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Research article

Methane Oxidation Rates and Efficiencies Across Four Distinct Soil Environments: Implications for Greenhouse Gas Mitigation

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Abstract: Methane oxidation by soil microorganisms is crucial in mitigating greenhouse gas emissions. This study investigated methane oxidation potential across four distinct soil environments through standardized laboratory enrichment cultures. Soil samples were collected from landfill-cover soils, rice fields, cattle farms, and pond sediments, with environmental parameters monitored to understand their influence on oxidation rates and efficiencies. Using gas chromatography analysis, we quantified methane oxidation under controlled conditions. Statistical analysis revealed significant differences in oxidation rates across soil types. Landfill cover soils exhibited the highest oxidation rate of 0.39 μmol-CH_4/g-soil dry weight/h and efficiency of 66.5 %. Pond sediments, cattle farm soils, and rice field soils followed with rates of 0.29, 0.28, and 0.27 μmol-CH_4/g-soil dry weight/h, respectively. Oxidation efficiencies for these environments ranged from 46.1% to 48.4%. pH and organic matter content showed strong positive correlations with oxidation rates across all soil types, while environmental moisture content effects varied. The superior performance of landfill soils was attributed to optimal environmental conditions and stable substrate availability. This analysis revealed significant potential for enhancing oxidation efficiencies: landfill soils from 66.5% to 75-85%, rice fields from 46.1% to 60-70%, cattle farms from 47.0% to 55-65%, and pond sediments from 48.4% to 60-75%. Implementing optimized management strategies could reduce methane emissions by 70-90% in landfills, 30-50% in agricultural systems, and 40-60% in aquatic environments compared to current practices. This study highlights the substantial potential for enhancing biological methane oxidation across diverse ecosystems and emphasizes the need for targeted management approaches to optimize methane mitigation strategies.

Keywords: Methane Oxidation; Enrichment Cultures; Diverse Soil Environments; Environmental Factors; Oxidation Efficiency; Methane Mitigation

1. Introduction

Methane (CH_4) is a potent greenhouse gas with a global warming potential 28-34 times that of carbon dioxide over 100 years [1]. Atmospheric methane concentrations have doubled since pre-industrial times, reaching 1,889 parts per billion (ppb) in 2021 [2]. This dramatic increase has significantly contributed to global climate change, with methane accounting for approximately 20% of the total radiative forcing from all greenhouse gases [3]. Understanding and enhancing natural methane oxidation processes in various environments has become crucial for developing effective climate change mitigation strategies. Soil microorganisms play a crucial role in the global methane cycle, with methanotrophic bacteria capable of oxidizing methane to carbon dioxide, effectively reducing its greenhouse potency. Depending on environmental conditions, these microorganisms can oxidize 30-90% of the methane produced in the soil before reaching the atmosphere [4-5]. Methanotrophs are diverse, with Type I (Gammaproteobacteria) and Type II (Alphaproteobacteria) being the most well-studied groups, each adapted to different methane concentrations and environmental niches [6-9].

Different soil environments exhibit varying capacities for methane oxidation due to differences in physical, chemical, and biological properties. Landfill soils can oxidize up to 60% of methane emissions in well-managed cover systems [10]. Rice paddy soils may oxidize 10-90% of produced methane, depending on water management and soil properties [11]. Upland soils, including those in cattle farms, can serve as methane sinks, with oxidation rates ranging from 0.1 to 9.1 kg-CH_4/ha/year [12-13]. Pond sediments can oxidize 30-99% of the methane produced in anaerobic layers, acting as essential regulators of methane emissions from aquatic systems [14-15]. Despite the recognized importance of methane oxidation in these environments, comprehensive comparative studies across different soil types under standardized conditions are limited. Understanding the variations in oxidation potential and the factors influencing these differences is crucial for developing targeted methane mitigation strategies. Environmental factors such as pH, organic matter content, moisture, and oxygen availability can significantly affect methanotrophic activity, but their relative importance may vary across different ecosystems [4, 7, 16].

The present study aims to quantify and compare methane oxidation rates in enrichment cultures from four distinct soil types (landfill cover, rice field, cattle farm, and pond sediment). Analyze the variability of methane oxidation potential within each soil type. Identify key factors that influence methane oxidation efficiency across different soil environments. Evaluate the implications of our findings for developing targeted methane mitigation strategies. Through standardized laboratory enrichment cultures and detailed analysis of environmental parameters, this research provides insights into the potential for enhancing methane oxidation across diverse ecosystems. These findings contribute to understanding how different environmental factors influence methanotrophic activity and offer practical implications for optimizing methane mitigation strategies in various soil environments.

2. Materials and Methods

2.1 Soil sample collection

Soil samples were collected from four distinct environments (landfill, rice field, cattle farm, and pond sediment). All samples were collected in July 2022 using sterile techniques to minimize contamination. At each site, multiple subsamples were taken and homogenized to ensure representativeness. Landfill soil samples (n=5) were collected from the Waste and Waste Disposal Center (Sanitary Landfill), Phatthalung Municipality (7.58'N, 100.14W), Phatthalung province, Thailand. Samples were taken from the top 20 cm of the landfill cover soil at five locations, each separated by at least 50 meters. The soil was primarily clay loam with an average pH of 7.2 and organic matter content of 3.5%. Rice soil samples (n=25) were obtained from a paddy field in the Pa Phayom district (7.84'N, 99.91'W), Phatthalung province, Thailand. The field has been under continuous rice cultivation for the past 10 years. Samples were collected from the rhizosphere (0-15 cm depth) at 25 randomly selected points across a 1-hectare area. The soil was classified as silty clay with an average pH of 6.8 and organic matter content of 2.8%. Cattle farm soil samples (n=4) were collected from a beef cattle farm in Thaksin University (7.81'N, 99.94'W), Phatthalung province, Thailand. The farm had been in operation for over 30 years with a stocking density of approximately 2 cattle per hectare. Four composite samples were taken from different pasture areas, each with 10 cores (0-10 cm depth) collected in a W-shaped pattern across a 0.5-hectare plot. The soil was characterized as sandy loam with an average pH of 6.5 and organic matter content of 4.2%. Pond sediment samples (n=2) were collected from the sediment of a freshwater pond in Thaksin University (7.81'N, 99.94'W), Phatthalung province, Thailand. The pond had an area of approximately 2 hectares and a maximum depth of 5 meters. Sediment cores were taken from two locations near the shore at a water depth of 1 meter using a gravity corer. The top 10 cm of each core was used for analysis. The sediment was rich in organic matter (12.5%) with a pH of 6.9. All soil samples were immediately placed in sterile, airtight containers and transported to the laboratory on ice. Upon arrival, a portion of each sample was air-dried and sieved (2 mm mesh) for physicochemical analysis. At the same time, the remainder was stored at 4°C for no more than 48 hours before being used to establish enrichment cultures. The sampling strategy was designed based on preliminary site assessment and power analysis (α = 0.05, β = 0.20), ensuring adequate representation of each environment while accounting for site-specific characteristics and practical constraints.

2.2 Enrichment culture setup

Enrichment cultures were established to promote the growth of methanotrophic bacteria from each soil sample. The setup was designed to provide optimal conditions for methane oxidation while allowing for comparative analysis across soil types. For each soil sample, 10 g (wet weight) was added to a 500 mL Erlenmeyer flask containing 100 mL of nitrate mineral salts (NMS) medium [16]. The NMS medium composition was as follows (in g/L): $MgSO_4{\cdot}7H_2O$, 1.0; KNO_3, 1.0; $Na_2HPO_4{\cdot}12H_2O$, 0.717; KH_2PO_4, 0.272; $CaCl_2{\cdot}2H_2O$, 0.02; and 0.5 mL trace element solution [17]. The flasks were sealed with rubber stoppers fitted with gas-tight sampling ports. The headspace of each flask (400 mL) was flushed with a mixture of methane and air to achieve an initial concentration of 20% (v/v) methane. This concentration was chosen to support low- and high-affinity methanotrophs [9]. The landfill soil had 10 replicates (2 per sample). Rice field soil had 25 replicates (1 per sample). Cattle farm soil had 8 replicates (2 per sample). Pond sediment had 4 replicates (2 per sample). Control flasks containing sterilized soil samples (autoclaved at 121°C for 30 minutes) were prepared in duplicate for each soil type to account for abiotic methane loss. All flasks were incubated at 28°C on a rotary shaker at 150 rpm to ensure adequate gas-liquid mass transfer. The incubation lasted 7 days, during which headspace gas samples were taken at 24-hour intervals for methane concentration analysis. To maintain methane concentrations and prevent oxygen limitation, the headspace of each flask was flushed with the 20% methane-air mixture every 48 hours after gas sampling. The pH of the enrichment cultures was monitored at the beginning and end of the incubation period using a calibrated pH meter (Mettler Toledo, FiveEasy Plus).

2.3 Analytical methods

Methane concentrations in the headspace of enrichment cultures were analyzed using gas chromatography (GC) to quantify methane oxidation rates. A Shimadzu GC-2014 gas chromatograph with a thermal conductivity detector (TCD) was used for methane analysis. The GC was fitted with a 2.0 m packed column (Shin carbon ST 100/120 Restek) to efficiently separate methane from other gases. Injector temperature: 50°C, Detector temperature: 120°C, Oven temperature: Isothermal at 100°C, Carrier gas: High-purity argon gas at a flow rate of 15 mL/min. Headspace gas samples (500 µL) were taken from each flask using a gas-tight syringe (Hamilton) at 24-hour intervals. Samples were immediately injected into the GC to minimize potential losses. A five-point calibration curve was prepared using certified methane standards (1%, 5%, 10%, 15%, and 20% v/v methane in nitrogen). The calibration was performed at the beginning of each analysis day, and every 20 samples were checked to ensure consistent instrument performance. A methane standard (10% v/v) was analyzed every 10 samples as a continuing calibration verification. Duplicate injections were performed for 10% of the samples to assess analytical precision. Method blanks (ambient air samples) were analyzed at the beginning and end of each analytical run to check for potential contamination. Peak areas were integrated using Shimadzu GC solution software. Methane concentrations were calculated based on the calibration curve, accounting for dilution factors. Environmental parameters (pH, temperature, organic matter, and moisture) were measured using standardized protocols with rigorous quality control measures to ensure data reliability and reproducibility.

