

EFFECT OF LAND-USE PATTERNS ON SOIL ENZYMATIC ACTIVITY AND MICROBIAL DYNAMICS IN CARBON, NITROGEN AND PHOSPHORUS CYCLING OF WETLANDS IN THE LESSER KHINGAN MOUNTAINS, NORTHEASTERN CHINA

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Abstract. Land use patterns significantly influence soil enzyme activities, microbial communities, and soil physicochemical properties. This study investigates the effects of land use changes on enzyme activity, soil properties, and bacterial communities in soil aggregates within reclaimed and no-tillage wetlands in the Lesser Khingan Mountains, Northeastern China. Forest wetlands converted to reclaimed and no-tillage systems showed declines in soil organic carbon (SOC), total nitrogen, total phosphorus, total potassium, cation exchange capacity, enzyme activities, and available phosphorus. Microbial diversity increased with changes in community composition, including higher relative abundances of Proteobacteria and Actinobacteria. Soil aggregate micro-environments modulated enzyme response intensity, with SOC negatively correlated with Firmicutes, Acidobacteriota, Chloroflexi, and Gemmatimonadota, while available phosphorus (AP) positively correlated with Nitrospirota, Latescibacterota, and Bacteroidota. The results reveal that land use changes have a more pronounced impact on microbial communities than aggregate size, emphasizing the critical role of sustainable land management in maintaining soil health. This study highlights the need for rational development of marshes in the Lesser Khingan Mountains, considering enzyme activities and microbial dynamics in nutrient cycling. These findings contribute to understanding the ecological implications of land use changes and support evidence-based conservation and sustainable management practices.

Keywords: *wetland ecosystem, land use change, carbon cycling, soil biodiversity, sustainable soil management*

Introduction

Wetlands with are critical environments for human survival, serving as key biological habitats and ecosystems. They also represent natural landscapes with rich biodiversity (Xu et al., 2020). Wetlands are crucial for various ecological functions such as water conservation, climate improvement, soil fixation, protection of fertilizer, purification of the environment, and maintenance of biodiversity. They are often referred to as the “kidney of the earth”, serving as a vital component of the global ecosystem. Furthermore, wetlands are considered the “cradle of life” as they provide a suitable habitat for numerous plant and animal species to thrive. Additionally, wetlands are referred to as the “species gene pool”, highlighting their importance in preserving genetic diversity and supporting ecosystem health (Ballut-Dajud et al., 2022). The degradation and pollution of wetlands have become a widespread phenomenon and a significant threat to ecological security. They are a reflection of environmental changes, resulting in reduced biodiversity and the degradation of ecosystem services (Liang et al., 2020; Dar et al., 2022). The causes of wetland functional degradation can be attributed to both natural and anthropogenic factors, with research indicating that human activities have a greater impact on wetlands than natural factors (Chi et al., 2021). Therefore, it is necessary to study the influence of different land use patterns on wetland ecological conditions.

Wetlands, as unique ecosystems located at the transition zone between aquatic and terrestrial environments, are widely recognized for their nutrient retention function and accumulation of organic matter (Liu and Li, 2020). The decomposition of soil organic matter by soil enzymes plays a vital role in the biogeochemical processes within wetland soils, including natural substance cycling (Yang et al., 2022). The activity of enzymes directly affects the rate of material transformation and cycling, thus exerting a significant influence on the functional aspects of wetland ecosystems (Li et al., 2024).

Soil aggregates, as primary carriers of soil elemental biogeochemical cycling, are essential components of soil structure due to their strong bonding forces compared to surrounding particles (Lavelle et al., 2020; Baiano et al., 2021). These aggregates form, stabilize, and decompose continuously, a process influenced by microbial communities (Han et al., 2021). Microorganisms play a key role in shaping the spatial arrangement of soil particles, stabilizing the structure with their cells and metabolic byproducts, and contributing to aggregate breakdown by degrading binding agents (Lakshmi et al., 2020). Additionally, soil microbial communities indirectly impact soil aggregate disintegration through interactions with mycorrhizal fungi, root-associated fungi, viruses, and bacteria, which can influence plants and plant communities (Liao et al., 2022).

The Lesser Khingan Mountains, situated in northeastern China, are known for their dense distribution of cold-region forest wetlands. These mountains possess significant carbon sequestration potential, with an average carbon density higher than that of natural forests in Heilongjiang Province and the national forest vegetation carbon density level (Bing et al., 2022). Human activities have had a notable impact on the forest wetlands in the Lesser Khingan Mountains since the 1970s. The primary forms of human disturbance include land reclamation, drainage, afforestation, and the abandonment of reclaimed land after cultivation. Variations in hydrological conditions and vegetation in natural marsh wetlands lead to differences in litter inputs and root exudates received by the soil, resulting in varying soil carbon pools, especially active carbon (Liu et al., 2022). Soil enzymes are crucial for decomposing and transforming active organic carbon, playing a key role in regulating soil biological processes (Lee et al., 2020). However, there is limited research on the relationship between land use practices and soil enzyme activities, as well as the composition of active carbon, nitrogen, and phosphorus components in natural marsh wetlands in the Lesser Khingan Mountains (Diao et al., 2020). Do different land-use patterns significantly influence the activity of enzymes involved in carbon, nitrogen, and phosphorus cycling in soil aggregates of wetlands in the Lesser Khingan Mountains, resulting in varying levels of enzymatic activity due to changes in soil structure, organic matter content, and microbial community composition? This study focuses on wetland soils in the cold regions of northeastern China, investigating how different land-use practices affect the distribution of soil aggregates and the characteristics of functional microbial communities involved in carbon, nitrogen, and phosphorus cycling. Currently, there is insufficient understanding of the mechanisms by which soil aggregates, enzyme activities, and microbial community responses interact in cold-region wetlands, particularly regarding how land-use changes regulate biochemical cycling through adjustments in aggregate size fractions. Accordingly, we hypothesize: (1) different land-use practices significantly influence the structure and stability of soil aggregates; and (2) variations in aggregate size lead to pronounced spatial differences in the activity of key nutrient-cycling enzymes and in the distribution of associated functional microbial communities. The main objectives of this study are to quantify the activities of enzymes related to C, N, and P cycling across various aggregate-size

fractions, identify and elucidate the functional microbial communities closely associated with these enzymes, and clarify how these processes interact under different land-use scenarios. Our findings will advance the understanding of nutrient-cycling mechanisms in cold-region wetland soils and provide a scientific basis for optimizing land-use and soil conservation practices.

Materials and methods

Site description

The research site is located in the middle section of the Lesser Khingan Mountains in northeastern Heilongjiang Province, China, specifically within the Hongxing Wetland National Nature Reserve in Yichun City (E 128°21'40"–128°53'30", N 48°41'20"–49°11'00"). The reserve covers an area of 111,995 hectares, featuring forests, rivers, ponds, and marshes, with a wetland area of 52,349 hectares and forest and other areas totaling 59,646 hectares. The Lesser Khingan Mountains' wetlands form a critical component of northern temperate forest ecosystems, playing an essential role in water regulation, carbon sequestration, and the maintenance of regional biodiversity. They also serve as important habitats and migration corridors for various wetland-dependent species, highlighting their broader ecological significance. As one of the most intact forested wetland regions in northeastern China, the Hongxing Wetland National Nature Reserve exhibits a high degree of typicality, primitiveness, and representativeness in terms of its vegetation and soil profile, making it pivotal for both ecological research and conservation efforts. These cold-region wetlands are increasingly recognized for their potential in mitigating climate change impacts through carbon storage, while also providing vital ecosystem services such as flood control and nutrient cycling. Therefore, understanding soil development and the underlying biogeochemical processes within these wetlands is of great importance for conservation and sustainable management. The climate of the reserve is characterized by an average annual temperature of 0.7 °C, with extreme minimum and maximum temperatures of –44.5 °C and 35 °C, respectively. The considerable temperature range throughout the year results in accumulated temperatures above 10 °C ranging from 1,700 to 2,000 °C, and the area receives an average of 2,287.8 hours of sunshine annually. Annual precipitation ranges from 500 to 610 mm, contributing to the hydrological dynamics that define wetland characteristics. Due to the high latitude and the combination of natural and human influences, soil development is variable across the landscape. The primary zonal soil type in the reserve is dark brown soil, while non-zonal soils include meadow soil, swamp soil, and peat soil (Ding et al., 2023). This diversity of soil types provides a rich context for examining the interactions between soil physicochemical properties, microbial communities, and wetland functionality under different land use practices.

