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Phenols, Tannins, and Total Colonies of Phenol-Degrading Bacteria in the Natural and Cultivated Peatlands

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ABSTRACT

Phenols play an important role in inhibiting the decomposition rate of peats. This research compares the total phenols, tannins, and colonies of phenol-degrading bacteria in natural and cultivated peatlands. The work was conducted from September to December 2023 on the peatlands in Pontianak, West Kalimantan. Samples of 60 cm^3 were collected from the peat surface. The samples were taken from 4 types of peats, namely natural peats covered by ferns (A), cultivated peat with raised beds (B), cultivated peat with raised beds ameliorated with lime and beef manure (C), and cultivated peat with raised beds ameliorated with lime, beef manure, and Jadam fertilizer (D). The total phenols content was measured using the Folin-Ciocalteu 10% method using a UV-Vis spectrophotometer at a wavelength of 765 nm. The total tannins content was measured using the Folin-Denis method using a UV-Vis spectrophotometer at a wavelength of 740 nm. Total bacterial colonies were counted using the Total Plate Count method on selective Mineral Salt Medium (MSM) agar with the addition of phenol solution. The results showed that the average total phenols and tannins content values were generally low in the cultivated peats, particularly in the raised bed (B). The results indicate that the cultivated peat accelerates the activity of phenol-degrading bacteria.

Keywords: Jadam fertilizer, phenols, phenol-degrading bacteria, peats, tannin

INTRODUCTION

Peatlands used for agriculture are a significant source of carbon emissions, contributing to global warming. The organic materials in peat, particularly phenolic compounds, have the potential to oxidize and release CO_2. However, the impact of agricultural activities such as bed formation and fertilizer application on the activity of phenol-degrading bacteria remains poorly documented and insufficiently understood.

Phenolic compounds are among the most abundant plant metabolites and are known to degrade more slowly in soil than other organic materials (Min *et al.* 2015). The degradation of phenols involves various enzymes such as phenol oxidase (Freeman *et al.* 2004; Pind *et al.* 1994), which are produced by microbes, primarily fungi (Min *et al.* 2015). Several bacteria, such as *Burkholderia* sp. and *Stenotrophomonas* sp., have been found to be capable of degrading phenolics in acidic and frequently waterlogged peatland environments (Kamardan *et al.* 2022). Phenolics can enhance plant resistance to certain pathogens and can be utilized as plant growth-promoting rhizobacteria (Lobato-Ureche *et al.* 2023).

The cultivation of plants in peatlands is typically accompanied by fertilization, such as the application of Jadam fertilizer derived from edamame soybean waste. This fertilizer has the potential to enhance the microbial activity involved in the decomposition of organic matter in peat soil (Riyani *et al.* 2021). Microorganisms from the rhizosphere of edamame plants simultaneously break down organic matter and metabolize phenolic acids into organic compounds, which serve as nutrient sources for plants. Consequently, these microbes play crucial roles in degrading phenolic compounds and improving soil nutrient availability.

This study aimed to compare the levels of total phenolics, tannins, and the total colony count of phenol-degrading bacteria under different peatland conditions, namely: (A) natural peat covered by ferns, (B) cultivated peat with raised beds, (C) cultivated peat with raised beds ameliorated with lime and beef manure, and (D) cultivated peat with raised beds ameliorated with lime, beef manure, and Jadam fertilizer.

METHODS

Research Sites

This study was conducted from September to December 2023 on peatlands located in the Southeast Pontianak District, Pontianak City, West Kalimantan Province. Soil samples were analyzed at the

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Laboratory of the Faculty of Agriculture, Tanjungpura University, Pontianak.

Materials and Equipment

The materials used included peat soil, Jadam fertilizer, dolomite lime, cattle manure, alcohol, pure phenol, distilled water, NaCl, methanol, and Mineral Salt Medium ($MgSO_3$, $CaCl_2$, KH_2PO_3, K_2HPO_3, NH_3NO_3, $FeCl_3$, and agar). Reagents for phenolics and tannin analysis included Folin-Ciocalteu, Folin-Denis, and Na_2CO_3.

The field equipment used consisted of a hoe, soil sample rings, PVC pipes, machete, measuring tape, and Ziplock plastic bags. The laboratory equipment included Petri dishes, an analytical balance, micropipette tips, a laminar flow hood, an autoclave, a UV-Vis spectrophotometer, a centrifuge, and a shaker.