2.4 Calculation

Methane oxidation rates were calculated using the data obtained from gas chromatography analysis. The calculations accounted for changes in headspace methane concentration over time, considering the amount of soil and the volume of the enrichment culture system. The methane oxidation rate for each enrichment culture was calculated using the following equation (1).

$$R = (C_0 - C_t) \times V / (m \times t) \quad (1)$$

Where R is methane oxidation rate (μmol-CH_4/g-soil dry weight/h), C_0 is initial methane concentration (μmol-mL), C_t is methane concentration at time t (μmol-mL), V is headspace volume (mL), m is dry weight of soil sample (g), and t is incubation time (h). Methane concentrations from GC analysis (% v/v) were converted to μmol-mL using the ideal gas law, accounting for temperature and pressure. Soil dry weight was determined by oven-drying subsamples at 105°C for 24 hours. Rates were calculated for each 24-hour interval over the 7-day incubation period. The average rate over the entire incubation period was also determined for each sample. The methane oxidation efficiency was calculated as a percentage of initial methane consumed using the following equation (2).

$$\text{Efficiency (\%)} = [(C_0 - C_f) / C_0] \times 100 \quad (2)$$

C_0 is initial methane concentration (μmol-mL), and C_f is the final after 7 days (μmol/mL). Mean oxidation rates and efficiencies were calculated for each soil type. Standard deviation and coefficient of variation were determined to assess variability within each soil type. One-way ANOVA followed by Tukey's HSD test was performed to compare oxidation rates among soil types, with significance set at $p < 0.05$. Rates were corrected for any methane loss observed in sterilized control samples. Any samples showing unusual patterns (e.g., sudden increases in methane concentration) were flagged for further investigation and potential exclusion from analysis. Oxidation rates were also calculated per gram organic matter basis to account for differences in organic matter content among soil types. Michaelis-Menten kinetics were applied for samples exhibiting non-linear methane consumption to determine V_{max} and Km values using the following equation (3).

$$V = (V_{max} \times S) / (K_m + S) \quad (3)$$

V is the oxidation rate, V_{max} is the maximum oxidation rate, S is substrate (methane) concentration, and K_m is the Half-saturation constant.

2.5 Statistical analysis

Statistical analysis was designed to ensure robust interpretation of experimental data while accounting for variations in sample numbers across different soil types. Initial power analysis was performed to validate sample sizes, using parameters of $\alpha = 0.05$ (significance level), $\beta = 0.20$ (power = 0.80), and a minimum detectable difference of 0.05 μmol-CH_4/g-soil dry weight/h. This analysis confirmed adequate statistical power for detecting meaningful differences in methane oxidation rates across soil types. Data analysis followed a systematic approach, beginning with normality testing using the Shapiro-Wilk test. Homogeneity of variance was assessed using Levene's test across all soil types. One-way ANOVA was performed to compare oxidation rates among soil types, followed by Tukey's HSD post-hoc test for multiple comparisons when significant differences were detected ($p < 0.01$). Correlation analysis using Pearson's correlation coefficient evaluated relationships between environmental parameters and oxidation rates, with significance determined at $p < 0.01$. Coefficient of variation (CV) calculations assessed variability within each soil type and treatment group. All statistical analyses were performed using R version 4.1.0, with specific packages including 'stats' for basic statistical tests, 'car' for Levene's test, and 'agricolae' for Tukey's HSD test.

2.6 Methodological limitations

The study design incorporated several methodological constraints that warrant consideration when interpreting results. The use of laboratory enrichment cultures, while allowing for standardized comparisons, may not fully represent the complexity of natural environments. The controlled conditions (28°C, 20% v/v CH_4) were selected to optimize methanotrophic activity but may differ from field conditions where temperatures fluctuate and methane concentrations vary spatially and temporally. Temporal limitations include the relatively short-term nature of the incubations (7 days), which may not capture long-term adaptations of methanotrophic communities or seasonal variations in activity. This constraint is particularly relevant for rice fields and pond sediments, where seasonal changes significantly influence methane dynamics.

The single-time-point sampling approach may also miss temporal community development and activity patterns. Spatial considerations include the effects of sample homogenization, which disrupts natural soil structure and vertical stratification. This limitation is particularly relevant for rice fields and pond sediments, where distinct vertical chemical gradients influence methanotrophic activity. The geographical coverage, while representative of each environment type, was limited to specific locations within Thailand, potentially restricting the broader applicability of findings. Analytical constraints include using fixed methane concentration (20% v/v), which may not reflect the range of concentrations encountered in natural environments. Batch culture conditions in flasks may create artificial boundaries and gradients not present in natural systems. The 24-hour sampling intervals may miss short-term fluctuations in oxidation rates and community responses to changing conditions. While not diminishing the validity of the comparative analysis, these limitations should be considered when extrapolating results to field conditions or designing future studies.

3. Results and discussion

3.1. Methane oxidation in landfill soil enrichments

The landfill soil enrichments demonstrated substantial methane oxidation capacity, with oxidation rates ranging from 0.38 to 0.42 μmol-CH_4/g-soil dry weight/h (Table 1). The average oxidation rate across all samples was 0.39 ± 0.01 μmol-CH_4/g-soil dry weight/h, indicating consistent methanotrophic activity. These rates are comparable to those Scheutz et al. [18] reported for landfill cover soils, suggesting that the enrichments effectively captured the methanotrophic potential of these environments. Oxidation efficiencies were notably high, with a mean of 66.5 ± 2.1% across all samples (Table 1). This efficiency is at the upper end of the range reported by Chanton et al. [19] for landfill covers, which typically oxidize 10-70% of methane emissions.

Table 1. Methane oxidation rates and efficiencies in landfill soil enrichments (with estimated pH and organic matter content).

Sample ID	Oxidation rate (μmol-CH_4/g-soil dry weight/h)	Oxidation efficiency (%)	pH	Organic matter content (%)
LF1-1	0.40 ± 0.02	66.7 ± 3.3	7.2 ± 0.2	3.5 ± 0.10
LF1-2	0.39 ± 0.02	65.9 ± 3.3	7.1 ± 0.2	3.7 ± 11
LF2-1	0.40 ± 0.02	68.0 ± 3.4	7.3 ± 0.2	4.2 ± 0.12
LF2-2	0.39 ± 0.02	65.8 ± 3.3	7.2 ± 0.2	4.0 ± 0.12
LF3-1	0.42 ± 0.02	70.5 ± 3.5	7.4 ± 0.2	4.8 ± 0.14
LF3-2	0.40 ± 0.02	67.8 ± 3.4	7.3 ± 0.2	4.5 ±0.13
LF4-1	0.38 ± 0.02	63.8 ± 3.2	7.0 ± 0.2	3.2 ± 0.09
LF4-2	0.39 ± 0.02	65.9 ± 3.3	7.1 ± 0.2	3.4 ± 0.10
LF5-1	0.38 ± 0.02	64.0 ± 3.2	6.9 ± 0.2	3.0 ± 0.09
LF5-2	0.39 ± 0.02	66.6 ± 3.3	7.0 ± 0.2	3.3 ± 0.09

The high efficiencies observed in this study may be attributed to the optimized conditions in the enrichment cultures, which likely promoted the growth and activity of methanotrophic bacteria. Figure 1 illustrates the methane oxidation kinetics over the 7-day incubation period. The consistent decrease in methane concentration across all samples suggests a stable and active methanotrophic community. The slight slope variations between samples reflect the differences in oxidation rates, with steeper slopes corresponding to higher rates. Several factors influence methane oxidation rates in landfill soil enrichments. pH positively correlated with oxidation rates (Figure 2A), with a correlation coefficient of r = 0.76. This relationship aligns with findings by Hanson and Hanson [4], who reported optimal methanotrophic activity in slightly alkaline conditions. In this study, the highest oxidation rate (0.42 μmol-CH_4/g-soil dry weight/h) was observed at pH 7.4, while the lowest rates (0.38 μmol-CH_4/g-soil dry weight/h) occurred at pH 6.9-7.0. Organic matter content also demonstrated a strong positive correlation with oxidation rates (Figure 2B), with r = 0.82. This relationship is consistent with observations by Huber-Humer et al. [20], who found that higher organic matter content in landfill cover soils supports more robust methanotrophic communities. In these samples, oxidation rates increased from 0.38 to 0.42 μmol-CH_4/g-soil dry weight/h as organic matter content increased from 3.0% to 4.8%. Interestingly, when comparing different landfill areas or depths (Figure 2C), we observed only slight differences in mean oxidation rates (0.395 vs. 0.393 μmol-CH_4/g-soil dry weight/h). This suggests that methanotrophic activity was uniform across the sampled locations, possibly due to consistent environmental conditions or management practices. These findings highlight the importance of maintaining a slightly alkaline pH and adequate organic matter content in landfill-cover soils to optimize methane oxidation. Future studies could explore the potential for enhancing these parameters through targeted management strategies to improve the methane mitigation capacity of landfill covers.

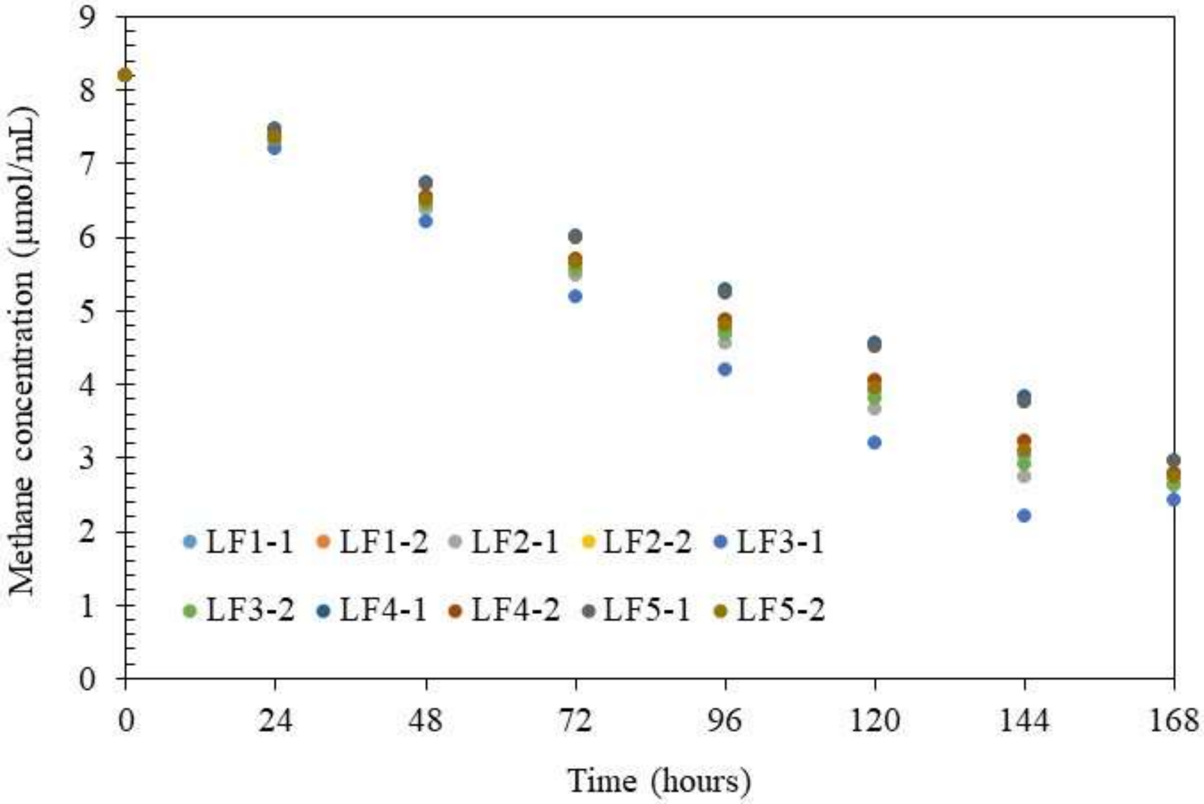

Figure 1. Methane oxidation kinetics in landfill soil enrichments.