Experimental design

This study was conducted from June 5 to September 10, 2023, in the Hongxing Wetland National Nature Reserve. During this period, the temperature ranged from 20–23 °C in June and July (with frequent rainfall) and dropped to around 18 °C in early September. Three 40-hectare plots were selected to represent different land-use types: (1) a natural forest swamp wetland (FW), dominated by Xing'an larch (*Larix gmelinii*) and serving as an undisturbed control site; (2) a reclaimed wetland (RW), converted for

soybean cultivation through drainage ditch excavation; and (3) a no-tillage plot (NT), also reclaimed from forest wetland but managed under no-tillage practices, aimed at assessing the effects of no-tillage agriculture on soil properties. To improve sample representativeness and capture spatial heterogeneity within each plot, an "S"-shaped sampling pattern was adopted, with five sampling points established along each transect. At each sampling point, three soil cores (0–20 cm depth) were collected using a shovel with a 15 cm diameter and mixed into one composite sample. Each plot produced five composite samples, resulting in a total of 15 samples across the three plots. After collection, soil samples were placed in clean plastic bags or containers, maintained at or below 4 °C, and transported to the laboratory within 24 hours. In the laboratory, plant residues, animal remains, and stones were removed, and each composite sample was divided into two portions. One portion was sieved through a 2 mm mesh and stored at 4 °C for enzyme activity and microbial diversity analyses, while the other portion was air-dried for measuring soil physicochemical properties (e.g., pH, bulk density, total carbon, and total nitrogen). Enzyme activity assays included polyphenol oxidase, lignin pero, endo-1,4-β-D-glucanohydrolase, xidase, β-1,4-glucosidase, xylanase and cellulase activity. Each composite sample was further split into three technical replicates to enhance the precision of measurements and ensure statistical reliability.

Soil sampling and aggregate size distribution analysis

The remaining soil samples will be air-dried and stored in a 4°C refrigerator for determining the soil aggregate content using an improved dry sieving method (Zhang et al., 2021; Ding et al., 2021). Soil moisture content will be monitored every 6 hours until it reaches approximately 10% to 15%. Once the soil moisture content is within this range, it will be sieved through an 8 mm sieve and then further sieved through nested sets of sieves with aperture sizes of 2 mm, 1 mm, and 0.25 mm, in that order. Each sieving process will involve 200 g of soil and will last 5 minutes. Following the model for aggregate hierarchy development in the reference (Tisdall and Oade, 1982; Ding et al., 2021), the fresh aggregate samples will be sorted into mega-aggregates (> 2 mm), macro-aggregates (2 ~ 0.25 mm), and micro-aggregates (< 0.25 mm). Parameters such as Mean Weight Diameter (MWD) and Geometric Mean Diameter (GMD) will be calculated using formulas from Li et al (2019). The Mean Weight Diameter (MWD) is a measure used to describe the aggregate stability of soil. It quantifies the average size of soil aggregates. The formula to calculate MWD is as follows:

$$\text{MWD} = \sum_{i=1}^n (x_i \cdot w_i) \quad (\text{Eq.1})$$

where:

x_i is the mean diameter of the i th size fraction of soil aggregates.

w_i is the weight fraction of the total sample that corresponds to the i th size fraction.

n is the number of size fractions.

The Geometric Mean Diameter (GMD) is another measure used to describe the average size of soil aggregates. Formula for GMD:

$$\text{GMD} = \exp \left(\frac{\sum_{i=1}^n (w_i \cdot \ln x_i)}{\sum_{i=1}^n w_i} \right) \quad (\text{Eq.2})$$

where:

x_i is the mean diameter of the i th size fraction of soil aggregates (in millimeters).

w_i is the weight fraction of the total sample that corresponds to the i th size fraction.

n is the number of size fractions.

exp denotes the exponential function.

After fractionation, samples for microbial analysis and long-term storage will be kept at $-80\text{ }^{\circ}\text{C}$ until DNA extraction within two days. Samples for chemical analysis will be air-dried, sieved, and stored at room temperature for subsequent analysis.

Soil chemical analysis

Soil pH was determined in a 2.5:1 water/soil suspension using a pH meter. Soil exchange capacity (EC) was determined by the BaCl_2 compulsive exchange method (Gillman and Sumpter, 1986). The Walkley-Black titration method was carried out to determine the soil's organic carbon (SOC) content (Faina et al., 2012). The methods of concentrated H_2SO_4 digestion and Kjeldahl were used to determine the total nitrogen content (TN) of the soil samples (Abujabhah et al., 2016). Total phosphorus content (TP) of the soil samples was determined by HClO_4 and H_2SO_4 digestion molybdenum antimony anti-colorimetry (Pan et al., 2016). Total potassium content (TK) was determined by atomic absorption spectrophotometry (Guan, 1986). The soil's available nitrogen (AN) was measured using the Alkali-diffusion method (Deng et al., 2016). Determination of the available phosphorus (AP) in soil was measured by using NaHCO_3 extraction- Mo-Sb Anti-colorimetry (Mehlich, 1984). Available potassium content (AK) was extracted with $1\text{ mol}\cdot\text{L}^{-1}$ NH_4OAc , and then determined by flame absorption spectroscopy (Bao, 2005). The activities of 16 enzymes were determined using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's instructions (Jiangsu Enzyme Immunoassay Co., Ltd.). The analyzed enzymes included polyphenol oxidase, lignin peroxidase, endo-1,4- β -D-glucanohydrolase, xylanase, β -1,4-glucosidase, cellulase, nitrogenase, ammonia monooxygenase, nitrite reductase, nitrate reductase, nitric oxide reductase, nitrous oxide reductase, N-acetyl-glucosaminidase, leucine aminopeptidase, alkaline phosphatase, fluorescein diacetate hydrolase, coenzyme F420 hydrogenase, and phosphodiesterase (Shi et al., 2023). For each assay, soil enzymes were extracted using a phosphate-buffered saline solution (PBS, pH 7.4) at a 1:5 (w/v) soil-to-buffer ratio. The mixture was homogenized and centrifuged at $10,000 \times g$ for 20 minutes at $4\text{ }^{\circ}\text{C}$, and the supernatant was collected as the enzyme extract. ELISA plates were coated with specific enzyme antibodies provided in the kit, followed by incubation with the enzyme extract. After washing to remove unbound materials, a substrate specific to the target enzyme was added, and the reaction was allowed to proceed under the conditions specified in the kit (e.g., $37\text{ }^{\circ}\text{C}$ for 30 minutes). Enzyme activity was quantified by measuring absorbance at the appropriate wavelength (e.g., 450 nm) using a microplate reader. The enzyme activity was calculated based on standard curves generated using known concentrations of the corresponding enzyme standards. The reaction velocity, proportional to the color intensity, was used to determine enzyme activity, which was expressed in specific activity units (e.g., μmol product formed per gram of soil per hour) or as activity relative to dry soil weight, depending on the enzyme and its function. For enzymes with ecological significance in nutrient cycling, such as nitrogenase and phosphatase, activity indices were normalized to account for soil organic matter content and microbial biomass, allowing for more accurate comparisons across samples. This

approach ensured precise quantification and comprehensive assessment of enzyme activities under different land-use practices.

DNA extraction and high-throughput 16S rRNA gene paired-end sequencing

Genomic DNA of the soil microorganisms was extracted with an Omega E.Z.N.A DNA Kit (Omega Bio-tek, Norcross, GA, USA). The extracted genomic DNA was detected by 1% agarose gel electrophoresis. The PCR was performed on a Geneamp 9700 PCR system (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). The universal primers 515f (5'-gtgccagcmgcg-3') and 907r (5'-ccgtcaattcmtragtt-3') were used to amplify the V3-V4 region of the bacterial 16S rRNA gene. The PCR products were quantified using a QuantiFluor® – ST fluorometer (Promega, Madison, WI, USA), and the samples were adjusted as needed for sequencing. Finally, they were sent to Shanghai Meiji Biotechnology Co., Ltd. (Shanghai, China) for high-throughput sequencing using an Illumina HiSeq 2500 PE250 platform (San Diego, CA, USA). Realtime quantitative PCR (RT-qPCR) analysis was conducted on 0.25 g of fresh soil. The DNA was extracted using the Mo Bio's PowerSoil® DNA Extraction Kit (Qiagen, Germany). The quality and concentration of extracted DNA were measured using NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE) (Horwath, 2017). By using an ABI7500 fluorescence quantitative PCR instrument (Applied Biosystems, USA) and SYBR® Premium Ex Taq Kit (Takara, Japan).