Sampling Plots

Four sampling locations were as follows: natural peat covered by ferns (A); cultivated peat with raised beds (B); cultivated peat with raised beds ameliorated with lime and beef manure (C); and cultivated peat with raised beds ameliorated with lime, beef manure, and Jadam fertilizer (D). Raised beds were constructed using a hoe, each measuring 80 cm × 180 cm, with a height of 30 cm. A total of eight raised beds were created, with 50 cm spacing between them.

The applied ameliorants included 720 g cattle manure, 1,780 g dolomite lime, and 500 mL Jadam fertilizer (15 mL/L) per plot. Jadam fertilizer was prepared from edamame waste, leaf litter, humus, salt, and water, then composted and incubated for 14 days. Fully mature Jadam fertilizer is characterized by a dark or blackened solution, white foam on the surface, and a reduced foul odor.

Samples were collected from 20 plots: 4 from site A, 8 from site B, 4 from site C, and 4 from site D. In each plot, four peat soil samples were taken using a 60 mL syringe at 10 cm depth. The samples were placed in PVC tubes lined with aluminum foil and sealed in labeled sample bags according to their collection points. Sampling was conducted under sterile (aseptic) conditions, with gloves and alcohol used to maintain sample integrity.

Total Phenolic Assay

A 1-gram soil sample was weighed, ground with liquid nitrogen, and mixed with 50 mL of absolute methanol. The sample was macerated using an orbital shaker at 150 rpm for 1 h. Afterward, the filtered solution was centrifuged at 3500 rpm for 3 min and further filtered using filter paper to obtain the extract. The filtrate was collected in a 100 mL vial wrapped in aluminum foil to protect it from light.

Total phenolics were assayed using the Folin-Ciocalteu method. The measurement was carried out by reacting 1 mL of the sample with 5 mL of distilled water, 2 mL of Folin-Ciocalteu reagent, and 2 mL of 10% saturated Na_2CO_3 solution. The sample was then homogenized and left to stand for 30 min. Finally, the absorbance of the sample was measured at 765 nm using a UV-Vis spectrophotometer (Lim *et al.* 2014).

Total Tannin Assay

A 0.2 mL aliquot of the macerated extract (after 1 h of maceration) was transferred into a test tube. Then, 1 mL of Folin-Denis reagent, 2 mL of 15% Na_2CO_3 solution, and 6.8 mL of distilled water were added. The test tube was then covered with aluminum foil and incubated for 60 min. After incubation, the absorbance of the sample was measured at 740 nm using a UV-Vis spectrophotometer.

Total Colony Count of Phenolics-Degrading Bacteria

The total colony count of phenolics-degrading bacteria was determined using Mineral Salt Medium (MSM) supplemented with 18 g of agar powder per 1000 mL of distilled water. The medium was sterilized using an autoclave at 121°C for 15 min. Phenol was added to the medium when the temperature reached approximately 40°C.

A 1 g soil sample was weighed and transferred into a test tube containing 9 mL of sterile physiological saline solution (0.85% NaCl), homogenized, and a serial dilution was performed up to 10^{-5}.

The pour plate method was used to count phenolic degrading bacteria, where 1 mL of each dilution was poured into Petri dishes before the application of nutrient agar medium. Microbial isolation from peat soil samples was conducted in duplicate with dilution factors of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. The samples were incubated at room temperature (25–27°C) for 5 d. Afterwards, the colonies that formed were counted, and the colony-forming units (CFU) per gram of the sample were determined. The principle of this method is that viable microbial cells grow in the medium, multiply, and form visible colonies. These colonies can be directly observed through clear zones that appear and counted without the use of a microscope. The total colony count was determined using petri dishes that met the Total Plate Count (TPC) criteria, where the number of colonies ranged from 25 to 250 per dish, following SNI 2897:2008 (Hartati 2016; Badan Standarisasi Nasional 2008). The formula used to calculate the colony count (CFU/mL) is as follows:

$$\text{CFU/mL} = \frac{\text{Average number of colonies on petri dish}}{\text{Dilution factor}}$$

CFU = colony forming units or the number of colonies

Data Analysis

The collected data were statistically analyzed using One-Way ANOVA. If the analysis of variance showed a significant effect, further comparison was conducted using the Bonferroni test at a 1% significance level to compare the levels of phenolics, tannins, and total

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colony counts of phenolic-degrading bacteria in the peat samples. The relationship among the total colony counts of phenolic-degrading bacteria, phenolics, tannins, moisture content, organic carbon (C-organic), and pH were analyzed using Pearson correlation analysis.