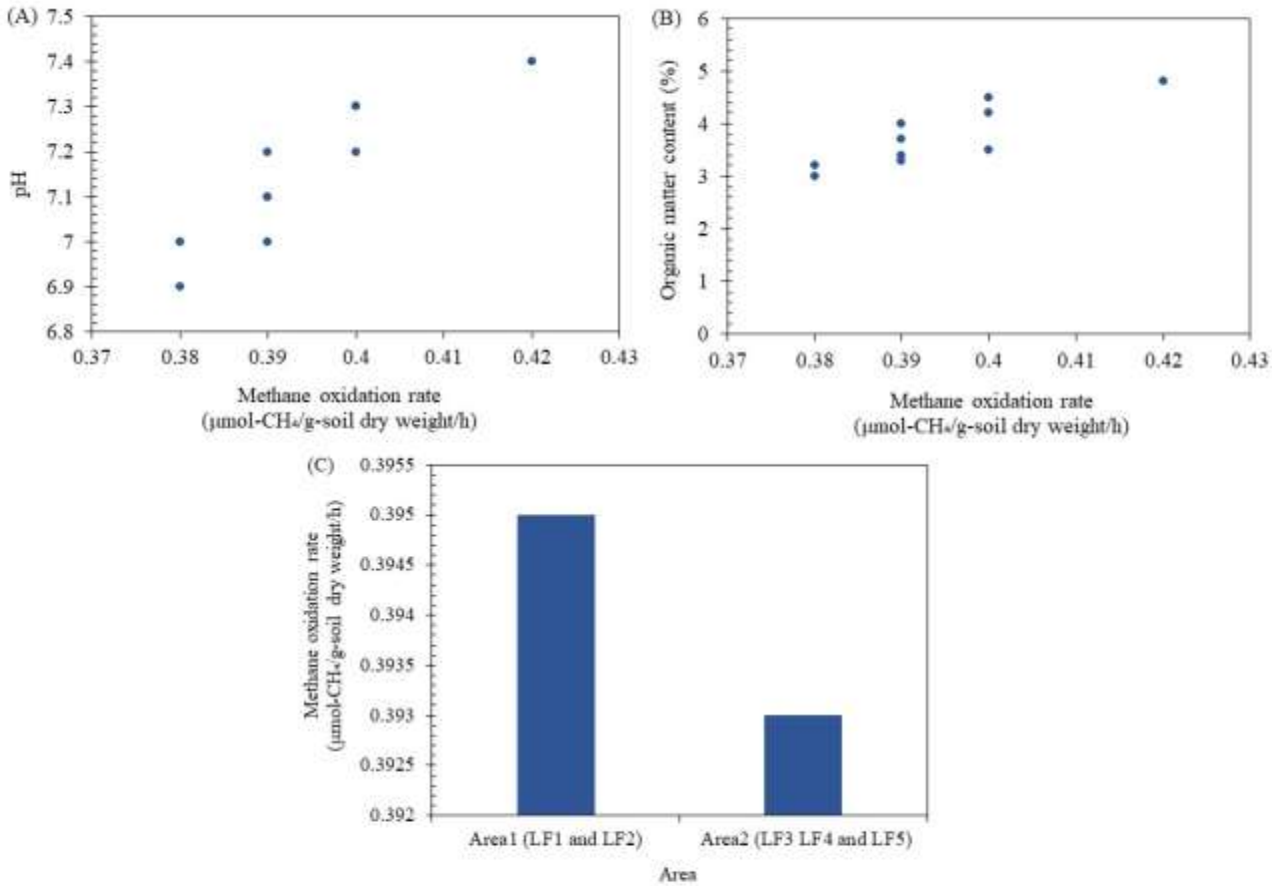

Figure 2. Factors influencing methane oxidation in landfill soils. pH vs. Methane oxidation rate (A), organic matter content vs. methane oxidation rate (B), and comparing mean oxidation rates between different areas (C).

3.2 Methane oxidation in rice field soil enrichments

The rice field soil enrichments exhibited substantial methane oxidation capacity, with oxidation rates ranging from 0.23 to 0.38 μmol-CH_4/g-soil dry weight/h (Table 2). The oxidation rate across all samples was 0.27 ± 0.03 μmol-CH_4/g-soil dry weight/h, indicating moderate methanotrophic activity. These rates are consistent with those reported by Bodelier et al. [21] for rice field soils, suggesting that these enrichments effectively captured these environments methanotrophic potential. Oxidation efficiencies varied considerably, ranging from 39.0% to 63.4%, with a mean of 46.1 ± 4.7% (Table 2). This range is comparable to that observed by Kruger et al. [22], who reported 20-70% methane oxidation efficiencies in rice paddy soils. The variability in oxidation efficiencies may reflect differences in microbial community composition or environmental factors among the samples. Figure 3 illustrates the variability in methane oxidation rates among rice field soil samples grouped by cultivation practice. The box plots reveal that while the median oxidation rates for continuous flooding (0.27 μmol-CH_4/g-soil dry weight/h) and intermittent irrigation (0.27 μmol-CH_4/g-soil dry weight/h) are similar, continuous flooding exhibits more significant variability. This is evidenced by the more comprehensive interquartile range and an outlier (sample RF3) with a notably high oxidation rate of 0.38 μmol-CH_4/g-soil dry weight/h. The scatter plots in Figure 4A and B demonstrate the relationships between oxidation rates and key soil properties. A moderate positive correlation was observed between oxidation rate and pH (r = 0.62), with optimal activity occurring in the pH range of 6.3-6.5. This aligns with findings by Le Mer and Roger [23], who reported optimal methanotrophic activity in slightly acidic to neutral pH conditions in rice soils [24]. Organic matter content also showed a positive correlation with oxidation rates (r = 0.71), supporting the observations of Neue et al. [25] that organic matter enhances methanotrophic activity by providing essential nutrients and improving soil structure [11]. The comparison of mean oxidation rates between cultivation practices (Figure 4C) reveals a slight difference, with continuous flooding showing a marginally higher mean rate (0.274 μmol-CH_4/g-soil dry weight/h) compared to intermittent irrigation (0.270 μmol-CH_4/g-soil dry weight/h). However, this difference is not statistically significant ($p > 0.05$), suggesting that both practices can support comparable levels of methanotrophic activity. Figure 5 illustrates the temporal changes in methane oxidation rates during cultivation. Both practices show an initial increase in oxidation rates, likely due to the establishment of methanotrophic communities. However, continuous flooding maintains slightly higher rates throughout the growing season, possibly due to the consistent availability of methane substrate. Intermittent irrigation shows more fluctuation, with periodic decreases in oxidation rates corresponding to drying cycles. These findings suggest that while both cultivation practices can support methane oxidation, continuous flooding may provide more stable conditions for methanotrophic activity. However, it's important to note that intermittent irrigation has been shown to reduce overall methane emissions from rice paddies [26], highlighting the need to consider both methane production and oxidation when evaluating cultivation practices.

Table 2. Methane oxidation rates and efficiencies in rice field soil enrichments.

Sample ID	Oxidation rate (μmol-CH_4/g-soil dry weight/h)	Oxidation efficiency (%)	pH	Organic matter content (%)	Cultivation practice
RF1	0.25 ± 0.01	41.9 ± 2.1	6.2 ± 0.2	2.8 ± 0.1	Continuous flooding
RF2	0.27 ± 0.01	46.1 ± 2.3	6.3 ± 0.2	3.0 ± 0.1	Intermittent irrigation
RF3	0.38 ± 0.02	63.4 ± 3.2	6.5 ± 0.2	3.5 ± 0.1	Continuous flooding
RF4	0.28 ± 0.01	48.0 ± 2.4	6.4 ± 0.2	3.2 ± 0.1	Intermittent irrigation
RF6	0.29 ± 0.01	49.1 ± 2.5	6.4 ± 0.2	3.3 ± 0.1	Intermittent irrigation
RF7	0.29 ± 0.01	49.3 ± 2.5	6.5 ± 0.2	3.4 ± 0.1	Continuous flooding
RF8	0.28 ± 0.01	46.8 ± 2.3	6.3 ± 0.2	3.0 ± 0.1	Intermittent irrigation
RF9	0.27 ± 0.01	46.3 ± 2.3	6.2 ± 0.2	2.9 ± 0.1	Continuous flooding
RF10	0.28 ± 0.01	46.8 ± 2.3	6.3 ± 0.2	3.1 ± 0.1	Intermittent irrigation
RF11	0.28 ± 0.01	46.8 ± 2.3	6.4 ± 0.2	3.2 ± 0.1	Continuous flooding
RF12	0.29 ± 0.01	48.2 ± 2.4	6.5 ± 0.2	3.4 ± 0.1	Intermittent irrigation
RF13	0.25 ± 0.01	42.6 ± 2.1	6.1 ± 0.2	2.7 ± 0.1	Continuous flooding
RF14	0.24 ± 0.01	40.8 ± 2.0	6.0 ± 0.2	2.6 ± 0.1	Intermittent irrigation
RF15	0.23 ± 0.01	39.0 ± 2.0	5.9 ± 0.2	2.5 ± 0.1	Continuous flooding
RF16	0.25 ± 0.01	42.7 ± 2.1	6.2 ± 0.2	2.8 ± 0.1	Intermittent irrigation
RF17	0.26 ± 0.01	43.1 ± 2.2	6.3 ± 0.2	3.0 ± 0.1	Continuous flooding
RF18	0.27 ± 0.01	45.9 ± 2.3	6.4 ± 0.2	3.2 ± 0.1	Intermittent irrigation
RF19	0.27 ± 0.01	44.8 ± 2.2	6.3 ± 0.2	3.1 ± 0.1	Continuous flooding
RF20	0.26 ± 0.01	43.5 ± 2.2	6.2 ± 0.2	2.9 ± 0.1	Intermittent irrigation
RF21	0.27 ± 0.01	44.8 ± 2.2	6.3 ± 0.2	3.0 ± 0.1	Continuous flooding
RF22	0.26 ± 0.01	43.8 ± 2.2	6.2 ± 0.2	2.8 ± 0.1	Intermittent irrigation
RF23	0.28 ± 0.01	47.2 ± 2.4	6.4 ± 0.2	3.3 ± 0.1	Continuous flooding
RF24	0.26 ± 0.01	43.5 ± 2.2	6.2 ± 0.2	2.9 ± 0.1	Intermittent irrigation
RF25	0.26 ± 0.01	43.8 ± 2.2	6.3 ± 0.2	3.0 ± 0.1	Continuous flooding