Statistical analysis

We used R v. 3.1.2 (R Core Team, 2013) and SPSS version 16.0 for Windows (SPSS Inc., USA) to generate thermographs and Venn diagrams and to perform one-way analysis of variance (ANOVA). Graphs were created using Origin 2019. Statistical significance was determined at a level of $\alpha = 0.05$ for testing main effects and interactions. Pairwise comparisons of means were conducted using Tukey's honestly significant difference (HSD) test, also with a significance level of $\alpha = 0.05$. To ensure consistency and accuracy in reporting results, statistical differences are explicitly presented only for findings meeting the significance threshold, with effect sizes provided to quantify the magnitude of differences between groups. The term "significant" is strictly used to describe statistically significant results, avoiding overinterpretation of trends or nonsignificant findings. Additionally, the geometric mean (GEA) of all enzyme activities in each soil sample was calculated as a composite enzyme activity index, representing overall soil quality (García-Ruiz et al., 2008). Redundancy analysis (RDA) was conducted using the vegan package in R to explore the relationships between soil enzyme activities and environmental variables. RDA was chosen for its ability to analyze multivariate datasets, enabling the joint assessment of variations in enzyme activities in response to multiple environmental factors. This method is particularly suitable for identifying patterns where dependent variables (enzyme activities) are influenced by several independent environmental variables. The RDA results are reported with a focus on statistically significant associations between the primary ordination axes and environmental factors, highlighting the key drivers of soil biochemical processes without extrapolating beyond the observed relationships. These analyses provide robust insights into the connections between environmental conditions and soil enzyme activity dynamics, offering a statistically grounded interpretation of the data.

Results

Characteristics of soil aggregate composition

The presence of mega-aggregate (> 2 mm) in soil is crucial for maintaining soil structure stability. The distribution of soil aggregates under various land use practices is depicted in *Table 1*. In the provided soil samples, mega-aggregate (> 2 mm) constitute the highest percentage, ranging from 36.33 % to 41.34 % of the total soil particle size distribution. Differences in the mean weight diameter (MWD) values of soil aggregates were observed between FW and NT compared to RW, while the geometric mean diameter (GMD) values showed an opposite trend following changes in land use.

Table 1. Land uses change on aggregate distribution and stability. Values are mean \pm standard deviation

Treatment	Mechanically stable aggregates				
	Me / %	Ma / %	Mi / %	MWD / mm	GMD / mm
FW	41.34 \pm 2.55 a	32.49 \pm 2.04 a	26.17 \pm 2.80 b	3.96 \pm 0.36 a	2.59 \pm 0.11 a
RW	36.33 \pm 2.04 b	30.74 \pm 1.61 a	32.93 \pm 3.78 a	3.32 \pm 0.41 b	2.28 \pm 0.13 b
NT	40.90 \pm 1.97 a	30.95 \pm 2.71 a	28.15 \pm 2.60 ab	4.02 \pm .027 a	2.41 \pm 0.16 a

Different lower letters in the same column indicate significant differences among treatments ($p < 0.05$). Me, mega-aggregate; Ma, macro-aggregate; Mi, micro-aggregate; GMD, geometric mean diameter; MWD, mean weight diameter

Soil physical and chemical properties

Table 2 presents the results of soil physical and chemical properties analysis. The soil sample provided has a slightly acidic pH, with consistent pH values ranging from 6.330 to 6.668 across different treatments. The two-way ANOVA results indicate that land use type had a significant impact on SOC content, while TP content was only significantly affected by RW treatment. Among the tested nutrients, the levels of TN, AN, TP, and TK followed the order: FW > NT > RW. AP content ranged from 70.125 mg·kg⁻¹ (NT) to 35.155 mg·kg⁻¹ (FW), with the RW showing higher AN content compared to FW. AK content was notably higher in abandoned land compared to other soil types. Additionally, EC was significantly higher in FW compared to RW and NT treatments.

Effects of land-use patterns on microbial alpha diversity

The diversity gradient was evident across the fractions, with mega-aggregates exhibiting the highest Chao1 and Shannon index values compared to micro-aggregates (*Table 3*). Community richness and evenness were lowest in the micro-aggregate fractions across all libraries. FW displayed higher aggregate-associated α -diversity than RW ($p < 0.05$), and the Shannon index difference among land use types was statistically significant. The Chao1 index in the micro-aggregate fraction was significantly higher in FW compared to RW ($p < 0.05$). Mega-aggregates consistently showed higher α -diversity than macro-aggregates and micro-aggregates, irrespective of land use type.

Table 2. Soil physical and chemical properties in land uses changes

Index	FW		RW		NT	
	F	P	F	P	F	P
pH	6.572	0.099	6.668	0.361	6.330	0.303
SOC (g·kg ⁻¹)	30.118	< 0.001	19.753	< 0.001	25.318	<0.001
TN (g·kg ⁻¹)	3.543	0.190	1.848	0.842	2.538	0.852
AN (mg·kg ⁻¹)	146.237	17.871	119.318	15.297	149.781	27.575
TP (g·kg ⁻¹)	0.330	0.083	0.058	< 0.001	0.225	0.029
AP (mg·kg ⁻¹)	35.155	2.779	70.125	1.674	50.403	3.235
TK (g·kg ⁻¹)	2.620	0.350	1.840	0.109	2.085	0.479
AK (mg·kg ⁻¹)	153.640	9.957	156.505	4.702	182.348	3.817
EC (mS·cm ⁻¹)	12.722	0.734	8.223	0.868	5.535	0.305

Analysis of variance (Duncan's multiple comparison test) was used to test the significance of differences. pH, the soil pH value. TN, the total nitrogen content of the soil samples. TP, the total phosphorus content of the soil samples. AN, the available nitrogen of the soil samples. AP, the available phosphorus content of the soil samples. SOC, the soil organic carbon content. EC, the exchange capacity in soils. TK, the total potassium content of the soil samples. AK, the available potassium content of the soil samples

Table 3. Diversity index of soil bacterial phylotype diversity of 16S rRNA gene libraries in different land-use patterns

Land Use Types	Aggregates size	Chao1	Shannon
FW	Me	3868.57 ± 12.44 a	6.92 ± 0.07 a
	Ma	3797.16 ± 40.94 b	6.63 ± 0.02 c
	Mi	3377.40 ± 30.17 c	6.58 ± 0.03 c
RW	Me	2605.40 ± 39.52 e	6.10 ± 0.04 d
	Ma	2335.19 ± 18.62 f	5.72 ± 0.06 e
	Mi	1684.86 ± 70.21 g	5.43 ± 0.02 f
NT	Me	3711.84 ± 18.75 b	6.79 ± 0.04 b
	Ma	3448.97 ± 90.03 c	6.21 ± 0.01 d
	Mi	3259.35 ± 15.62 d	6.16 ± 0.07 d

Mean values (means ± SD, n = 3) followed (a, b, c, d, e, f, g) indicate significant difference between land-use types at the p < 0.05 level. FW, Natural Forest Swamp Wetland; RW, Reclaimed wetland; NT, No-Tillage; Me, mega-aggregate; Ma, macro-aggregate; Mi, micro-aggregate

Enzyme activity of soil carbon cycle

The enzyme activities related to carbon cycle in soil aggregates of varying particle sizes under different land use practices are illustrated in *Figure 1*. The FW treatment notably boosts the activities of polyphenol oxidase, lignin peroxidase, and β-1,4-glucosidase in total soil aggregates compared to RW and NT treatments. Specifically, soil aggregates in < 0.25 mm (Mi), the FW treatment shows a significant increase in endo-1,4-β-D-glucanohydrolase activity compared to RW and NT treatments, with increments of 16.32 % and 29.44 %, respectively. Moreover, RW and NT treatments increase xylanase and cellulase activity in < 0.25 mm soil aggregates compared to FW treatment, but decrease these activities in soil aggregates of 0.25 ~ 2 mm (Ma) and > 2 mm (Me).

