EFFECTS OF VEGETATION ON SOIL MICROBIAL BIOMASS CARBON/NITROGEN AND SOIL ENZYME ACTIVITIES IN THE ZHEGAO RIVER WETLAND IN DIFFERENT RESTORATION YEARS

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Abstract. Vegetation restoration is the primary strategy employed for the purification and restoration of habitats in the Zhegao River wetlands. To investigate the changing characteristics of soil microbial biomass carbon/nitrogen (MBC/MBN) and soil enzyme activities in wetlands undergoing different vegetation restoration treatments, those involving reed and willow restoration were studied in 2014 (WR8) and 2021 (WR1), with a native vegetation wetland (NW) used as a control. This study examined changes in the MBC/MBN, sucrase (Suc), urease (Ure), and acid phosphatase (Acp) in these wetlands. The results indicated that during the restoration of Zhegao River wetlands, reed vegetation had a stronger positive effect on soil properties after one year of restoration than that of willow vegetation. After eight years, the effects of willow vegetation restoration were greater than those of reed vegetation restoration. The activities of MBC, MBN, Suc, Ure, and Acp in the reed and willow wetland soils for different years of vegetation restoration decreased with greater soil depth. Thus, reeds and willows have distinct contributions toward enhancing the ecological service functions of the Zhegao River wetlands for different restoration years, which provides a scientific basis for the selection and protection of ecological wetland restoration.

Keywords: *vegetation restoration, soil property, soil depth*

Introduction

Wetlands comprising 6% of the Earth's surface, are the most biodiverse of ecosystems, and provide significant ecological and economic value (Dar et al., 2020). Serious issues resulting from intensifying global climate change and anthropogenic interference encompass reduced wetland areas (Hu et al., 2017), the degradation of system functions (Costanza et al., 2014), and decreased biodiversity (Liu et al., 2019). Consequently, wetland conservation and restoration have garnered broad attention worldwide (Mo et al., 2023). Chaohu Lake, which is one of the five largest freshwater lakes in China, has a watershed area of 12,938 km^2, and is situated between two cities and three counties. Urbanization and other human activities have exerted significant pressures on maintaining the integrity and functions of the Chaohu Lake wetland ecosystem. Among the ten wetland parks that surround Chaohu Lake. Zhegao River Wetland Park boasts the largest natural wetland area and the greatest variety of wetland types. Biorepair measures are primarily implemented to restore ecological functions in degraded wetlands, including vegetation and soil.

Assessing the impacts of various vegetation types on soil quality is critical in wetland ecosystems (Wang et al., 2020). Soil-plant interactions play crucial roles in nutrient cycling, vegetation growth, and long-term sustainability of wetland ecosystems (Baldi, 2021). Alterniflora in the Yancheng Tidal Flats wetland had a greater effect on soil organic carbon (C), total nitrogen (N), and bacterial communities compared to reeds and suaeda salsa (Fang et al., 2020). In contrast, the reed-cattail community demonstrated a stronger capacity to enhance the total soil N and phosphorus (P) enrichment (Ge et al., 2017); thus, alleviating soil eutrophication. Soil quality indices such as soil organic C, total porosity, and bulk density were higher in the robinia community and sahara saesa community of the wetlands in the Yellow River Delta compared to the tamarix community (Zhang et al., 2022). Subsequent to a review of 24,034 publications from 80 websites worldwide, several researchers found that the introduction of trees in wetland communities may significantly enhance soil enzyme activities and promote soil microbial diversity (Huang et al., 2022). Vegetation remediation in wetlands can enhance the soil organic C content, facilitate the restoration of soil microbial communities (Cui, Li et al., 2018), increase soil microbial biomass, and drive wetland vegetation succession (Song et al., 2019). Spatially, wetlands restored using vegetation such as reed, tamarix, and suaeda salsa exhibited a gradual decline in soil quality from inland areas to the coastline (Sun et al., 2022).

Soil microbial biomass serves as the driving force behind organic matter and nutrient cycle turnover (Deng et al., 2019), while soil enzymes play a vital role in the catalytic decomposition of complex organic matter via microorganisms (Yang et al., 2023). Both factors have been extensively investigated to elucidate the functional states of soil ecosystems through the analysis of microbial mass and changes in enzyme activities. For example, Spanish researchers examined variations in soil invertase, urease, β-glucosidase, and other enzyme activities to assess the level of functional rejuvenation in the soil of abandoned irrigation jungle systems (Ananbeh et al., 2019). Other investigators used changes in soil sucrase, urease, alkaline phosphatase activity, and organic matter content to analyze the impacts of saltwater intrusion on soil quality in coastal wetlands (Xian et al., 2019). The relationships between soil physicochemical properties, enzyme activities, and recovery times are often uncertain.

By evaluating the activities of urease, sucrase, and alkaline phosphatase in reed wetland soil after both 3 y and 11 y of restoration, the researchers revealed that the soil quality was improved and its structure was more stable after the restoration of the Yellow River Delta wetland (Ge et al., 2023). In Changting County, China, the soil microbial biomass in degraded wetland ecosystems increased significantly after 4 y of vegetation restoration. However, even after 35 y of vegetation cover, the soil microbial biomass remained lower than that of the native vegetation state (Bai et al., 2019). The soil enzyme activities of a sub-humid desert grassland significantly reduced for one year, after which they and the organic C increased after 4 y of recovery (Gou et al., 2019).