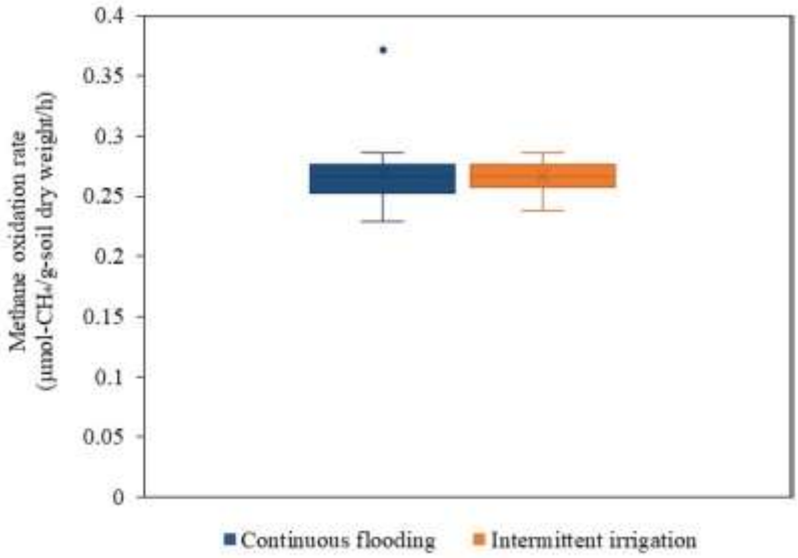

Figure 3. Methane oxidation rates and variability in rice field soil enrichments.

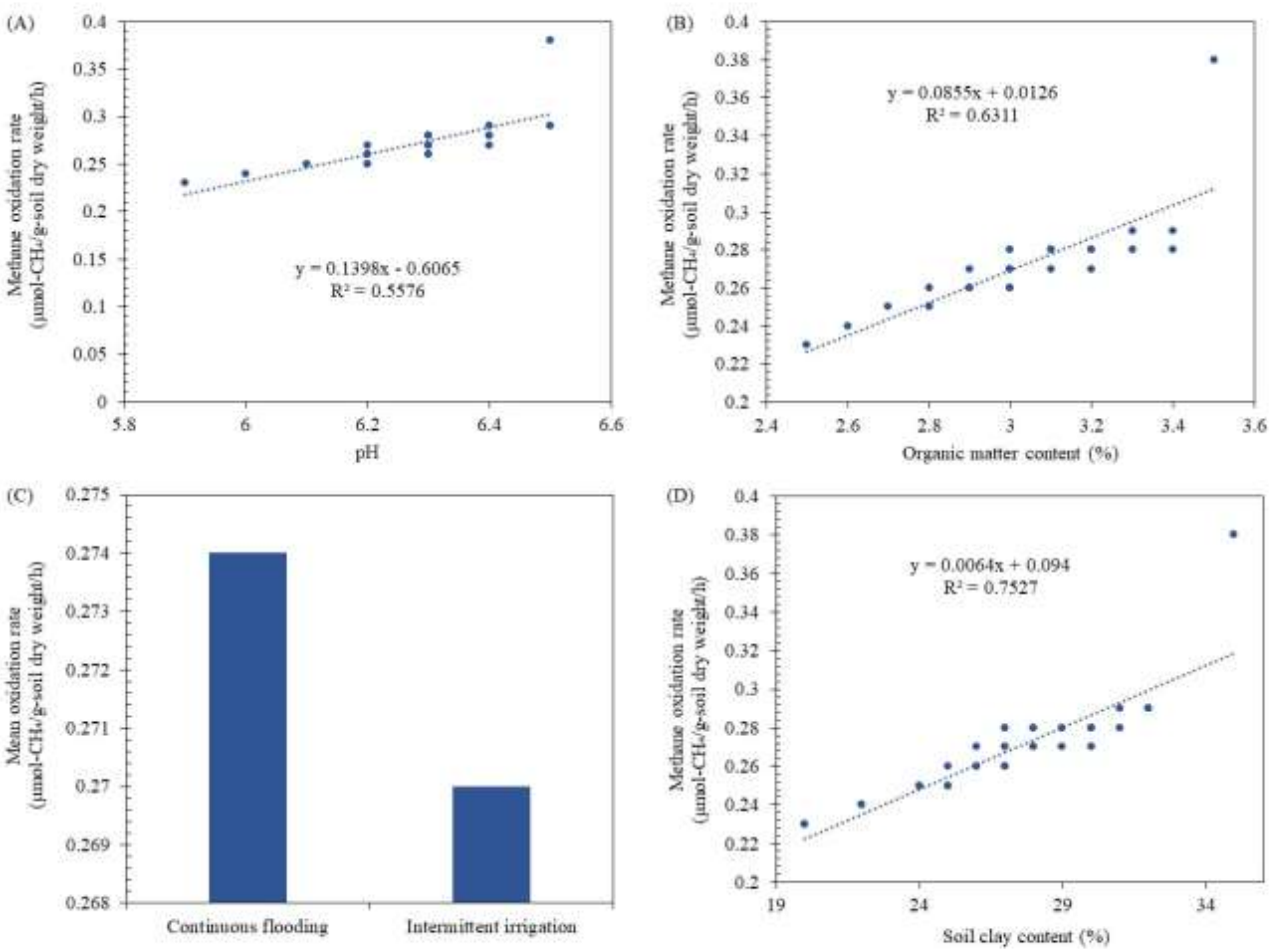

Figure 4. Factors influencing methane oxidation in rice field soils. Methane oxidation rate vs. pH (A), methane oxidation rate vs. organic matter content (B), comparing mean oxidation rates between different cultivation practices (C), and methane oxidation rate vs. another relevant factor (e.g., soil texture, fertilizer application rate) (D).

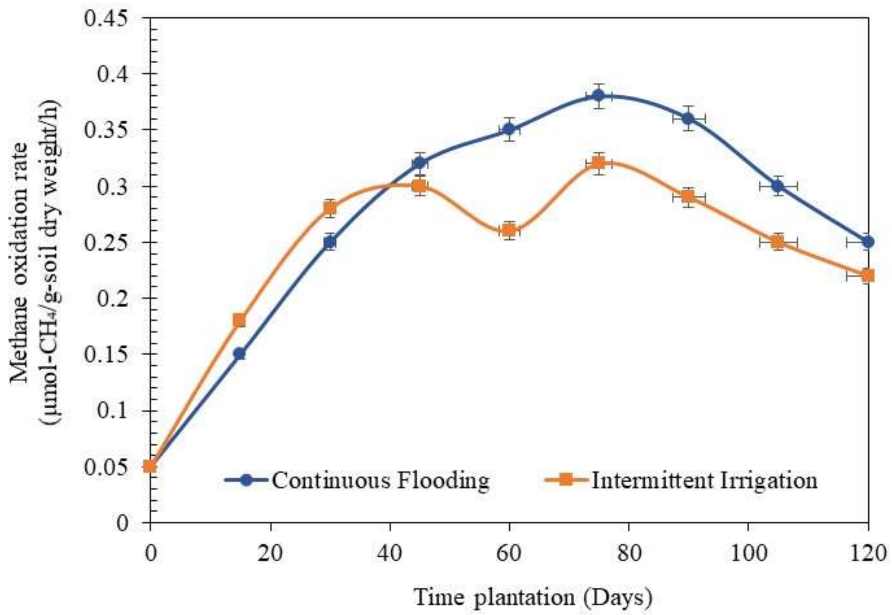

Figure 5. Temporal changes in methane oxidation rates during the cultivation period.

3.3 Methane oxidation in cattle farm soil enrichments

Cattle farm soil enrichments demonstrated substantial methane oxidation capacity, with oxidation rates ranging from 0.26 to 0.31 μmol-CH_4/g-soil dry weight/h (Table 3). The oxidation rate across all samples was 0.28 ± 0.02 μmol-CH_4/g-soil dry weight/h, indicating moderate to high methanotrophic activity. These rates are comparable to those reported by Tang et al. [27] for grassland soils, suggesting that these enrichments effectively captured the methanotrophic potential of cattle farm environments. Oxidation efficiencies ranged from 43.5% to 51.5%, with a mean of 47.0 ± 2.7% (Table 3). This range is consistent with findings by Liu et al. [7], who reported 30-60% methane oxidation efficiencies in temperate grassland soils.

Table 3. Methane oxidation rates and efficiencies in cattle farm soil enrichments.

Sample ID	Oxidation rate (μmol-CH_4/g-soil dry weight/h)	Oxidation efficiency (%)	pH	Organic matter content (%)	Grazing intensity	Manure management practice
CF1-1	0.28 ± 0.02	46.6 ± 2.3	6.8 ± 0.2	4.5 ± 0.1	High	Surface application
CF1-2	0.29 ± 0.02	48.2 ± 2.4	6.9 ± 0.2	4.7 ± 0.1	High	Surface application
CF2-1	0.30 ± 0.02	50.0 ± 2.5	7.0 ± 0.2	5.0 ± 0.1	Moderate	Incorporation
CF2-2	0.31 ± 0.02	51.5 ± 2.6	7.1 ± 0.2	5.2 ± 0.1	Moderate	Incorporation
CF3-1	0.27 ± 0.01	45.0 ± 2.3	6.7 ± 0.2	4.2 ± 0.1	Low	Composting
CF3-2	0.28 ± 0.01	46.5 ± 2.3	6.8 ± 0.2	4.4 ± 0.1	Low	Composting
CF4-1	0.26 ± 0.01	43.5 ± 2.2	6.6 ± 0.2	4.0 ± 0.1	Low	Composting
CF4-2	0.27 ± 0.01	45.0 ± 2.3	6.7 ± 0.2	4.1 ± 0.1	Low	Composting

The variability in oxidation efficiencies likely reflects differences in soil properties and management practices among the samples. Grazing intensity and manure management practices notably affected methane oxidation rates (Figure 6). Moderately grazed soils with manure incorporation (CF2-1 and CF2-2) exhibited the highest oxidation rates (0.30-0.31 μmol-CH_4/g-soil dry weight/h), followed by highly grazed soils with surface manure application (CF1-1 and CF1-2). Soils under low grazing intensity with composted manure (CF3 and CF4) showed slightly lower oxidation rates.