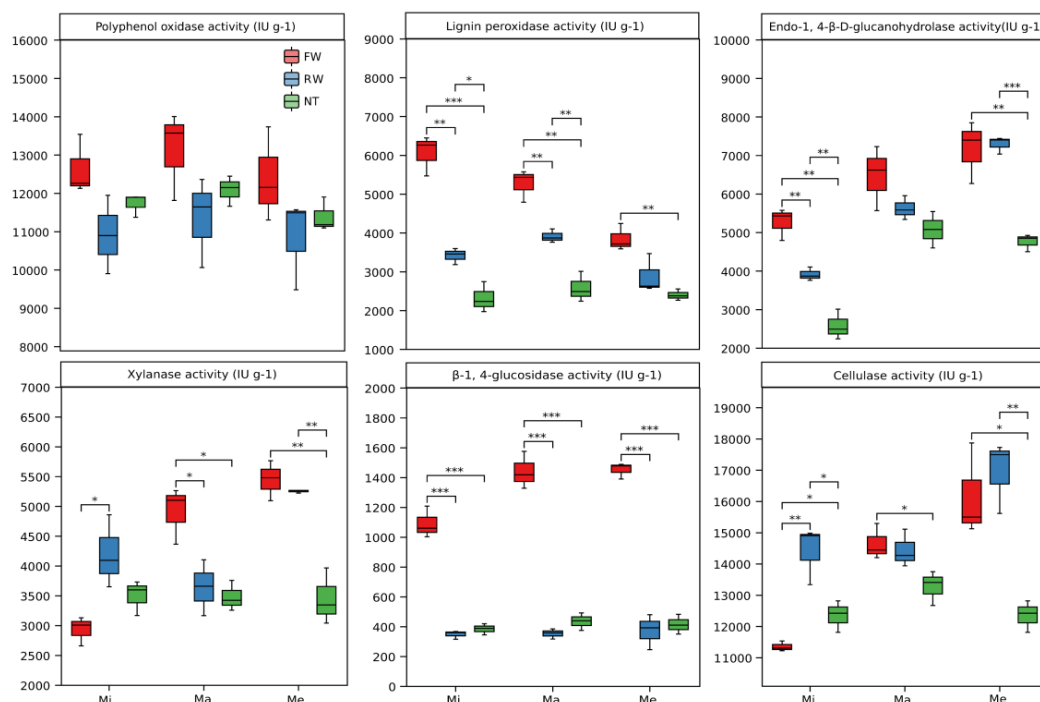


Figure 1. Soil carbon cycle enzyme activities under land-uses patterns. “*” indicates a significant interaction at the 0.05 level; “**” indicates a significant interaction at the 0.01 level; “***” indicates a significant interaction at the 0.001 level. Different letters above the bars indicate statistical differences among treatments in the same size, the horizontal line indicates the difference between two groups. FW, Natural Forest Swamp Wetland; RW, Reclaimed wetland; NT, No-Tillage; Me, mega-aggregate; Ma, macro-aggregate; Mi, micro-aggregate

Enzymes activity of soil nitrogen cycle

Key enzymes such as nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase play a crucial role in controlling the denitrification processes in soil. In this study (Figure 2), nitrogenase activity, ammonia monooxygenase activity, and nitrate reductase activity in total soil aggregates were significantly increased with the NT treatment compared to FW and RW. Similarly, a notable increase in nitrite reductase activity was observed in the macro-aggregate and mega-aggregate under the NT treatment, showing enhancements of 10.78%, 29.86%, 42.40%, and 24.21%, respectively. Moreover, nitric oxide reductase activity and n-acetyl-glucosaminidase activity in the mega-aggregate exhibited significant increases of 33.06 %, 31.38%, 56.27%, and 32.15% with the NT treatment compared to FW and RW treatments. Furthermore, FW and RW treatments resulted in significantly higher leucine aminopeptidase activity in total soil aggregates compared to the NT treatment.

Enzymes activity of soil phosphorus cycle

As shown in Figure 3, the NT treatment significantly increased alkaline phosphatase activity in the mega-aggregate by 17.35% compared to FW treatment and by 13.84% compared to RW treatment. Conversely, the FW treatment significantly increased alkaline phosphatase activity by 15.04% and 13.06% compared to the RW and NT

treatments, respectively. On the other hand, the RW treatment resulted in significant increases in fluorescein diacetate activity and coenzyme F420 hydrogenase activity in both the mega-aggregate and macro-aggregate when compared to FW and NT treatments, with increases of 19.19%, 35.29%, 19.84%, and 28.63% respectively. Furthermore, FW treatment exhibited significantly higher phosphodiesterase activity in the total soil aggregates compared to RW and NT treatments.

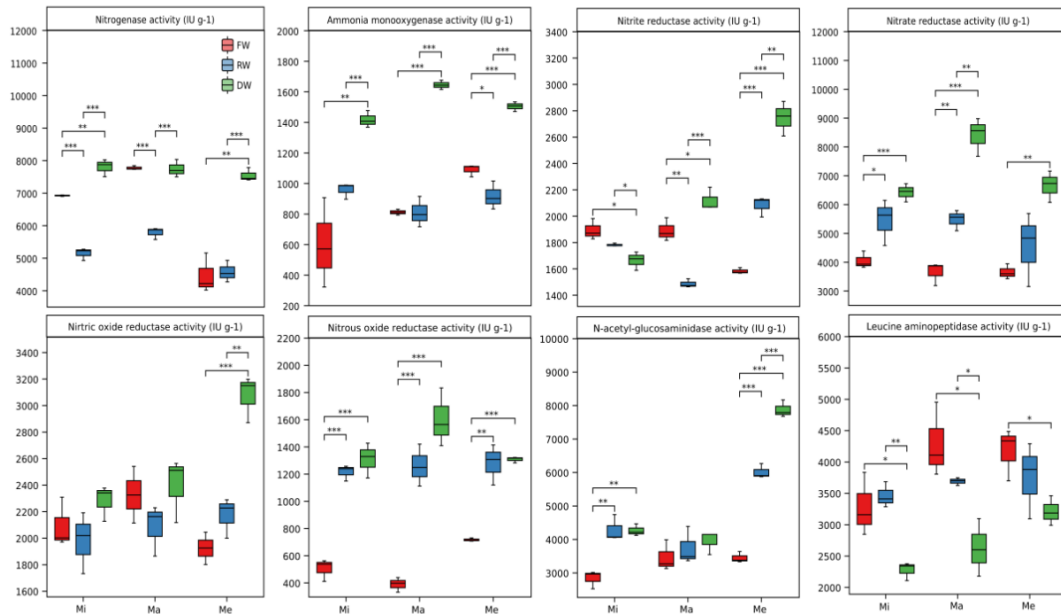


Figure 2. Soil nitrogen cycle enzyme activities under land-uses patterns. FW, Natural Forest Swamp Wetland; RW, Reclaimed wetland; NT, No-Tillage; Me, mega-aggregate; Ma, macro-aggregate; Mi, micro-aggregate. “*” indicates a significant interaction at the 0.05 level; “**” indicates a significant interaction at the 0.01 level; “***” indicates a significant interaction at the 0.001 level

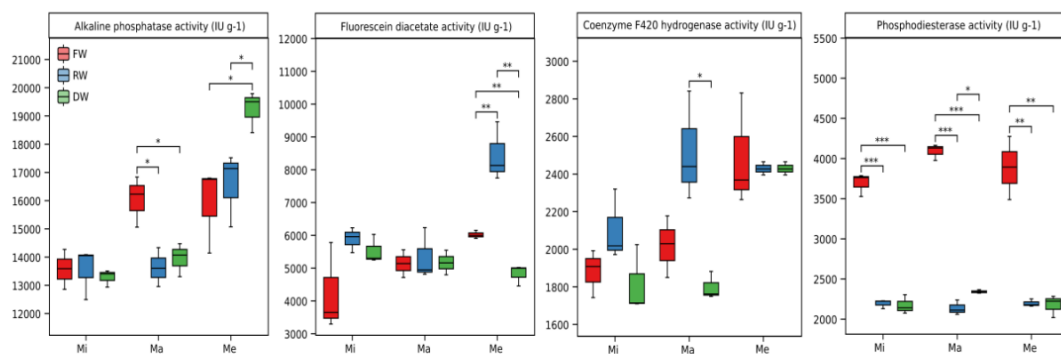


Figure 3. Activities of Alkaline phosphatase, Fluorescein diacetate activity, Coenzyme activity and Phosphodiesterase activity under land uses patterns. FW, Natural Forest Swamp Wetland; RW, Reclaimed wetland; NT, No-Tillage; Me, mega-aggregate; Ma, macro-aggregate; Mi, micro-aggregate. “*” indicates a significant interaction at the 0.05 level; “**” indicates a significant interaction at the 0.01 level; “***” indicates a significant interaction at the 0.001 level

Microbial community composition

As shown in *Figure 4*, high-throughput Illumina sequencing of the V4 region in soil samples generated 1,652,617 reads after quality filtering. A total of 8897 OTUs were detected at a 3% dissimilarity threshold. The sequences were classified into 83 known and 24 potential bacterial phyla, with Proteobacteria and Actinobacteria being prevalent in all soil samples. Variations in the abundances of Acidobacteriota, Chloroflexi, Firmicutes, and Gemmatimonadota were noted in soil mega-aggregates, with MAFW showing higher levels. Nitrospirota, Planctomycetota, Latescibacterota, and MBNT15 phyla were sporadically present at low levels. Additionally, Firmicutes, Gemmatimonadota, and Myxococcaota in the RW group (MeRW, MaRW, and MiRW) exhibited significant differences compared to the FW and NT groups (MeFW, MaFW, MiFW, MeNT, MaNT, and MiNT). Moreover, Bacteroidota had higher abundances in the MiNT group.