RESULTS AND DISCUSSION

Total Phenolics

The average total phenolic content ranged from 1.38 mg to 2.70 mg GAE/g at sites A, B, C, and D (Figure 1). A one-way ANOVA analysis showed that the site had a significant effect on the total phenolic content ($p < 0.05$). Further analysis using the Bonferroni post-hoc test indicated a significant difference between sites A and C, whereas no significant differences were observed among sites A, B, and D.

Total Tannins

The average total tannin content ranged from 0.35 mg to 0.74 mg TAE/g at sites A, B, C, and D (Figure 2). Based on the one-way ANOVA analysis, site location significantly affected the total tannin content ($p < 0.05$). The Bonferroni post hoc test showed that site A had the highest tannin content (0.74 mg TAE/g), which was significantly different from sites B, C, and D. Additionally, site B exhibited significant differences from sites C and D, while plots C and D showed no significant differences.

Total Colony Count of Phenolic-Degrading Bacteria

The phenol-degrading bacterial colonies was enumerated in dilution series 10^{-3} and 10^{-4}. The average total colony count ranged from 7.62×10^3 to 41.9×10^4 CFU/g, with variations observed across sites

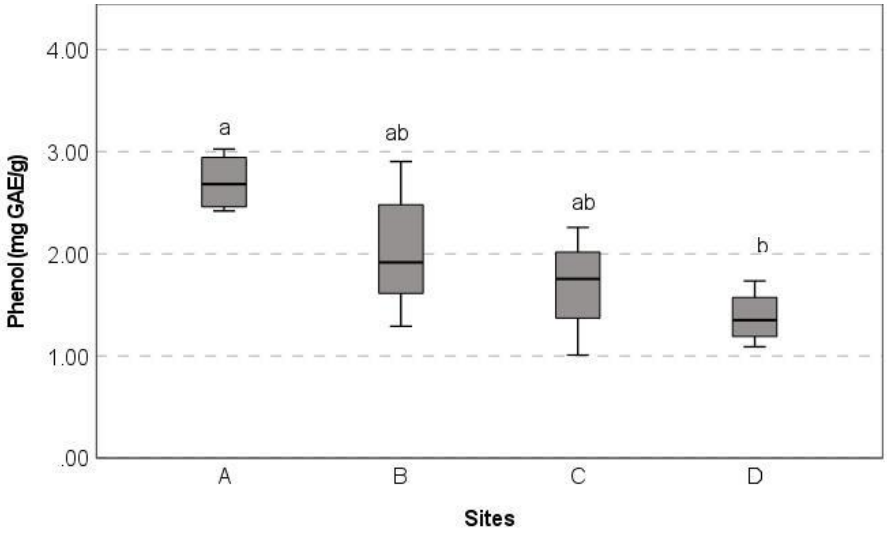

Figure 1 Total phenolic content in sites A, B, C, and D. Identical notations indicate no significant difference (<0.01) based on the Bonferroni test at a 1% significance level.

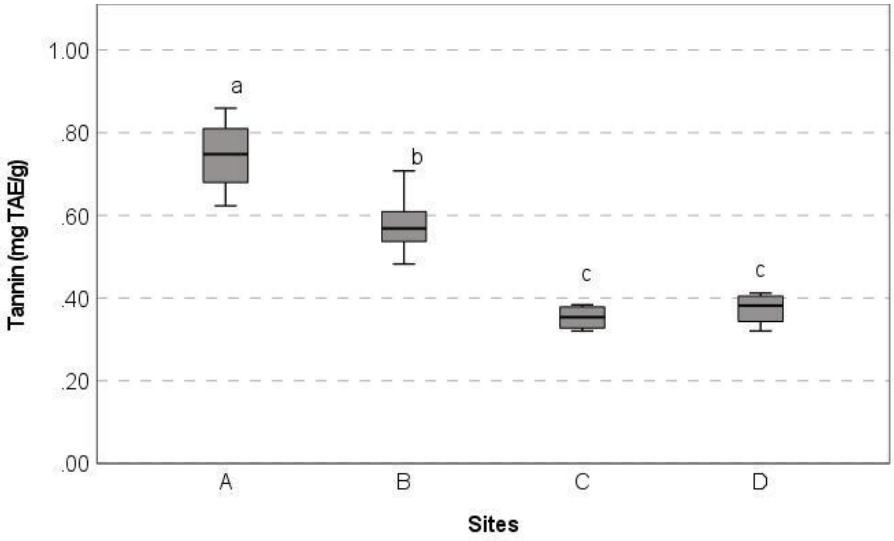

Figure 2 Total tannin content in sites A, B, C, and D. Identical notations indicate no significant difference (<0.01) based on the Bonferroni test at a 1% significance level.