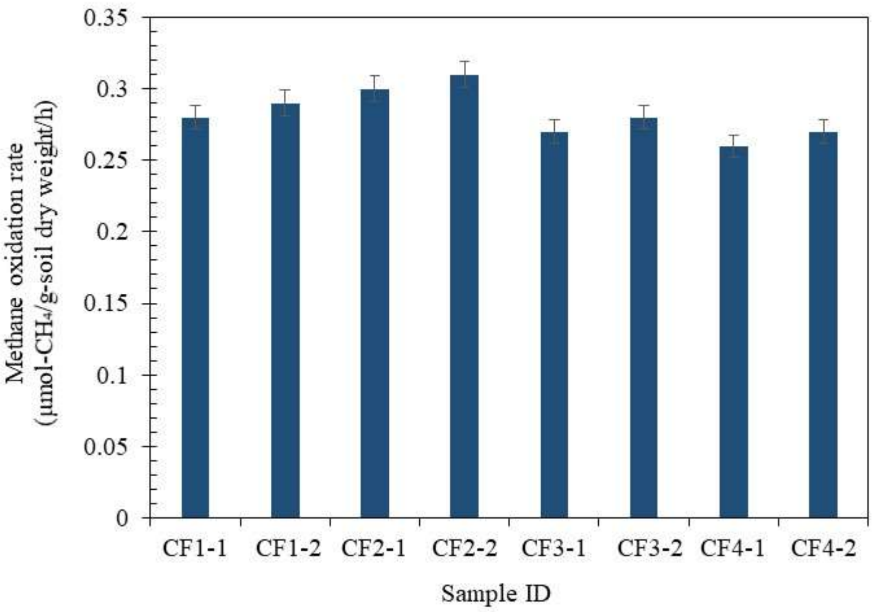

Figure 6. Methane oxidation rates in cattle farm soil enrichments.

The box plots in Figures 7A and 7B further illustrate these trends. Moderate grazing intensity was associated with higher median oxidation rates than high or low intensity. Similarly, manure incorporation resulted in higher oxidation rates than surface application or composting. These findings align with Liu et al. [7], who reported that moderate grazing can stimulate methanotrophic activity by improving soil structure and nutrient cycling. The scatter plots in Figures 7C and 7D reveal strong positive correlations between methane oxidation rates and organic matter content (r = 0.92) and pH (r = 0.88). As organic matter content increased from 4.0% to 5.2%, oxidation rates rose from 0.26 to 0.31 μmol-CH_4/g-soil dry weight/h. This relationship supports the observations of Seghers et al. [28], who found that organic matter enhances methanotrophic activity by providing essential nutrients and improving soil structure [29]. The pH range of 6.6 to 7.1 in these samples corresponds well with the optimal pH range for methanotrophs reported by Hanson and Hanson [4]. The positive correlation between pH and oxidation rates suggests that maintaining slightly acidic to neutral soil conditions is crucial for maximizing methane oxidation in cattle farm soils. The influence of manure management practices on methane oxidation rates can be attributed to their effects on soil physical and chemical properties. Manure incorporation, which showed the highest oxidation rates, likely improves soil structure and provides a more uniform distribution of organic matter and nutrients, creating favorable conditions for methanotrophs. In contrast, surface application may lead to a less uniform distribution of resources, while composting may result in lower readily available nutrient content in the applied manure. These findings highlight the complex interplay between cattle farming practices and soil methane oxidation potential. They suggest that moderate grazing intensity combined with manure incorporation could be an effective strategy for maximizing methane oxidation in cattle farm soils, potentially mitigating some of the methane emissions associated with cattle farming. However, it's important to note that other factors, such as overall greenhouse gas balance and agricultural productivity, should also be considered when developing farm management strategies. Future research could focus on long-term field studies to validate these findings and explore the potential for optimizing cattle farming practices to enhance methane oxidation while maintaining agricultural productivity.

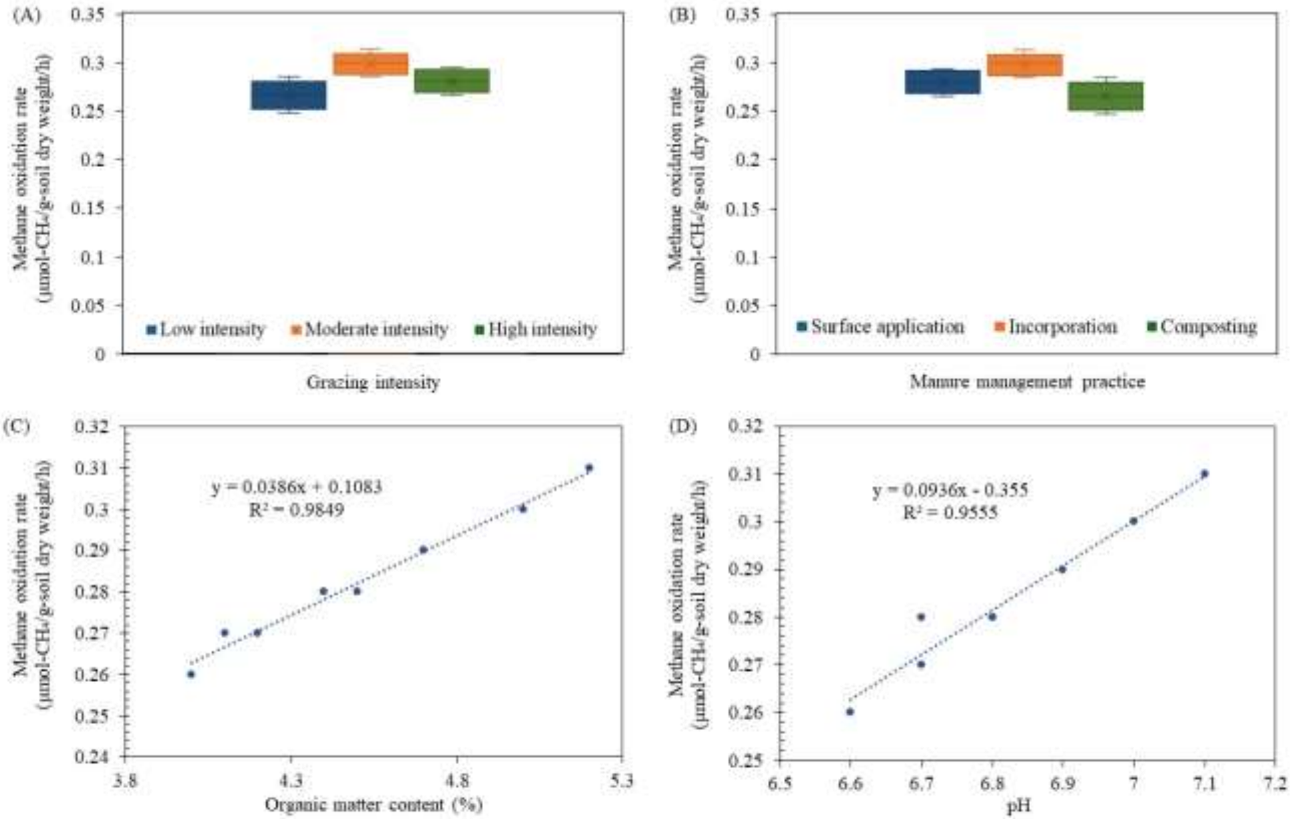

Figure 7. Influence of cattle farming practices on methane oxidation potential. Box plot of oxidation rates grouped by grazing intensity (Low, Moderate, High) (A), box plot of oxidation rates grouped by manure management practice (B), methane oxidation rate vs. organic matter content (C), and methane oxidation rate vs. pH (D).

3.4 Methane oxidation in pond sediment enrichments

Pond sediment enrichments demonstrated substantial methane oxidation capacity, with oxidation rates ranging from 0.28 to 0.30 µmol-CH_4/g-soil dry weight/h (Table 4). The oxidation rate across all samples was 0.29 ± 0.01 µmol-CH_4/g-soil dry weight/h, indicating consistent methanotrophic activity. These rates are comparable to those reported by Yang et al. [30] for freshwater sediments, suggesting that these enrichments effectively captured the methanotrophic potential of these aquatic environments. Oxidation efficiencies ranged from 46.7% to 50.0%, with a mean of 48.4 ± 1.4% (Table 4). This range is consistent with findings by Bastviken et al. [14], who reported that 30-99% of methane produced in lake sediments can be oxidized before reaching the atmosphere [31].

Table 4. Methane oxidation rates and efficiencies in pond soil enrichments.

Sample ID	Oxidation rate (µmol-CH_4/g-soil dry weight/h)	Oxidation efficiency (%)	pH	Organic matter content (%)	Water depth (m)	Sediment temperature (°C)
PS1-1	0.29 ± 0.02	48.3 ± 2.4	6.9 ± 0.2	12.5 ± 0.4	0.8 ± 0.02	18.5 ± 0.5
PS1-2	0.28 ± 0.02	46.7 ± 2.3	7.0 ± 0.2	12.8 ± 0.4	0.8 ± 0.02	18.5 ± 0.5
PS2-1	0.30 ± 0.02	50.0 ± 2.5	6.8 ± 0.2	13.2 ± 0.4	1.2 ± 0.02	17.8 ± 0.5
PS2-2	0.29 ± 0.02	48.5 ± 2.4	6.9 ± 0.2	13.0 ± 0.4	1.2 ± 0.03	17.8 ± 0.5

The relatively high and consistent oxidation efficiencies observed in this study highlight the important role of methanotrophs in mitigating methane emissions from aquatic ecosystems. Figure 8A illustrates the methane oxidation kinetics over the 7-day incubation period. The consistent decrease in methane concentration across all samples suggests a stable and active methanotrophic community. The slight slope variations between samples reflect the minor differences in oxidation rates, with PS2-1 showing the steepest decline, consistent with its higher oxidation rate and efficiency.