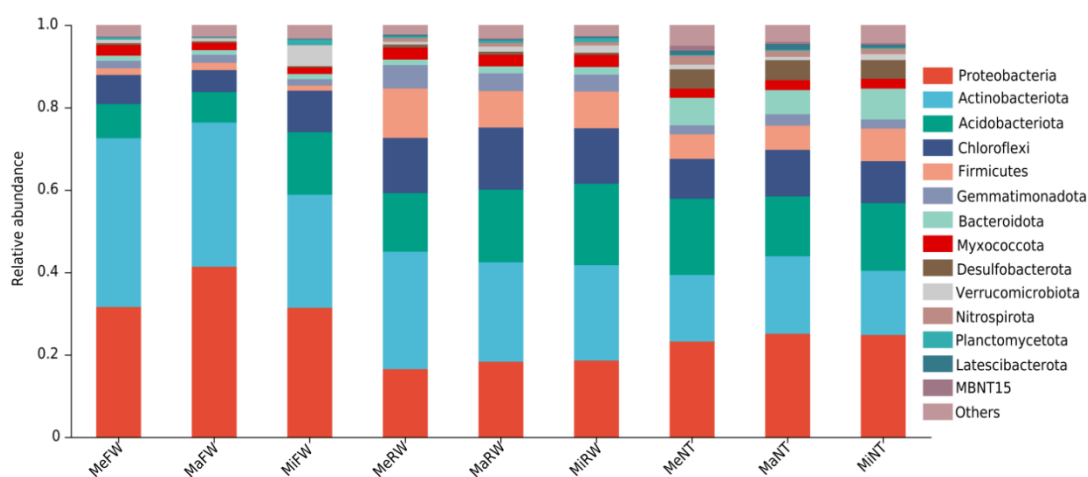


Figure 4. Relative abundance of dominant bacteria phyla on the size of soil aggregates in land uses patterns. Taxonomic classification of OTUs at level of phyla. Abbreviations: MeFW, mega-aggregate of forest wetland; MaFW, macro-aggregate of forest wetland; MiFW, micro-aggregate of forest wetland; MeRW, mega-aggregate of reclamation wetland; MaRW, macro-aggregate of reclamation wetland; MiRW, micro-aggregate of reclamation wetland; MeNT, mega-aggregate of no-tillage; MaNT, macro-aggregate of no-tillage; MiNT, micro-aggregate of no-tillage

Environmental determinants of soil bacteria community

The Redundancy Analysis (RDA) clearly distinguished between different land use patterns (*Figure 5*) and was utilized to analyze the OTU composition of soil bacteria. The cumulative contribution rate of both axes was 84.17% (*Figure 5*). The reclamation wetland groups were found to be distributed in the positive direction of the first sorting axis, whereas the abandoned land groups were distributed in the negative direction of the first sorting axis, indicating that cultivation and natural restoration were the primary influencing factors. Forest wetland with large aggregates (MeFW and MaFW) were positioned in the positive direction of the second sorting axis, while micro aggregates of forest wetland (MiFW) were in the negative direction. Parameters such as AP, AK, and EC showed a positive correlation with the first sorting axis, while SOC, TN, TP, TK, AN,

and pH displayed a negative correlation. The permutation test highlighted that EC, SOC, AP, pH, TN, and AN had the most significant impacts on the structure of the soil bacterial community.

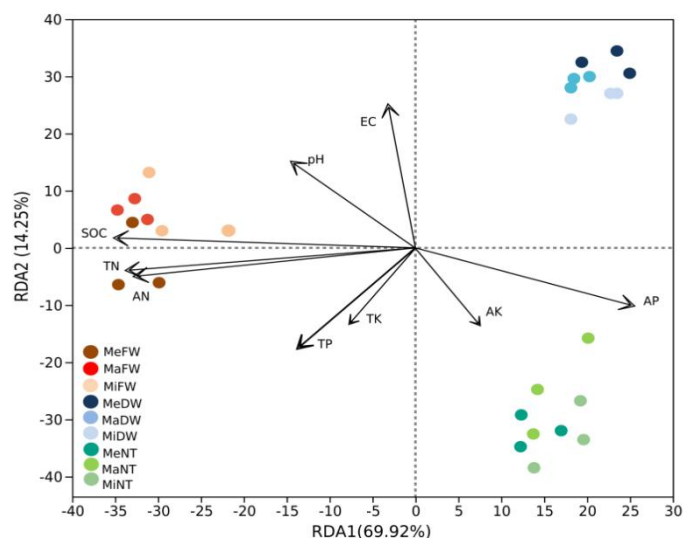


Figure 5. Redundancy analysis (RDA) and spearman correlation heatmap analysis of soil bacteria community in relation to environmental factors in land uses patterns. Abbreviations: MeFW, mega-aggregate of forest wetland; MaFW, maro-aggregate of forest wetland; MiFW, micro-aggregate of forest wetland; MeRW, mega-aggregate of reclamation wetland; MaRW, macro-aggregate of reclamation wetland; MiRW, micro-aggregate of reclamation wetland; MeNT, mega-aggregate of no-tillage; MaNT, macro-aggregate of no-tillage; MiNT, micro-aggregate of no-tillage. pH, the soil pH value. TN, the total nitrogen content of the soil samples. TP, the total phosphorus content of the soil samples. AN, the available nitrogen of the soil samples. AP, the available phosphorus content of the soil samples. SOC, the soil organic carbon content. EC, the exchange capacity in soils. TK, the total potassium content. AK, the available potassium content

Environmental determinants of soil bacteria community

As shown in Figure 6, the correlation heatmap analysis results showed that Proteobacteria was significantly positively correlated with SOC, TN ($r=0.74$, $p=0.0003$), and TP ($r=0.72$, $p=0.0007$). AP was significantly positively correlated with Nitrospirota, Latescibacterota, Bacteroidota, and Desulfobacteriota, while negatively correlated with Actinobacteriota ($r=-0.49$, $p=0.0389$); SOC is negatively correlated with Firmicutes, Acidobacteriota, Chloroflexi, and Gemmatimonadota; TN is negatively correlated with Firmicutes and Latescibacterota.

Mantel test analysis of soil enzyme activity with soil physicochemical properties and Microbial community composition

As shown in Figure 7, AN, Actinobacteriota, Proteobacteria, Bacteroidota and Firmicutes had highly significantly positive correlation with the soil C-cycle, but pH, TP, AP had negative correlation. TK, Actinobacteriota, Bacteroidota and Desulfobacteriota had a positive correlation with the soil N-cycling, but AK, Chloroflexi, Verrucomicrobiota had negative correlation. EC, TN, Proteobacteria and Firmicutes had significant positive correlation with the soil P-cycle, but pH, SOC, TP, AP, Acidobacteriota and Chloroflexi had negative correlation.

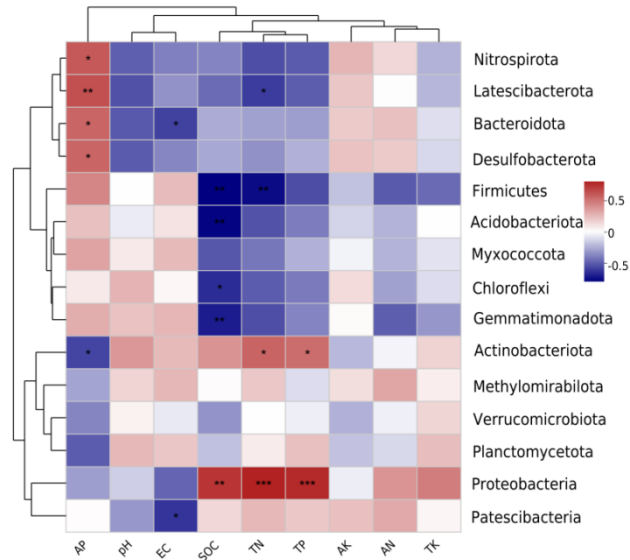


Figure 6. Spearman correlation heatmap analysis of soil bacteria community in relation to environmental factors in land uses patterns. Abbreviations: pH, the soil pH value. TN, the total nitrogen content of the soil samples. TP, the total phosphorus content of the soil samples. AN, the available nitrogen of the soil samples. AP, the available phosphorus content of the soil samples. SOC, the soil organic carbon content. EC, the exchange capacity in soils. TK, the total potassium content. AK, the available potassium content. “*” indicates a significant interaction at the 0.05 level; “**” indicates a significant interaction at the 0.01 level; “***” indicates a significant interaction at the 0.001 level