The Zhegao River Wetland Park is among the top ten wetlands that surround Chaohu Lake. Serving as an ecological barrier on the north bank of Chaohu Lake, it features diverse wetland types, including forests, marshes, and riverbanks. Since 2008, the Zhegao River Wetland has prioritized the implementation of vegetation restoration measures to address various levels of wetland degradation. This study aimed to investigate the correlations between vegetation remediation and soil health bioindicators. Specifically, the willow and reed wetlands, which were restored in, 2014 and, 2021, respectively, were selected as research models to address the following questions: (1) How does diverse vegetation in wetland ecosystems influence the changes in soil microbial mass, C, N, and enzyme activities over time? (2) What are the patterns of spatial transfer of soil microbial mass, C, N, and enzyme activities in wetlands with different restoration timeframes? (3) What are the primary factors that drive changes in soil microbial mass, C, N, and enzyme activities in wetlands? This study aims to provide fundamental data on the dynamic changes of soil in the Chaohu wetland toward the establishment of a scientific foundation for ecological environmental management.

Materials and methods

Study area

The Zhegao River Wetland (117°43’39”-117°49’12” E, 31°35’58”-31°39’36” N) is situated in Chaohu City, of Anhui Province, Chia (*Fig. 1*). It falls within the North subtropical humid monsoon climate region, with an average annual temperature that ranges from 15°C to 16°C. The area experiences a >200 days frost-free period and an average annual precipitation of, 1000 mm. The wetland soil is comprised mainly of paddy soil and yellow-brown soil, with a pH range of from 5-8. The total wetland area covers 323.87 hm^2, which represents a wetland rate of 72.51%. Among these, lake wetlands encompass an area of 32.67 hm^2 that accounts for 10.09% of the total wetland area. These lake wetlands are predominantly found along the shores of Chaohu Lake. The dominant plant species include willow, metasequoia, poplar, reed, small fluffy grass, wild alfalfa, annual canopy, etc. (Cao et al., 2019).

Sample plot settings

In September 2022, an investigation was conducted in the Zhegao River wetland, which was divided into three categories: native vegetation wetland (NW), 1-year restoration wetland (WR1), and 8-year restoration wetland (WR8). Willows and reeds were planted in the WR1 and WR8 wetlands. According to the classification standard of wetland in Convention on Wetlands, the sample land is divided into reed wetland and willow wetland. A total of 18 plots, consisting of three survey plots each in the willow and reed wetlands of NW, WR1, and WR8, were established. The survey plots dimensions were 20 m × 20 m, which were spaced more than 100 m apart.

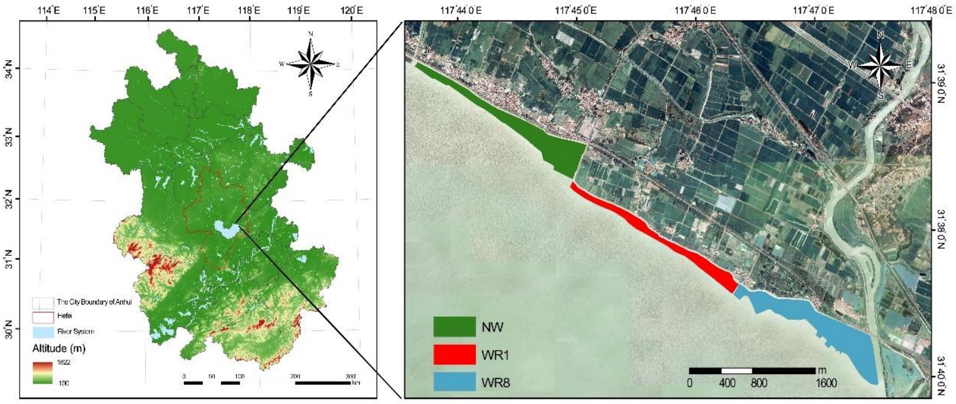

Figure 1. *Location of the study area*

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Sample collection and analysis

In September 2022, soil samples were collected from 18 quadrats. Within each quadrat, five sampling points were selected along an S-line to represent five depth layers: 0-20 cm, 20-40 cm, 40-60 cm, 60-80 cm, and 80-100 cm. Soil drilling was employed for sample extraction, and a total of 90 samples from the same depth layer were combined. The samples were transferred to the laboratory where plant roots, stones, and other debris were removed. A 500 g fresh soil subsample was stored in a refrigerator at 4°C for the determination of microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and water content (WC). The remaining soil samples were air-dried, ground, and sieved through a 2 mm mesh for the determination of soil enzyme activities, soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), and other parameters.