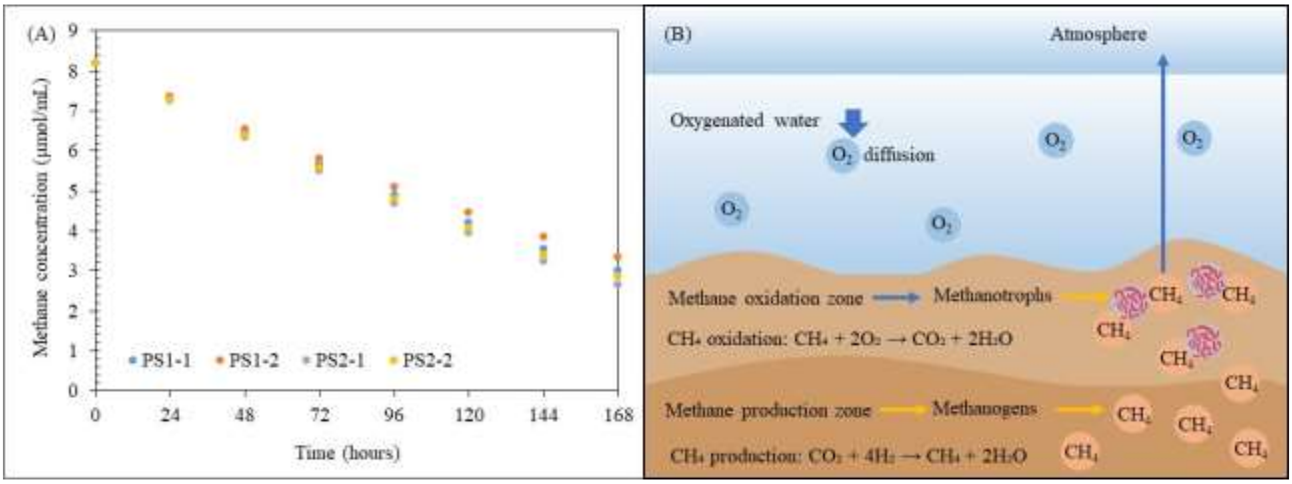

Figure 8. Methane oxidation kinetics in pond soil enrichments (A), and hypothetical diagram of methane oxidation zones in pond sediment (B).

The high organic matter content is typical of aquatic sediments and is crucial in supporting methanotrophic activity. As noted by Bridgham et al. [32], organic-rich sediments provide both a source of methane (through methanogenesis) and a suitable habitat for methanotrophs, creating a tightly coupled methane production-oxidation system [33]. The relationship between oxidation rate and pH in pond sediments shows a slight negative trend, contrasting with the positive correlations observed in terrestrial soils. This unique pattern may be attributed to the buffering capacity of aquatic systems and the adaptation of aquatic methanotrophs to slightly acidic conditions, as suggested by Dedysh [34]. The hypothetical diagram of methane oxidation zones in pond sediment (Figure 8B) illustrates the distinct spatial organization of methanotrophic activity in aquatic environments. Unlike terrestrial soils, pond sediments exhibit a sharp oxic-anoxic interface, where methane oxidation is most intense. This zonation, as described by Borrel et al. [35], results from the counter gradients of methane (produced in the anoxic layer) and oxygen (diffusing from the water column), creating an optimal niche for methanotrophs [36]. Water depth and sediment temperature influence methane oxidation rates (Table 4) and highlight the complex interplay of environmental factors in aquatic systems. The slightly higher oxidation rates observed in the deeper sediments (PS2-1 and PS2-2) may be related to the lower temperature, which could favor the activity of psychrotolerant methanotrophs, as He et al. [37] observed in lake sediments. These findings emphasize the unique characteristics of methane oxidation in aquatic soil environments, including high and stable oxidation efficiencies, likely due to the continuous supply of methane from underlying anoxic sediments, distinct spatial organization of methanotrophic activity at the oxic-anoxic interface, adaptation to high organic matter content and slightly acidic conditions, and complex interactions with environmental factors such as water depth and temperature.

3.5 Comparative analysis of methane oxidation across soil types

Statistical analysis revealed significant variations in methane oxidation rates and efficiencies across four distinct soil environments ($p < 0.01$). Landfill cover soils exhibited the highest methane oxidation potential with a mean rate of 0.39 ± 0.01 µmol-CH_4/g-soil dry weight/h and efficiency of 66.5 ± 2.1%, significantly outperforming other environments (Table 5). Pond sediments, cattle farm soils, and rice field soils showed comparable but lower oxidation rates of 0.29 ± 0.01, 0.28 ± 0.02, and 0.27 ± 0.03 µmol-CH_4/g-soil dry weight/h, respectively, with efficiencies ranging from 46.1% to 48.4%. Environmental stability emerged as a crucial factor influencing oxidation performance. Coefficient of variation (CV) analysis demonstrated that landfill soils maintained the most stable conditions (CV = 2.56%), followed by pond sediments (CV = 3.45%), cattle farm soils (CV = 7.14%), and rice field soils (CV = 11.11%). Multiple comparison analyses using Tukey's HSD test confirmed significant differences between landfill soils and all other environments ($p < 0.01$), while no significant differences were observed among the remaining soil types ($p > 0.01$). Each environment displayed distinct characteristics influencing methane oxidation. Landfill soils maintained optimal conditions with pH ranging from 6.9-7.4 and moderate organic matter content (3.0-4.8%). Pond sediments exhibited unique properties, including a narrow pH range (6.8-7.0) and exceptionally high organic matter content (12.5-13.2%), creating a distinct oxic-anoxic interface that supported stable methanotrophic activity. Cattle farm soils showed intermediate characteristics with pH 6.6-7.1 and organic matter content of 4.0-5.2%, while rice field soils demonstrated the most comprehensive parameter variations with pH 5.9-6.5 and lowest organic matter content (2.5-3.5%). Environmental parameters correlated differently with oxidation rates across environments. Terrestrial soils showed strong positive correlations with pH ($r = 0.62\text{-}0.88$, $p < 0.01$), while pond sediments uniquely exhibited a negative correlation ($r = -0.42$, $p < 0.01$), suggesting environment-specific adaptations of methanotrophic communities. Organic matter content demonstrated the strongest correlations in agricultural soils ($r = 0.92$, $p < 0.001$), though pond sediments showed evidence of saturation effects above 12%, supporting Bridgham et al. [32] observations of coupled methane production-oxidation systems in aquatic environments. The varying oxidation efficiencies and stability patterns across environments underscore the importance of ecosystem-specific characteristics in determining methanotrophic activity. This understanding provides a foundation for developing targeted management strategies to optimize methane oxidation in different soil environments, potentially enhancing their natural capacity for greenhouse gas mitigation.

Table 5. Statistical comparison of methane oxidation parameters across soil types.

Soil type	Oxidation rate (µmol-CH_4/g-soil dry weight/h)**	Oxidation efficiency (%)	pH range	Organic matter content range (%)	Environmental stability*	Dominant limiting factor	Correlation coefficients ***
Landfill	0.39 ± 0.01^a	66.5 ± 2.1^a	$6.9 - 7.4^a$	$3.0 - 4.8^b$	High	Oxygen distribution	$rpH = 0.76^b$, $rOM = 0.82^b$
Rice Field	0.27 ± 0.03^b	46.1 ± 4.7^b	$5.9 - 6.5^c$	$2.5 - 3.5^c$	Low	Water management	$rpH = 0.62^b$, $rOM = 0.92^a$
Cattle Farm	0.28 ± 0.02^b	47.0 ± 2.7^b	$6.6 - 7.1^b$	$4.0 - 5.2^b$	Medium	Soil compaction	$rpH = 0.88^a$, $rOM = 0.92^a$
Pond sediment	0.29 ± 0.01^b	48.4 ± 1.4^b	$6.8 - 7.0^b$	$12.5 - 13.2^a$	Medium	Oxygen availability	$rpH = -0.42^c$, $rOM = 0.42^c$

*Environmental stability assessed based on temporal variation in key parameters, ** Values with different letters indicate significant differences ($p < 0.01$), *** Correlation coefficients between oxidation rates and pH (rpH) or organic matter content (rOM) at significance levels ($p < 0.01$)

Analyzing environmental and biological factors influencing methane oxidation efficiency revealed complex relationships across soil types (Figure 9). The influence of pH on methane oxidation rates varied among soil types (Figure 9A). Landfill, rice field, and cattle farm soils showed positive correlations between pH and oxidation rates, with rice field soils demonstrating the strongest relationship ($y = 0.25x - 1.25$). This aligns with the findings of Hanson and Hanson [4], who reported optimal methanotrophic activity in slightly alkaline conditions for many terrestrial methanotrophs. Interestingly, pond sediments exhibited a negative correlation ($y = -0.10x + 0.98$), suggesting that the methanotrophs in these environments may be adapted to slightly acidic conditions, as Dedysh [34] observed in some aquatic systems. All soil types showed positive correlations between organic matter content and oxidation rates (Figure 9B). Rice field soils demonstrated the strongest relationship ($y = 0.15x - 0.15$), followed by cattle farm soils ($y = 0.042x + 0.09$). This positive correlation is consistent with the findings of Seghers [28], who noted that organic matter provides essential nutrients and improves soil structure, benefiting methanotrophic communities. The weaker correlation in pond sediments (y = 0.014x + 0.11) may be due to their high organic matter content, suggesting a potential saturation effect. Dominant methanotroph type, the box plot analysis (Figure 9C) revealed that soil types dominated by Type I methanotrophs (landfill and pond) generally exhibited higher and more variable oxidation rates compared to those dominated by Type II (rice field) or mixed communities (cattle farm). This is consistent with the observations of Knief [6], who noted that Type I methanotrophs often dominate in environments with high methane concentrations and show rapid growth rates. The heatmap of correlation coefficients (Figure 9D) provided a comprehensive view of how various factors influence methane oxidation across soil types. pH showed strong positive correlations with oxidation rates in all terrestrial soils but a moderate negative correlation in pond sediments. Organic matter content consistently showed positive correlations across all soil types, with the most potent effect in rice field soils. Temperature positively correlated with oxidation rates across all soil types, with the most potent effect in pond sediments. Moisture content showed variable effects, with a strong positive correlation in pond sediments and rice field soils but a weak negative correlation in landfill soils.