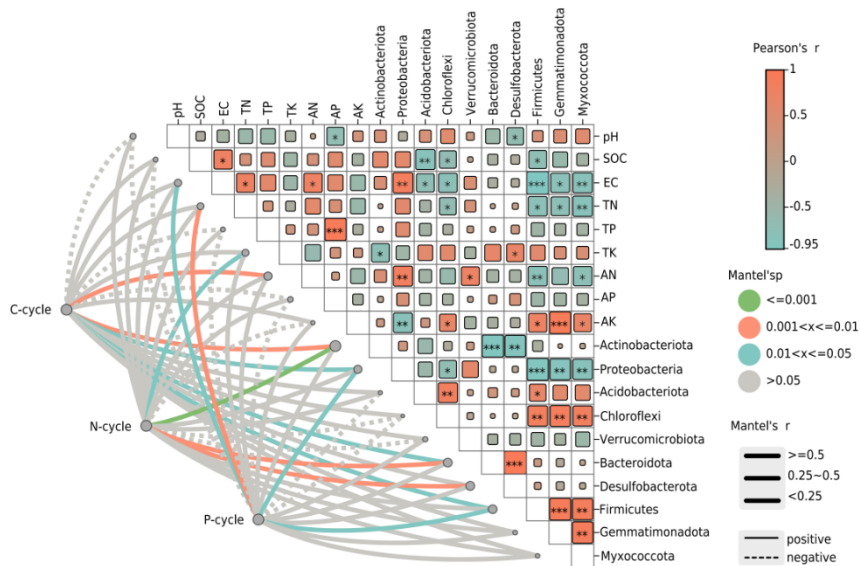


Figure 7. Mantel test analysis based on the relative activity of key enzymes of the soil nutrient cycle. Abbreviations: pH, the soil pH value. TN, the total nitrogen content of the soil samples. TP, the total phosphorus content of the soil samples. AN, the available nitrogen of the soil samples. AP, the available phosphorus content of the soil samples. SOC, the soil organic carbon content. EC, the exchange capacity in soils. TK, the total potassium content. AK, the available potassium content. “*” indicates a significant interaction at the 0.05 level; “**” indicates a significant interaction at the 0.01 level; “***” indicates a significant interaction at the 0.001 level

Discussion

Soil aggregates, as the fundamental unit of soil structure, are porous formations resulting from the clustering and binding of soil mineral particles (Cates et al., 2016). Their stability is crucial for soil structural properties, nutrient cycling, and microbial activities, thereby influencing key nutrient transformation processes such as carbon, nitrogen, and phosphorus recycling (Pinheim et al., 2004; Cosmas and Johan, 2019). Land use patterns directly impact soil aggregates, with forest swamp reclamation decreasing aggregate stability and cultivated land abandonment enhancing it (Zhuang et al., 2017; Chen et al., 2019). Soil aggregate stability, reflecting soil structure stability, directly influences other soil physical and chemical properties (Nath and Rattan, 2017). The average weight diameter (MWD) and geometric mean diameter (GMD) of aggregates are key indicators of stability, with higher values indicating greater aggregation and stability (Cao et al., 2021). Soil organic carbon, a vital cementing material in aggregate formation, is influenced by different land use patterns, subsequently affecting aggregate stability. The levels of aggregates > 0.25 mm are known as soil aggregate structures and are crucial for maintaining soil structure stability. This study observed that the average soil organic carbon content was highest in forest wetlands, followed by abandoned land and cultivated land. Converting forestland to cultivated land led to a decrease in macro-aggregates (> 0.25 mm), as well as a significant reduction in mean weight diameter (MWD) and geometric mean diameter (GMD) values ($P < 0.05$). The conversion of forestland to farmland resulted in a decrease in soil organic carbon content, leading to the dispersion of soil macro-aggregates. Conversely, leaving farmland as abandoned land promoted the formation of intermediate aggregates with larger particle sizes, thereby enhancing soil structure stability. Abandoning sloping farmland decreased the content of micro-aggregates (< 0.25 mm) while promoting the formation of macro-aggregate (2 mm ~ 0.25 mm), ultimately improving soil aggregate stability. These findings align with previous research. Cultivated land must enhance and safeguard soil organic carbon levels through various management practices, such as using organic fertilizer alongside chemical fertilizer, returning straw to the field, and implementing protective farming measures. These actions aim to boost the presence of macro-aggregates in the soil and bolster soil structure stability. In comparison to forest wetlands, cultivated soil exhibits a notable decrease in organic carbon content, along with reduced levels of aggregates > 0.25 mm, as well as MWD and GMD values. This decline may be attributed to factors like root exudates. The lower surface vegetation density and shorter cultivation periods on cultivated land, in contrast to forest land, likely lead to fewer underground root systems. Plants influence soil physical properties and microorganisms through root exudate release and growth activities, impacting soil aggregation. Plant root exudates produce polymer clay that strongly adheres to soil particles, facilitating aggregate formation. In farmland, the limited amount of root exudates results in fewer soil adhesives that promote aggregation, leading to decreased aggregate content and reduced soil aggregate stability.

Previous research has indicated that the composition of soil microbial communities in wetland ecosystems is influenced by a range of physicochemical properties, including soil porosity, temperature, water content, pH, and salinity (Cheung et al., 2018; Xie et al., 2021). The physicochemical characteristics of wetland soil, along with the cycling processes of biological activity, are particularly sensitive to water conditions and the types of surface vegetation present (Zhang et al., 2019). Forest wetlands, as observed in this study, demonstrated higher levels of total nitrogen and total phosphorus compared to

other land use types (Tan et al., 2022). This difference can be attributed to the notable water retention capacity of forest wetlands, which promotes the proliferation and metabolic activity of aerobic microorganisms in the soil, ultimately resulting in a positive accumulation of nutrients in the soil. Arable farmland soils exhibited higher available phosphorus content, potentially due to lower microbial activity. The conversion efficiency of insoluble phosphorus is largely influenced by surface biomass, leading to inadequate absorption and utilization of phosphorus, resulting in its accumulation in the soil (Ibrahim et al., 2022). Studies have shown that wetland and marsh meadow soils in high-altitude cold river wetlands on the Qinghai-Tibet Plateau had significantly higher total nitrogen, total phosphorus, and alkaline nitrogen content compared to other vegetation types (Wang et al., 2020). This is attributed to the seasonal climate changes in northern regions, creating alternating wet and dry conditions in wetland soils that promote microbial decomposition and transformation of soil organic residues, thereby enhancing the utilization of organic nitrogen, phosphorus, and other nutrients (Ding et al., 2020). Soil electrical conductivity is a crucial indicator for assessing soil fertility, influenced by factors such as soil moisture, temperature, organic matter content, salinity, and soil texture (Javed et al., 2022). In forest wetlands, the soil electrical conductivity was notably higher compared to other land use types, attributed to the elevated levels of soil moisture and organic carbon content characteristic of wetland environments (Liu and Li, 2015).

The research indicates that β -1,4-glucosidase activity is susceptible to environmental factors, with a positive correlation observed between its activity and soil organic matter content. Land use practices were found to have a significant impact on soil enzyme activity, with β -1,4-glucosidase playing a crucial role in soil carbon cycle and organic matter decomposition. Soil aggregates from forest wetlands exhibited higher β -1,4-glucosidase activity, likely due to the abundant vegetation in these areas, which includes trees and wetland plants. These plants contribute substantial amounts of organic matter rich in cellulose and other polysaccharides, including β -1,4-glucosidic bonds. Consequently, forest wetland soils provide a greater organic matter pool for β -1,4-glucosidase degradation, leading to enhanced enzyme activity. Lignin peroxidase is essential in soil processes such as organic matter decomposition, lignin degradation, heavy metal removal, and degradation of harmful compounds. It plays a key role in facilitating the cycling and degradation of soil organic matter, thus maintaining soil health and ecosystem functionality. Research analyzing soil organic carbon content and microbial alpha diversity has shown that higher organic matter content in forest wetland soils provides more substrate for lignin peroxidase to catalyze lignin degradation reactions. In contrast, agricultural and fallow lands generally have lower organic matter content due to crop harvesting and soil management practices, leading to organic matter loss. Forest wetland soils host more abundant and diverse microbial communities, including fungi and bacteria capable of producing lignin peroxidase. These rich microbial communities result in more lignin peroxidase producers, thereby enhancing the enzyme's activity. Xylanase plays a critical role in soil carbon cycling by breaking down xylan, a major polysaccharide in plant cell walls, into xylose and glucose monomers. This released glucose serves as a carbon source for soil microorganisms, stimulating microbial growth and activity. The level of xylanase activity can significantly impact the rate and mechanisms of carbon transformation in soil. Higher xylanase activity can expedite xylan degradation, providing more carbon sources for soil microorganisms. This process is vital for organic matter decomposition and carbon cycling in soil, ultimately affecting the stability and persistence of organic carbon. Xylanase exhibits its primary activity in soil