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A, B, C, and D (Figure 3). Statistical analysis using one-way ANOVA demonstrated that the land preparation treatments had a significant impact on the total colony count ($p < 0.05$). Further Bonferroni post hoc testing confirmed a significant difference between site A and the other sites (B, C, and D)

Interpretation

The total phenolic and tannin contents in peatlands are significantly influenced by soil microbial activity, which plays a crucial role in the degradation of phenolics. Phenolics and tannins, as significant components of peatland organic matter, decompose slowly owing to their complex chemical structures, which are difficult to break down. This study indicates that the degradation rate of phenolic compounds is slower at site A than at other sites. This condition is attributed to environmental factors at site A, such as low soil pH, high water content, and limited aeration, which hinder microbial activity. Conversely, soil management and the addition of ameliorants at sites B, C, and D significantly improved the physical and chemical qualities of the peat soil. Based on the findings (Table 1), there are significant differences in the physical properties (e.g., gravimetric water content) and chemical properties (such as organic carbon content and pH) between site A and sites B, C, and D.

The application of manure and lime to sites C and D has been shown to positively impact soil pH, which is a key factor in supporting microbial activity (Kang *et al.* 2018; Nor Suhaila *et al.* 2010). In this study, the soil pH in sites C and D reached 5.69 and 5.45, respectively, which is significantly higher than the pH of site A at only 3.84. An increase in pH is positively correlated with the degradation rate of phenolics by specific bacterial groups (Bing *et al.* 2024), such as *Bacillus* spp. (Zhang *et al.* 2023). Phenol oxidase activity in peatlands occurs optimally within a pH range of 5–6 (Kim *et al.* 2024). The study also revealed that the bacterial colony count at sites B, C, and D was higher than that at site A, which had the lowest pH (<4).

Environmental factors simultaneously influence the activity of phenol-degrading microbes. Table 2 presents the correlation between the number of phenol-degrading bacterial colonies, total phenols, tannins, pH, organic carbon content, and gravimetric water content. Pearson correlation analysis between total bacterial colonies and phenolics content showed a weak and insignificant negative correlation, whereas the correlation with tannin content was significantly negative at −0.59, at the 1% significance level. This implied a moderately strong relationship between tannin content and total bacterial colonies, meaning that the total bacterial colonies decreased as tannin content increased. This finding aligns with that of Olchowik-Grabarek *et al*. (2022), that high concentrations of phenolics (phenols and tannins) can penetrate and disrupt bacterial cell walls and precipitate proteins within the cells. At lower concentrations, phenolics form complex bonds with

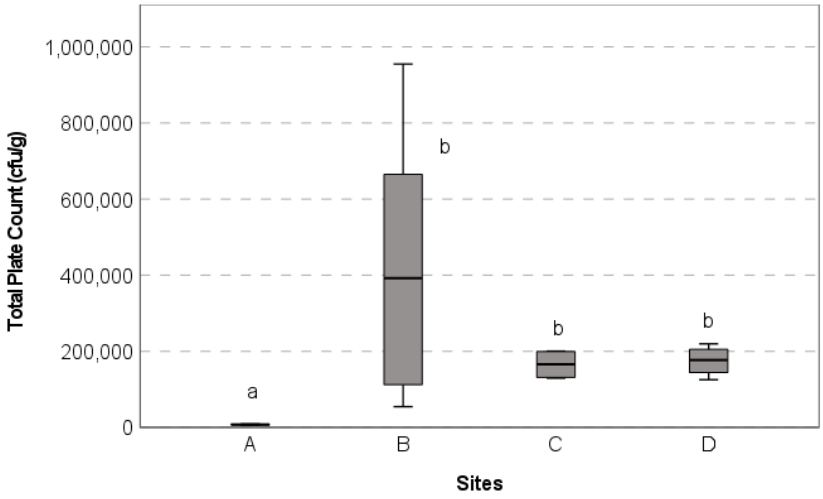

Figure 3 Total colonies of phenol-degrading bacteria in A, B, C, and D sites. Identical notations indicate no significant difference (<0.01) based on the Bonferroni test at a 1% significance level.

