenesis following the depletion of alternative electron acceptors (Sapkota et al., 2020). The positive correlation between CH₄ and H₂ also reflects greater H₂ availability as an electron donor for hydrogenotrophic methanogenesis. However, the strong inverse relationship between ORP and H₂ ($r = -0.98$, $p < 0.001$) indicates that these two variables represent closely coupled aspects of the same redox transition rather than independent predictors. This collinearity should

therefore be considered when interpreting their individual correlation with CH₄ flux.

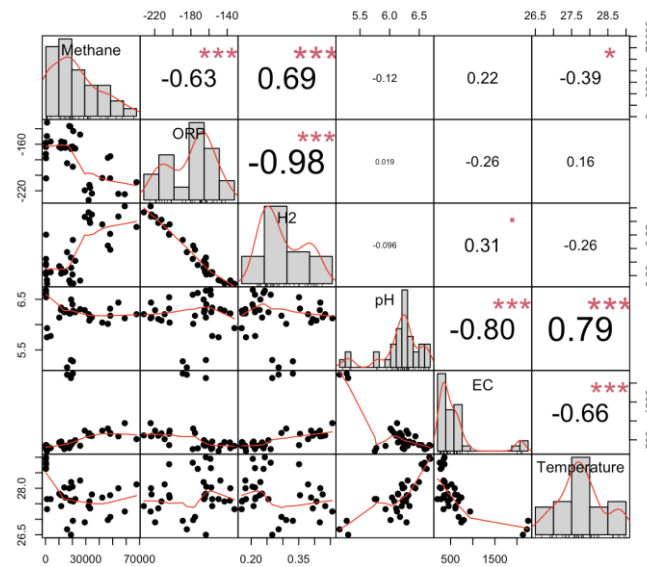


Figure 2. Relationships between CH₄ emissions and soil environmental drivers of methanogenesis during flooded soil incubation

The contrasting response of S+Fe and S+Cu provided further insight into how amendment-specific processes may have interacted with the evolving redox conditions during straw decomposition. S+Fe strongly suppressed CH₄ flux at day 7 but showed higher CH₄ flux at day 15, suggesting that Fe's suppressive effect was transient. One possible explanation is progressive Fe(III) reduction during anaerobic decomposition. As Fe(III) becomes progressively reduced, its competition with methanogenesis for electron donors may diminish (Hu et al., 2024), allowing CH₄ flux to increase despite the absence of the most strongly reducing ORP. However, this study did not directly measure Fe redox transformation, so this explanation remains inferential.

S+Cu showed a contrasting response. Despite exhibiting the most negative ORP and relatively high H₂ concentration at day 15, S+Cu maintained lower CH₄ flux than other amended treatments and achieved the highest average suppression over the incubation. This suggests that establishing strongly reducing conditions did not necessarily translate into greater CH₄ production in the presence of CuSO₄. Previous anaerobic-digestion studies have shown that Cu can affect CH₄ flux in a concentration- and exposure-dependent manner, potentially by interfering with microbial and methanogenic activity (Fang et al., 2024; Sun et al., 2024). Such metal-mediated microbial constraints provide a plausible explanation for the persistent suppression observed under S+Cu, although the present experiment did not directly measure microbial activity or methanogenic abundance. These results suggest that amendment performance during post-harvest straw decomposition was influenced by the interaction between evolving redox conditions and amendment-specific processes, rather than by redox status alone. The contrasting temporal trajectories of FeCl₃ and CuSO₄ further suggest that a single-endpoint measurement may not adequately capture amendment performance during straw-return practice.

CONCLUSIONS

This study demonstrated that straw return under flooded conditions increased CH₄ emissions, highlighting the importance of post-harvest straw management for short-term CH₄

mitigation. Amendment addition substantially modified this response, but its effectiveness varied with amendment type and incubation stage. Among the tested amendments, CuSO_4 reduced CH_4 by 45.2% over the 15-day incubation and provided the most consistent short-term mitigation, whereas FeCl_3 showed strong initial suppression of 91.9% at day 7 but showed weaker suppression by day 15. The significant relationships between CH_4 flux and ORP and dissolved H_2 indicate that changes in the anaerobic redox environment were associated with CH_4 flux, although amendment-specific responses suggest that redox status alone did not determine mitigation performance.

LIMITATIONS & FURTHER RESEARCH

This study was limited by its short-term laboratory incubation and the use of a single paddy soil, which may limit direct extrapolation to field conditions. In addition, Fe redox transformation, carbon substrate transformation, and methanogenic activity were not directly measured. Therefore, the proposed mechanisms explaining the contrasting responses to FeCl_3 and CuSO_4 remain inferential. These findings suggest that amendment selection for post-harvest straw management should consider not only the initial CH_4 suppression level but also its persistence during anaerobic decomposition. Further research should directly evaluate Fe redox transformation, carbon substrate dynamics, H_2 availability, and methanogenic activity over longer incubation periods to clarify the mechanisms governing sustained CH_4 mitigation and provide a basis for subsequent field validation.

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