aggregates with particle sizes between 0.25~2 mm (Ma) and >2 mm (Me) in FW. High organic matter content in forest soils provides a greater substrate for enzyme reactions, leading to increased cellulase activity. Furthermore, forest wetland soils typically exhibit a more porous texture compared to agricultural and fallow lands. Smaller to medium-sized soil aggregates offer a larger particle surface area, which enhances enzyme diffusion and substrate contact, thus supporting cellulase activity (Xu et al., 2019). In contrast, agricultural and fallow lands often contain chemical substances like fertilizers, pesticides, and herbicides, which can potentially suppress cellulase activity (Meena et al., 2020).

Soil nitrogenase, the key enzyme in biological nitrogen fixation, consists of ferredoxin and molybdenum-iron protein complexes, with most microorganisms utilizing the molybdenum-iron nitrogenase system. This enzyme plays a crucial role in converting molecular nitrogen (N_2) into ammonia (NH_3), serving as a nitrogen source for organisms and contributing to the synthesis of amino acids and proteins (Mikha and Rice, 2004). Research has shown that high oxygen levels in black soil can reduce nitrogenase activity (Marhan et al., 2007). In this study, it was observed that aggregates with particle sizes 0.25 mm), possibly due to smaller pore size and lower oxygen content in smaller aggregates. Fallow land was found to increase the activity of nitrite reductase and nitrate reductase in aggregates of different sizes, while also affecting the activity of nitric oxide reductase and leucine aminopeptidase (Cayuela et al., 2014). The changes in enzyme activity suggest that land use patterns, particularly under fallow land, have a significant impact on soil denitrification, influencing the structure and function of the soil microbial community. These alterations in the microbial community can impact denitrification processes and microbial interactions, ultimately affecting the efficiency of denitrification in fallow land. Both N-acetyl- β -D-glucosaminidase and leucine aminopeptidase are classified as nitrogen assimilation enzymes. This research demonstrated that N-acetyl- β -D-glucosaminidase exhibited higher activity in micro-aggregate, aligning with findings from Ma et al (2021). This discrepancy in activity levels could be attributed to the enzymes' distinct hydrolytic substrates, influencing microbial community dynamics. Essentially, smaller aggregates may favor microorganisms producing N-acetyl- β -D-glucosaminidase, while medium-sized aggregates may be more conducive to microorganisms generating leucine aminopeptidase. As per the Michaelis-Menten theory, the enzymatic activity is influenced by substrate availability, suggesting that variations in substrate availability across different aggregate sizes could be a contributing factor (Min and Suseela, 2020). Ammonia monooxygenase is crucial in the initial step of nitrification, converting ammonium (NH_4^+) to nitrite (NO_2^-). Denitrification in soil primarily involves nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase, with nitrous oxide (N_2O) emissions mainly stemming from this process. This research uncovered noteworthy variations in the activities of these denitrification enzymes across different aggregate sizes, although no clear trends were evident. Prior studies have demonstrated that elevated soil organic carbon levels enhance the growth of denitrifying microorganisms, with the soil organic carbon content strongly linked to the composition of *nirK* gene communities and the abundance of *nirS* genes in paddy soils (Yang et al., 2021). Interestingly, alkaline phosphatase exhibited greater activity in larger aggregates, indicating reduced substrate availability for phosphatase in such aggregates, necessitating increased enzyme production by microorganisms. This also implies higher phosphorus availability in larger aggregates. Coenzyme F420 and FDA hydrolytic enzymes serve as reliable indicators of changes in soil microorganisms and overall microbial activity. In cultivated farmland, aggregates with particle sizes on < 2 mm (Me) and 0.25 ~ 2 mm (Ma)

displayed heightened activity of these enzymes, suggesting that increased coenzyme F420 and FDA hydrolytic enzyme activity may signal greater abundance and activity of methanotrophic bacteria in the soil (Wang et al., 2020). This, in turn, promotes the breakdown and transformation of organic carbon, resulting in lower organic carbon content in farmland compared to forest wetlands and fallow land (Zhu et al., 2020).

The dominance of Proteobacteria and Actinobacteriota in the soils was expected, as these groups are typically abundant in farmland and wetland soils. However, shifts in land use types markedly influenced the composition of the bacterial community. Following the transition from forest wetlands to farmland and no-tillage systems, there was a significant increase in the relative abundance of Acidobacteriota, Chloroflexi, and Firmicutes. This shift can be attributed to the effects of land use on microbial community composition through factors such as root exudates, litter chemistry, and soil management practices (Ji et al., 2020). Proteobacteria are recognized as one of the most abundant bacterial groups in soils due to their metabolic versatility and role in nutrient cycling (Kalam et al., 2020). The positive relationship observed between Actinobacteria and SOC, TN and TP underscores their ecological importance. Actinobacteria are key decomposers, capable of breaking down complex organic matter, such as plant residues and animal carcasses, into simpler compounds. This microbial activity releases carbon, nitrogen, and phosphorus, which become available for other microorganisms and plants (García-Ruiz et al., 2008). Moreover, Actinobacteria produce extracellular polysaccharides that bind soil particles, enhancing soil structure and forming stable aggregates. These ecological functions make Actinobacteria critical for maintaining soil health and nutrient cycling. Among the Actinobacteria, *Streptomyces* plays a vital role in soil ecosystems through its production of secondary metabolites, which regulate soil microbial communities, promote plant growth, aid nitrogen fixation, and suppress harmful microorganisms (Koza et al., 2020). Many Actinobacteria strains, including *Streptomyces* and phosphate-solubilizing bacteria, exhibit the ability to fix atmospheric nitrogen and solubilize organic and inorganic phosphorus. These processes contribute to increased TN and TP levels in the soil, improving nutrient availability for plants. Actinobacteria also form symbiotic relationships with plant roots, utilizing root exudates and mycorrhizal structures to retain nutrients in the rhizosphere. This symbiosis helps reduce nutrient loss through leaching, thereby stabilizing and enhancing soil nutrient pools (Asghar et al., 2024). Beyond Actinobacteria, other phyla such as Latescibacterota, Nitrospirota, and Bacteroidota also play critical roles in soil nutrient cycling. These groups participate in nitrogen and phosphorus cycling through processes such as mineralization, dissolution, and transformation of phosphorus, converting organic phosphorus into forms readily absorbed by plants. Their interactions with plant roots and rhizosphere processes enhance phosphorus availability, directly linking their activity to soil phosphorus content and plant uptake efficiency (Ding et al., 2021). Different land-use practices, such as agricultural croplands and no-tillage systems, significantly influence the structure and function of soil microbial communities. For instance, management practices like fertilizer application, organic amendments, and pesticide use can indirectly alter the abundance and activity of microbial taxa such as Latescibacterota, Nitrospirota, Bacteroidota, and Desulfobacterota, which are essential for phosphorus cycling. These microbial communities interact with plant roots, influencing nutrient cycling, plant productivity, and overall ecosystem functioning (Liu et al., 2024). In the Lesser Khingan wetlands, changes in land use patterns are expected to affect soil physicochemical properties, leading to shifts in microbial community composition.

Variations in indicators such as SOC, TN, TP, and pH under different land use types influence microbial functional groups and their ecological roles. For example, agricultural practices may enhance the abundance of specific taxa involved in nitrogen and phosphorus cycling, while reducing the diversity or activity of other important microbial groups. These findings highlight the need for sustainable land management practices to maintain soil microbial diversity and optimize ecosystem services, such as nutrient cycling and soil fertility (Teague and Kreuter, 2020).