Table 2 Peat properties at the study site

Site	Gravimetric water content (%)	C-organic (%)	pH
A	400 ± 27 a	51.28 ± 0.15 a	3.84 ± 0.20 a
B	212 ± 42 b	46.92 ± 1.33 b	4.90 ± 0.37 b
C	199 ± 75 b	40.48 ± 8.16 ab	5.69 ± 0.59 b
D	164 ± 40 b	41.80 ± 3.19 b	5.45 ±0.28 b

Description: Different letter notations in the gravimetric water content and pH columns indicate significant differences based on the 1% Bonferroni test, whereas in the Organic Carbon (C organic) column, they indicate significant differences based on the 5% Games-Howell test.

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Table 3 Pearson correlation test among research parameters

Variable count bacteria	Analysis	Phenols	Tannins	Soil pH	C-organic	Water content
Total plate	*r*	–0.32	–0.59**	0.65**	–0.41	–0.88**
	p-value	0.17	0.006	0.002	0.075	<0.001
Phenolics	*r*		0.80**	-0.73**	0.54*	0.40
	p-value		<0.001	<0.001	0.015	0.081
T annins	*r*			-0.82**	0.70**	0.65**
	p-value			<0.001	0.001	0.002
Soil pH	*r*				–0.70**	–0.67**
	p-value				0.001	0.001
C-organic	r					0.61**
	p-value					0.005

Description: * and ** indicate significance at the 0.05 (5%) and 0.01 (1%) levels, respectively, based on Pearson correlation test.

proteins before penetrating cells, leading to protein precipitation and denaturation, ultimately inactivating essential bacterial enzyme systems. The insignificant relationship between phenolic content and total bacterial colonies was due to the statistically similar phenols content (Figure 1) at sites B, C, and D, rendering its effect on bacterial colony count negligible. Environmental changes affect bacterial growth and survival, as bacteria that are unable to adapt to new conditions die due to unsuitable metabolic conditions (Fruci and Poole 2016).

Pearson correlation analysis between total bacterial colonies and soil pH showed a strong positive correlation of 0.65 at the 1% significance level. This suggests a strong relationship between bacterial colony count and soil pH, meaning that a higher soil pH corresponds to an increase in bacterial colonies. This finding is consistent with that of Irfan (2014), that pH plays a crucial role in nutrient availability for soil microorganisms and enzymatic activity. The optimal pH for most bacteria ranges from a minimum of 4 to a maximum of 9, although some species can thrive under acidic or alkaline conditions. Additionally, pH significantly influences soil microbial activity and development, as microbial activity decreases with decreasing pH. However, in this study, soil pH ranged between 3.84 and 5.69, categorized as strongly acidic to slightly acidic, meaning that bacterial colony counts increased as pH rose.

Pearson correlation analysis between total bacterial colonies and water content revealed a strong negative correlation of –0.88 at the 1% significance level. This indicates a strong inverse relationship, meaning that as the bacterial colony count increased, the water content decreased to a certain threshold in this study. This trend was associated with the peatland management practices at the study site. Prolonged peatland cultivation leads to decreased soil water content and peat thickness. High water content affects phenols-degrading bacterial colonies, as bacterial populations in anaerobic conditions are much lower than those in aerobic conditions. This finding supports the report by Zhong *et al*. (2024), that decreasing water content transforms peatland conditions from anaerobic to aerobic, leading to an increase in bacterial populations.

Pearson correlation analysis between phenol content and soil pH showed a strong negative correlation of –0.73 at the 1% significance level, while the correlation between tannin content and soil pH was significantly negative at –0.82 at the same significance level. This reveals a strong and very strong inverse relationship between phenols and tannins content and soil pH, meaning that these parameters corresponds to lower soil pH (Figure 4). The acidic reaction (pH) of peat soil with a high organic content is due to organic acid compounds, such as humic acid and fulvic acid. Carboxyl (–COOH) and phenol ($-C_6H_5OH$) functional groups in these organic acids contribute to lowering peat soil pH (Ivanov *et al.* 2016; Chahardoli *et al.* 2020). The acidic pH of peat soil is likely unfavorable for the degradation of phenolic compounds. Low soil pH also reduces enzymatic activity, damages enzymes, and inhibits enzymatic processes (Puissant *et al.* 2019).