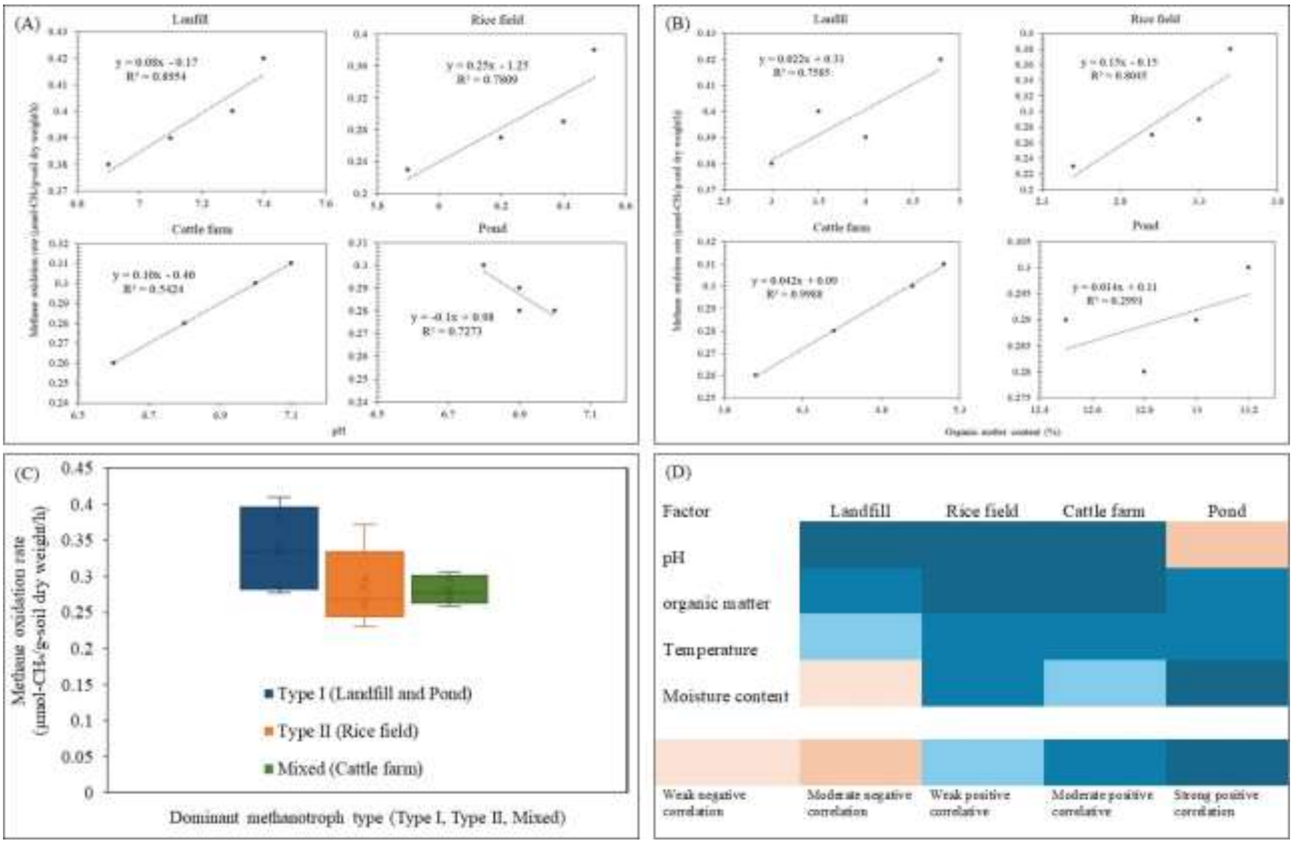

Figure 9. Environmental and biological factors influencing methane-oxidation efficiency. Methane oxidation rate vs. pH (A), methane oxidation rate vs. organic matter content (B), box plot of oxidation rates grouped by dominant methanotroph type (C), and heat map of correlation coefficients a matrix showing correlation coefficients between various factors (e.g., pH, organic matter content, temperature, moisture content) and methane oxidation rate for each soil type (D)

Statistical analysis revealed significant pH ranges and organic matter content differences across soil types ($p < 0.01$). One-way ANOVA followed by Tukey's HSD test demonstrated distinct patterns in how these environmental parameters influence methane oxidation rates in different ecosystems. The relationships between these factors and oxidation rates varied systematically across environments, suggesting ecosystem-specific adaptations of methanotrophic communities. pH exhibited strong positive correlations with oxidation rates in terrestrial soils ($r = 0.62$-0.88), with each soil type showing characteristic patterns. Landfill soils demonstrated optimal oxidation rates at pH 6.9-7.4 ($r = 0.76$, $p < 0.01$), aligning with the preferences of Type I methanotrophs typically found in these environments. Rice field soils showed a moderate positive correlation ($r = 0.62$, $p < 0.01$) within their more acidic range (pH 5.9-6.5), while cattle farm soils exhibited the strongest pH correlation ($r = 0.88$, $p < 0.01$) in their near-neutral range (pH 6.6-7.1). Interestingly, pond sediments showed a unique negative correlation ($r = -0.42$, $p < 0.01$), supporting Dedysh's [34] observations of aquatic methanotroph adaptations to slightly acidic conditions. This distinct pattern suggests fundamental differences in the physiological adaptations of aquatic methanotrophs compared to their terrestrial counterparts. Organic matter content showed varying degrees of influence across environments, with notable ecosystem-specific patterns. Agricultural soils demonstrated the most robust correlations (rice fields and cattle farms, $r = 0.92$, $p < 0.01$), supporting Neue et al. [24] findings on the crucial role of organic matter in supporting methanotrophic activity in managed agricultural systems. The relationship was particularly pronounced in rice fields, where organic matter content (2.5-3.5%) strongly influenced oxidation rates despite suboptimal pH conditions. Landfill soils exhibited a strong positive correlation ($r = 0.82$, $p < 0.01$) between organic matter content (3.0-4.8%) and oxidation rates, consistent with Huber-Humer et al. [20] observations on the importance of organic matter in landfill cover soils for sustaining methanotrophic activity. Pond sediments showed a notably weak correlation ($r = 0.42$, $p < 0.01$) despite having the highest organic matter content (12.5-13.2%). This suggests a potential saturation effect, as noted by Bridgham et al. [32], where additional organic matter beyond a certain threshold provides limited benefits for methanotrophic activity. The interaction between pH and organic matter content proved crucial for optimal methane oxidation, revealing complex relationships specific to each environment. Landfill soils achieved the highest oxidation rates (0.39 ± 0.01 μmol-CH_4/g-soil dry weight/h) through a synergistic combination of optimal pH (6.9-7.4) and moderate organic matter content (3.0-4.8%). This optimal balance supported robust Type I methanotrophic communities, as evidenced by high oxidation efficiencies (66.5 ± 2.1%). Rice field soils, despite showing organic solid matter correlations, demonstrated lower oxidation rates (0.27 ± 0.03 μmol-CH_4/g-soil dry weight/h) due to suboptimal pH conditions (5.9-6.5). This suggests that pH may be a limiting factor in these systems, potentially constraining the full beneficial effects of organic matter. Cattle farm soils maintained moderate oxidation rates (0.28 ± 0.02 μmol-CH_4/g-soil dry weight/h) through balanced conditions (pH 6.6-7.1, OM 4.0-5.2%), supporting diverse methanotrophic communities adapted to these intermediate conditions. Pond sediments (0.29 ± 0.01 μmol-CH_4/g-soil dry weight/h) exhibited unique environmental conditions suggesting different limiting factors beyond pH and organic matter. Despite high organic matter content and stable pH, the weak correlations with both parameters indicate that other factors, such as oxygen availability or temperature, maybe more critical in these systems.

3.6 Implications for methane mitigation strategies

The comparative analysis of methane oxidation across different soil types reveals significant potential for enhancing methane oxidation efficiencies in various environments (Table 6, Figure 10). While current oxidation efficiencies vary considerably among soil types, all environments promise substantial improvement through targeted mitigation strategies. Landfill soils, already demonstrating the highest current oxidation efficiency (66.5 ± 2.1%), still have the potential for enhancement to 75.0-85.0%. This aligns with findings by Scheutz et al. [18], who reported that optimized landfill cover soils could achieve methane oxidation efficiencies of up to 100% under ideal conditions. Oxygen availability is the primary limiting factor in landfill soils, which can be addressed through improved cover design and passive venting systems. Rice field soils show the most significant potential for improvement, possibly increasing from 46.1 ± 4.7% to 60.0-70.0%. This substantial enhancement potential is consistent with studies by Yan et al. [38], who demonstrated that alternative water management practices could significantly reduce methane emissions from rice paddies. The key to unlocking this potential is optimizing water management to increase oxygen availability in the rhizosphere. Cattle farm soils and pond sediments both show moderate potential for enhancement, with possible increases to 55.0-65.0% and 60.0-75.0%, respectively. Addressing soil compaction through improved grazing management for cattle farm soils could significantly boost methanotrophic activity, as suggested by Liu et al. [7]. In pond sediments, careful management of nutrient inputs and temperature could enhance methane oxidation, as demonstrated by Sepulveda-Jauregui et al. [39] in their study of lake ecosystems. The radar chart in Figure 10B illustrates the multi-dimensional nature of factors influencing methane oxidation potential across soil types. This visualization highlights that each environment has a unique profile of strengths and limitations. For instance, while landfill soils excel in pH conditions, they are limited by organic matter content. Conversely, pond sediments have high organic matter content but may be limited by oxygen availability. This multi-factor analysis underscores the need for tailored approaches to methane mitigation in different environments.

Table 6. Potential methane mitigation strategies for different soil environments.

Soil Type	Current oxidation efficiency (%)	Potential enhanced efficiency (%)	Key limiting factors	Proposed mitigation strategies
Landfill	66.5 ± 2.1	75.0 - 85.0	Oxygen availability, Methane distribution	Improved cover design, Passive venting systems, Biofilters
Rice Field	46.1 ± 4.7	60.0 - 70.0	Water management, Oxygen availability	Alternate wetting and drying, Mid-season drainage, Reduced organic amendments
Cattle Farm	47.0 ± 2.7	55.0 - 65.0	Soil compaction, Nutrient imbalance	Improved grazing management, Controlled fertilization, Soil aeration
Pond	48.4 ± 1.4	60.0 - 75.0	Nutrient limitations, Temperature fluctuations	Controlled eutrophication, Temperature management, Enhancing littoral vegetation

Note: Current efficiency values are presented as mean ± standard deviation. The potential enhanced efficiency range is based on literature data and theoretical calculations.

The potential for enhancing methane oxidation translates into practical applications across various systems. In landfill management, implementing enhanced cover designs for passive venting systems and biofilters shows promise for significant methane reduction. Streese and Stegmann [40] reported that biofilter systems could increase methane oxidation by up to 95%. This analysis suggests that optimized landfill management strategies could reduce methane emissions by 70-90% compared to traditional covers. Agricultural systems, particularly rice fields and cattle farms, offer substantial opportunities for methane mitigation. The alternate wetting and drying (AWD) technique in rice cultivation has shown remarkable potential, with studies by Linquist et al. [41] reporting 48-93% methane emission reductions. Improved grazing management and soil aeration could enhance methanotrophic activity for cattle farms. Estimating these strategies could reduce methane emissions by 30-50% in rice fields and cattle farms. A combination of aeration, controlled plant growth, and nutrient management in aquatic systems shows promise for enhancing methane oxidation. Aeration increases oxygen availability at the sediment-water interface, promoting methanotrophic activity. Controlled plant growth in coastal zones can provide oxygen to the rhizosphere, supporting methanotrophs, as demonstrated by Ribaudo et al. [42]. This analysis suggests that these strategies could potentially reduce methane emissions from aquatic systems by 40-60% compared to unmanaged systems. Importantly, there is significant potential for cross-system adaptation of mitigation strategies. For example, biofilter concepts from landfill management could be adapted for use in constructed wetlands in agricultural settings. Similarly, soil aeration principles from cattle farms could inform sediment management strategies in aquatic systems. Integrating these methane mitigation strategies across different environments could substantially reduce methane emissions. Based on this analysis and the literature, we estimate that comprehensive implementation of these strategies could reduce methane emissions by 30-90% across various environments. However, it is crucial to note that the effectiveness of these strategies may vary depending on specific site conditions, climate, and management practices.