In wetland ecosystems, plants, soil, and microorganisms are intricately connected, interacting to form a unified organic whole through material cycling and energy flow (Silva and Lambers, 2021). Soil microorganisms are key regulators of soil nutrient status and quality, playing a central role in driving nutrient cycling processes (Yang et al., 2016). The essential elements carbon (C), nitrogen (N), and phosphorus (P) exhibit strong coupling interactions, functioning not only within individual plants but also across entire ecosystems (Lynn et al., 2017). Soil carbon cycling, a crucial metabolic process in global biogeochemical cycles, is closely linked to climate regulation (Deng et al., 2017; Hu et al., 2019). Microorganisms drive soil carbon cycling through activities such as organic carbon fixation, decomposition, methane metabolism, and interconnected processes of carbon migration and transformation, thereby facilitating energy flow and material cycling within ecosystems (Kuypers et al., 2018). Land-use changes significantly influence soil microbial community structure, enzyme activity, and nutrient cycling dynamics. In this study, we observed that shifts in land use from natural wetlands to farmland and no-tillage systems led to significant changes in microbial diversity and enzyme activities associated with carbon cycling. Specifically, bacterial taxa such as Actinobacteriota, Proteobacteria, Bacteroidota, and Firmicutes were strongly correlated with enzyme activities that mediate organic matter decomposition, respiration, and carbon sequestration. These bacteria play critical roles in the breakdown of organic matter, releasing carbon stored in plant residues and soil organic matter, while also contributing to biological carbon fixation and storage (Chen et al., 2017). The observed increase in carbon-related enzyme activities, such as β -glucosidase and cellulase, under no-tillage management suggests that reduced soil disturbance promotes microbial communities capable of maintaining soil organic carbon pools. Our findings align with previous research indicating that microbial taxa like Actinobacteriota and Proteobacteria are essential drivers of soil nutrient cycling, including carbon and nitrogen dynamics (Yang et al., 2016). These microbial groups are particularly sensitive to land-use changes, as they respond to variations in soil organic carbon content, root exudates, and litter chemistry. Furthermore, the coupling of carbon and nitrogen cycles is mediated by these microbial communities, which regulate key processes such as nitrification, denitrification, and organic matter mineralization, processes closely tied to enzyme activity. Enzyme activity serves as a biochemical indicator of microbial function, reflecting the capacity of soil microbial communities to adapt to and process the inputs resulting from land-use changes. By integrating our results with existing literature, we highlight that microbial dynamics and enzyme activities are shaped by land-use practices, which determine soil carbon quality and stability. Practices like no-tillage, which enhance soil organic matter and reduce erosion, promote the activity of functional microbial groups involved in carbon cycling. These findings underscore the importance of sustainable land management in maintaining microbial diversity and optimizing soil carbon cycling, ultimately contributing to improved ecosystem resilience and soil health. This study contributes to a growing body of evidence linking land-use changes to microbial

community structure and enzyme activity, emphasizing the interconnectedness of microbial dynamics and biogeochemical cycles in wetland ecosystems.

Nitrogen cycling is a critical process in terrestrial ecosystems, impacting ecosystem stability and functions (Li et al., 2022). Soil nitrogen cycling involves various interconnected processes driven by microorganisms, including biological nitrogen fixation, mineralization, nitrification, and denitrification (Shukla et al., 2021). Bacterial communities like Actinobacteriota, Bacteroidota, and Desulfobacterota in the soil use nitrate as an electron acceptor through specific enzyme systems and metabolic pathways, converting nitrate to nitrite, nitric oxide, and nitrogen gas (Zhang et al., 2022). These denitrification processes help regulate nitrogen levels in the soil, releasing it into the atmosphere and maintaining nitrogen balance (Liu et al., 2023). The activity of these bacteria is vital for soil health, plant growth, and the global nitrogen cycle (Wang et al., 2022). This study reveals a negative correlation between nitrogen cycling processes and soil pH. Actinobacteriota, Bacteroidota, and Desulfobacterota are key microbial taxa involved in soil denitrification, with soil pH being a primary factor influencing the distribution of soil microbial communities. Avşar and Aras observed a significant negative correlation between pH and the abundance of the *nirK* gene, suggesting that alkaline soil environments inhibit the growth of denitrifying microorganisms, aligning with the current study's findings (Yang et al., 2017; Avşar and Aras, 2020).

Phosphorus, as an essential macronutrient, plays a crucial role in crop growth and ecosystem functioning (Turner et al., 2015). Microorganisms are key regulators of soil phosphorus cycling and availability, solubilizing mineral phosphates and transforming soil-bound phosphorus into forms accessible to plants and microbes (Bergkemper et al., 2016; Li et al., 2021). This study found a positive correlation between soil phosphorus cycling and EC, TN, Proteobacteria, and Firmicutes. Phosphorus primarily exists in the form of phosphate in soil, directly available to plants. EC and TN can impact phosphorus adsorption and desorption processes, with high levels associated with increased ion concentrations and organic matter content. This may lead to competitive adsorption of anions and organic matter onto soil particles, potentially increasing phosphate release into the soil solution and influencing phosphorus cycling (Dai et al., 2020). Microorganisms like Proteobacteria and Firmicutes are important in soil phosphorus cycling, with EC and TN affecting their community structure and activity, thus impacting phosphorus microbial transformations (Li et al., 2020). EC and TN levels in the soil can create favorable environmental conditions that enhance microbial growth and activity. Microorganisms play a crucial role in phosphorus cycling by breaking down organic matter, releasing enzymes, and interacting with plant roots (Zhang et al., 2021; Jiang et al., 2023; Hu et al., 2023). As a result, increased EC and TN levels can boost microbial activity, ultimately affecting phosphorus transformation and cycling. Certain microorganisms like Proteobacteria and Firmicutes have the ability to produce enzymes like phosphatases, which break down organic phosphorus compounds or convert inorganic phosphates into forms that plants can absorb and use (Wang et al., 2023). These bacteria can use both organic and inorganic phosphates as sources of phosphorus and release them into the soil through phosphatase activity (Yu et al., 2021). Some strains within Proteobacteria and Firmicutes can establish symbiotic relationships with plant roots, such as rhizobia and legume nodulation (Liu et al., 2023). Through these relationships, these bacteria interact with plant roots and provide plants with essential phosphorus and nitrogen nutrients through nitrogen fixation and phosphorus

solubilization. This symbiotic interaction plays a key role in promoting phosphorus cycling and enhancing plant uptake of phosphorus (Bergkemper et al., 2016).

The findings of this study emphasize the critical role of microbial communities and enzyme activities in regulating soil carbon cycling and overall soil health under different land-use practices. Sustainable land management practices, such as no-tillage agriculture, not only preserve soil organic matter but also enhance the activity of microbial taxa involved in carbon and nutrient cycling, thereby improving soil fertility and ecosystem resilience. By promoting functional microbial diversity and reducing soil disturbance, these practices can mitigate the negative impacts of land-use changes on soil quality. Incorporating organic amendments, minimizing chemical inputs, and adopting crop rotation systems can further support microbial communities and maintain soil functionality, particularly in wetlands undergoing agricultural conversion. To optimize nutrient cycling in wetlands of the Lesser Khingan Mountains, sustainable development strategies should be implemented. The results also carry important implications for policymakers aiming to balance agricultural productivity with environmental conservation. Policies that incentivize sustainable practices, such as no-tillage, organic farming, and wetland preservation, can encourage the adoption of land management strategies that enhance soil health and carbon sequestration. Additionally, integrating microbial and enzymatic indicators into national soil health monitoring programs could provide a more comprehensive assessment of land-use impacts on soil quality and inform data-driven decision-making. Policymakers should prioritize initiatives that promote education and training for land managers on the benefits of sustainable practices and support research on land-use impacts on soil microbiomes to guide evidence-based conservation and restoration efforts. By aligning agricultural and conservation policies, governments can foster long-term soil sustainability and contribute to global efforts to mitigate climate change.

Conclusions

This study demonstrates that different land-use patterns in the Lesser Khingan Mountains significantly affect soil physicochemical properties, aggregate composition, enzyme activities, and microbial community structure. The conversion of forest wetlands to farmland and no-tillage systems introduces notable anthropogenic disturbances, leading to changes in vegetation types, soil aggregate structure, and microbial community composition. Key bacterial groups such as Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Firmicutes, and Diplomycetes were identified as being sensitive to land-use changes, with their abundance varying by land-use type and aggregate size. Notably, the results indicate that land-use changes primarily influence the structure of soil microbial communities rather than their overall abundance. These findings provide actionable insights for conservation and land-use planning. The development of marshes in the Lesser Khingan Mountains should be approached with caution to preserve soil health and promote a balanced ecosystem. Sustainable land management practices, such as no-tillage and organic amendments, should be prioritized to mitigate the impacts of land-use changes and maintain the functionality of soil microbial communities. Future research should focus on the long-term effects of land-use changes and assess the interactions between land-use strategies, climate change, and soil health to guide evidence-based conservation and sustainable development efforts.

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