Pearson correlation analysis between phenolic content and organic carbon (C-organic) showed a significant positive correlation of 0.54 at the 5% significance level, while the correlation between tannin content and C-organic was significantly positive at 0.70 at the 1% significance level. This indicates moderately strong and strong relationships, meaning that higher phenol and tannin contents correspond to higher C-organic levels. This is because of the high organic acid content, which is closely linked to C-organic in the peat soil. C-organic serves as an indicator of organic matter quality, which is strongly associated with the decomposition rate (Jayasekara *et al.* 2024). C-organic is closely related to soil organic matter; a higher organic matter content results in a higher C-organic, indicating a lower peat decomposition rate. Peat at the sapric maturity stage had a lower C-organic due to advanced

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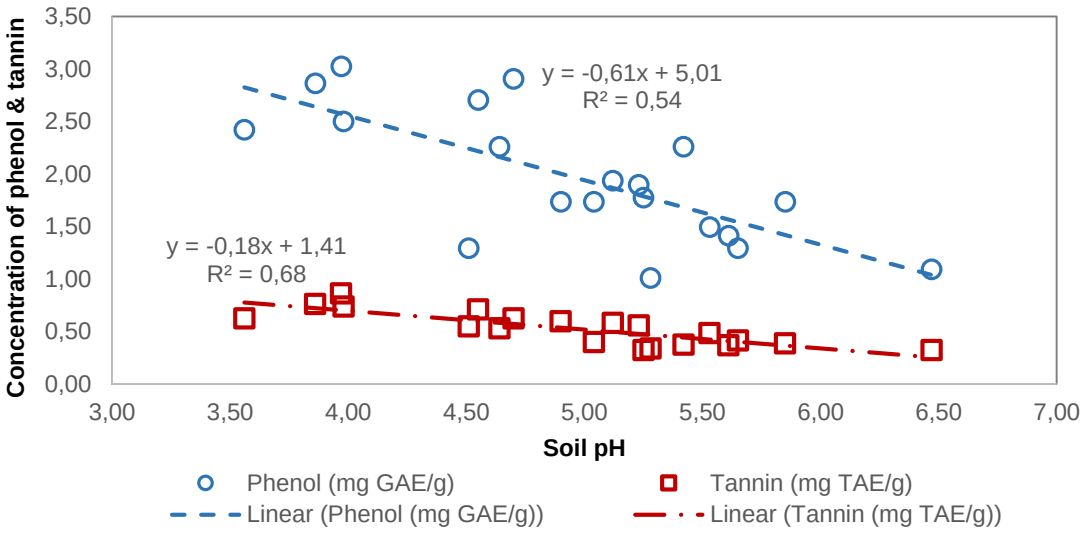

Figure 4 Scatter plot of phenol and tannin concentrations in relation to pH.

decomposition, leading to reduced microbial activity in breaking down organic matter. Phenolics are primarily produced by plants and can enter the subsurface system via root exudates, vegetation leachates, or litter decomposition. Once in the subsurface, these compounds can inhibit microbial activity and alter carbon release dynamics (Fenner and Freeman 2011; Zeng *et al.* 2021).

Although this study did not specifically identify bacterial species in the samples, the literature suggests that the dominant bacteria in peatlands managed for cultivation likely belong to lignocellulolytic groups from the phyla Proteobacteria, Actinobacteria, and Acidobacteria (Ayob *et al.* 2024). The presence of these bacteria is supported by gravimetric water content data, indicating aerobic conditions in the cultivated sites (B, C, and D), which favor the growth of phenolic-degrading bacteria. Specifically, Hadi *et al*. (2024) reported the dominance of phenolic-degrading bacteria over fungi in oil palm cultivation on peatlands. Pearson correlation analysis between water content and soil pH showed a strong negative correlation of −0.67 at the 1% significance level. This suggests a strong relationship, meaning that a higher water content corresponds to lower soil pH. This finding aligns with that of Zhong *et al*. (2024), that decreasing soil water content leads to increasing peat soil pH.

This study indicates that peat soil amelioration to enhance agricultural productivity may negatively affect climate change. Improvements in the physical and chemical properties of peat soil stimulate microbial activity in organic matter decomposition, ultimately increasing carbon emissions. Therefore, optimal water table management and prudent soil amelioration are essential strategies for balancing agricultural productivity with acceptable carbon emissions.

CONCLUSION

The lowest phenolic compound content was found in the ridges and ameliorated sites. Specifically, site C had the lowest total phenol content (1.38 mg GAE/g), while site D had the lowest total tannin content (0.35 mg TAE/g). This was associated with a relatively higher population of phenol-degrading bacteria in these areas. The findings of this study suggest that soil management practices can enhance the activity of phenolics-degrading bacteria, thereby accelerating peat organic decomposition.

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