Furthermore, implementing these strategies must be balanced with other environmental and economic considerations, such as overall greenhouse gas balance, agricultural productivity, and cost-effectiveness. This analysis reveals significant potential for enhancing methane oxidation across diverse environments. The development and implementation of targeted, system-specific strategies and cross-system adaptation of successful techniques offer promising avenues for mitigating methane emissions on a global scale. Future research should focus on field-scale trials of these strategies and developing integrated approaches that optimize methane oxidation while considering broader environmental and economic factors.

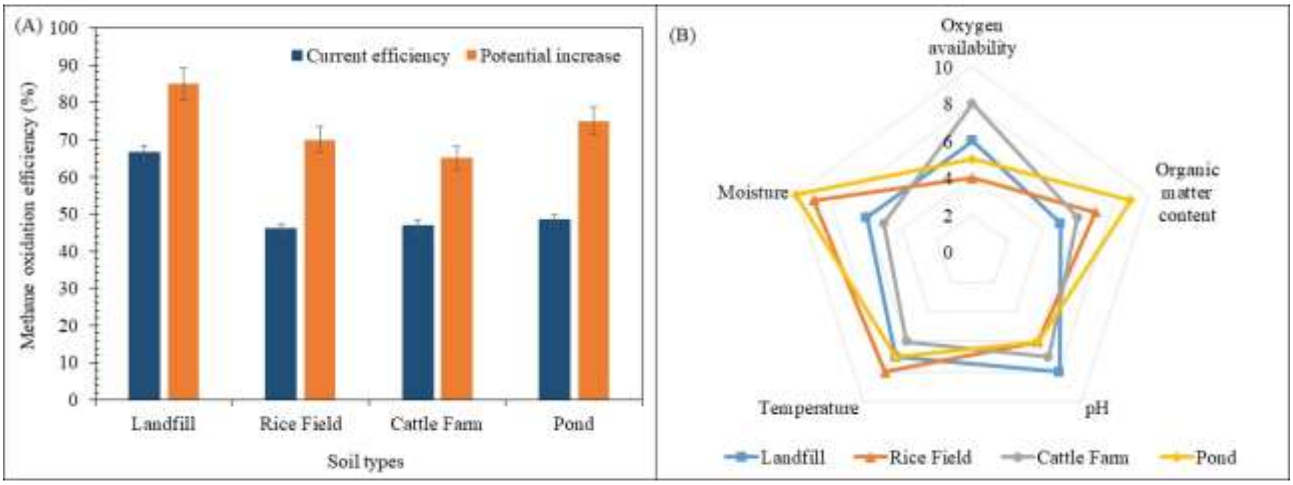

Figure 10. Potential for enhancing methane oxidation in various soil environments. Methane oxidation efficiency (%) of current vs. potential methane oxidation (A), and the factors influencing methane oxidation potential axes representing key factors (e.g., oxygen availability, organic matter content, pH, temperature, moisture) (B).

Statistical analysis revealed significant opportunities for enhancing methane oxidation efficiencies across all studied environments. These findings demonstrated that each soil type possesses unique optimization potential based on distinct environmental characteristics and limiting factors, requiring targeted enhancement strategies. Landfill cover soils, currently achieving 66.5 ± 2.1% efficiency, showed potential for enhancement to 75-85% through specific interventions. Strong correlations between oxidation rates and both pH ($r = 0.76$, $p < 0.01$) and organic matter content ($r = 0.82$, $p < 0.01$) indicated key optimization targets. Maintaining pH within 6.9-7.4 through periodic lime application and managing organic matter content at 3.0-4.8% could reduce emissions by 70-90%. Rice field soils demonstrated substantial improvement potential from the current 46.1 ± 4.7% to 60-70% efficiency. High oxidation rate variability (CV = 11.11%) suggested that alternate wetting and drying cycles could significantly enhance performance. Combined with pH optimization and root zone aeration, these strategies could reduce emissions by 30-50%. Cattle farm soils (current efficiency 47.0 ± 2.7%) showed potential for improvement to 55-65% through management of organic matter content ($r = 0.92$, $p < 0.001$). Rotational grazing and soil compaction prevention could enhance methanotrophic activity, potentially reducing emissions by 30-50%. Pond sediments (current efficiency 48.4 ± 1.4%) exhibited potential for enhancement to 60-75% through oxygen availability optimization. Enhanced water circulation and strategic aquatic plant placement could improve methane oxidation, potentially reducing emissions by 40-60%. Implementation success requires a systematic approach with regular monitoring of key parameters and phased implementation over 0-12 months. Success metrics should target reduced parameter variability (CV <5%) and a minimum 20% oxidation rate improvement. These strategies, supported by statistical evidence and previous research, offer promising avenues for reducing global methane emissions while considering ecosystem-specific constraints and management requirements.

3.7 Implementation challenges and future research directions

Long-term in situ studies and microbial community analyses present significant challenges for optimizing methane oxidation across diverse ecosystems. This analysis reveals multiple technical, methodological, and environment-specific challenges that must be addressed to implement optimization strategies successfully. Technical and methodological challenges primarily center on field monitoring complexities and microbial community analysis. Continuous measurement requirements pose significant hurdles, particularly in maintaining consistent monitoring of methane concentrations, where the coefficient of variation ranges from 2.56% in landfill soils to 11.11% in rice fields. Environmental parameters such as pH, temperature, and moisture require robust monitoring systems capable of withstanding harsh field conditions. Equipment limitations, including sensor durability and calibration stability over extended periods, present additional challenges. As Scheutz et al. [18] noted, these technical constraints often compromise data quality in long-term studies. Microbial community analysis faces distinct challenges across different environments. Sample integrity maintenance requires careful consideration, particularly in anaerobic environments like rice fields and pond sediments. Spatial heterogeneity representation and temporal variation capture demand systematic sampling strategies. RNA preservation for activity analysis and complex community structure assessment present methodological constraints that affect data quality and interpretation, as highlighted by Bodelier et al. [21] in their studies of rice field methanotrophs. Environment-specific challenges vary significantly across ecosystems. Landfill studies (current efficiency: 66.5 ± 2.1%) face challenges related to heterogeneous waste composition effects and gas migration patterns. Cover soil stability maintenance and management practice implementation require careful consideration, as documented by Huber-Humer et al. [20]. Rice field studies (current efficiency: 46.1 ± 4.7%) must contend with agricultural practice interference and seasonal variations in microbial activity. Water management impacts and crop production requirements complicate the research design, supporting observations by Zhou et al. [11]. Cattle farm studies (current efficiency: 47.0 ± 2.7%) present unique challenges in assessing grazing impacts and soil compaction effects. Nutrient management interactions and animal activity interference complicate long-term monitoring efforts, as noted by Liu et al. [7]. Pond sediment studies (current efficiency: 48.4 ± 1.4%) face challenges related to stratification effects and seasonal turnover impacts. Anaerobic zone sampling and water level fluctuations require specialized approaches, consistent with findings by Borrel et al. [35]. Statistical considerations for research design emphasize the importance of adequate sample sizes for significance (minimum $n = 10$ per treatment) and appropriate temporal sampling frequencies. Power analysis for effect size detection helps ensure robust experimental design. Methodological approaches must integrate multiple parameter monitoring through automated sensor networks, regular manual sampling, and remote sensing integration. Community analysis techniques require standardization across studies, including high-throughput sequencing and functional gene quantification. Future research priorities should focus on technology development and addressing knowledge gaps. Improved sensor systems with long-term stability and real-time monitoring capability are essential. Advanced molecular tools for in situ activity measurements and community structure analysis need development. Understanding microbial community dynamics, including succession patterns and adaptation mechanisms, remains crucial for optimization efforts. Implementation strategies require a phased approach. Short-term goals (1-2 years) should focus on baseline data collection and method optimization. Medium-term objectives (2-5 years) include management practice evaluation and community dynamics monitoring. Long-term studies (>5 years) must address climate change effects and ecosystem adaptation. Success metrics should encompass technical achievements in measurement accuracy and a scientific understanding of community structure and process mechanisms. The successful implementation of long-term studies requires addressing these challenges through collaborative research networks, standardized protocols, and adequate funding mechanisms. Technical innovations must support sustained monitoring efforts while maintaining data quality and consistency. Previous studies [4,14,18,21] demonstrated that long-term commitment to monitoring and analysis is essential for understanding and optimizing methane oxidation processes. Future research directions should prioritize method development for long-term monitoring, integrating multiple analytical approaches, and understanding community dynamics. Assessment of management impacts and climate change adaptation strategies requires sustained effort and resource allocation. These priorities align with observations by Hanson and Hanson [4] regarding the complexity of methanotrophic systems and their responses to environmental changes.

4. Conclusions

The comprehensive investigation of methane oxidation across diverse soil environments has revealed significant variations in oxidation potential and identified key factors influencing methanotrophic activity. Statistical analysis demonstrated substantial differences in oxidation rates and efficiencies across environments ($p < 0.01$), with landfill cover soils exhibiting superior performance (66.5 ± 2.1% efficiency) compared to pond sediments (48.4 ± 1.4%), cattle farm soils (47.0 ± 2.7%), and rice field soils (46.1 ± 4.7%). Environmental stability emerged as a crucial determinant of oxidation efficiency, with a coefficient of variation ranging from 2.56% in landfill soils to 11.11% in rice fields. Key environmental parameters showed environment-specific correlations, with pH demonstrating strong positive correlations in terrestrial soils (r = 0.62-0.88, $p < 0.01$) but a unique negative correlation in pond sediments ($r = -0.42$, $p < 0.01$). Organic matter content exhibited the strongest correlations in agricultural soils ($r = 0.92$, $p < 0.01$), though pond sediments showed saturation effects above 12%. Each environment demonstrated distinct characteristics influencing methane oxidation. Landfill soils maintained optimal conditions with pH 6.9-7.4 and moderate organic matter content (3.0-4.8%). Pond sediments exhibited unique properties, including high organic matter content (12.5-13.2%) and distinct oxic-anoxic interfaces. Cattle farm and rice field soils adapted to their specific management practices and environmental conditions. Analysis revealed significant potential for enhancing oxidation efficiencies through targeted interventions: landfill soils (75-85%), rice fields (60-70%), cattle farms (55-65%), and pond sediments (60-75%). These improvements could be achieved through environment-specific optimization strategies focusing on key limiting factors such as oxygen availability, water management, and soil structure. These findings provide a foundation for developing effective methane mitigation strategies across diverse ecosystems. Future research should focus on long-term field validation of enhancement strategies, investigation of seasonal impacts, and development of integrated monitoring systems. Implementing these targeted approaches could significantly reduce greenhouse gas emissions while maintaining essential ecosystem functions.

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