The determination of the soil water content (WC) was performed using the drying method, while the soil bulk density (BD) was measured using the ring knife technique. The soil organic carbon (SOC) was quantified by external heating with concentrated sulfuric acid-potassium dichromate. Further, the TN and TP were measured using concentrated sulfuric acid-hydrogen peroxide deboiling and an automatic discontinuous chemical analyzer (Yu et al., 2016). Chloroform fumigation extraction was used to determine the MBC and MBN (Makarov et al., 2016). The soil urease (Ure) was determined using the phenol-sodium hypochlorite colorimetric technique; soil sucrase (Suc) using the 3,5-dinitrosalicylic acid colorimetric method; and soil acid phosphatase (Acp) via the phenyldisodium phosphate colorimetric procedure (Zheng et al., 2022).

Statistics analysis

Data statistical analysis and processing were conducted using Excel and SPSS 26.0 software. To determine the significance of soil indicators under different remediation years, independent sample t-tests, one-way ANOVA. The effects of vegetation, restoration years, soil layer and their interactions on soil indexes were analyzed by Repeated-measures ANOVAs. Pearson correlation analysis was utilized to investigate the relationships between the basic soil physicochemical properties, microbial biomass, and enzyme activities. Redundancy analysis was performed to examine the relationships between the soil physicochemical factors, microbial biomass, and enzyme activities, using Canoco 5 software. The results were visualized with Origin 2022 mapping software.

Results

Changes in soil physicochemical properties in willow and reed wetlands under different restoration years

Changes in the soil pH, WC, BD, SOC, TN, and TP contents in willow and reed wetlands under different restoration years are shown in *Table 1* and *Figure 2*. There were no significant differences observed in the soil pH between the reed and willow wetlands ($p > 0.05$). However, the soil WC was significantly higher in the reed wetlands compared to that of the willow wetlands ($p < 0.05$). Similarly, there were no significant differences identified in the soil BD between the two wetland types ($p > 0.05$), except for the reed wetlands after one year of restoration, when it was significantly lower. The soil pH, WC, and BD exhibited increases with greater soil depth.

Moreover, the SOC and TN contents of the willow and reed wetlands after one (WR1) and eight (WR8) years of restoration were significantly lower compared to the natural wetland (NW) plots ($p < 0.05$). The TN content of the 20-40 cm soil layer (except for the WR1 plots in the reed wetlands) was typically higher than that in the NW plots. The willow wetlands exhibited higher SOC and TN contents in WR8 than in WR1. In contrast, the reed wetlands demonstrated higher SOC and TN contents in WR1 than in WR8. Additionally, the TP content of the WR1 plots in both the willow and reed wetlands was significantly higher than that in the NW plots. In the reed wetlands, the TP content of the 0-20 cm soil layer of the WR8 plots was significantly higher than that in the NW and WR1 plots; however, it decreased significantly in the 20-40 cm soil layer. Moreover, the TP content of the 40-100 cm soil layer was lower than that in the NW and WR1 plots. Overall, the SOC, TN, and TP contents of each soil layer in the wetlands under different restoration years exhibited a downward trend with greater soil depth.

Table 1. *Changes of physical and chemical properties of soil in willow and reed wetlands with different restoration years*

Wetland	Sample plot	Soil depth (cm)	pH	BD (g·m^{-3})	WC (%)	SOC (g·kg^{-1})	TN (g·kg^{-1})	TP (g·kg^{-1})
Willow wetland	NW	0-20	6.27±0.14 Bd	2.00±0.10 Bc	6.69±0.30 Bd	96.90±4.25 Aa	2.59±0.55 Aa	8.44±0.44 Aa
		20-40	6.69±0.05 Bc	2.06±0.08 Bbc	7.16±0.20 Bd	97.39±5.49 Aa	2.27±0.39 Aa	8.10±0.21 Ba
		40-60	7.34±0.07 Ab	2.16±0.12 Aab	9.10±0.25 Bc	89.98±3.89 Aa	1.99±0.50 Aa	8.14±0.29 Aa
		60-80	7.56±0.17 Aab	2.24±0.09 ABab	13.66±0.36 Bb	67.24±5.02Ab	1.83±0.29 Aa	7.87±0.20 Aba
		80-100	7.71±0.18 Aa	2.30±0.05 ABa	15.79±0.27 Ba	66.25±8.06 Ab	1.66±0.16 Aa	7.70±0.41 Aa
	WR1	0-20	5.52±0.13 Ad	2.29±0.04 Aa	5.75±0.32 Cd	70.20±5.46 Ba	1.36±0.04 Ca	9.02±0.20 Aa
		20-40	6.73±0.07 Ac	2.33±0.03 Aa	6.66±0.19 Bc	38.56±2.37 Cb	1.20±0.11 Bab	8.71±0.24 Aa
		40-60	7.67±0.16 Aa	2.30±0.04 Aa	7.64±0.25 Cb	31.64±2.82 Cbc	1.15±0.07 Bab	8.27±0.20 Ab
		60-80	7.35±0.11 Ab	2.34±0.05 Aa	8.69±0.25 Ca	26.70±2.24 Cc	1.13±0.15 Bab	8.13±0.07 Ab
		80-100	7.63±0.12 Aa	2.34±0.03 Aa	8.90±0.12 Ca	24.22±2.52 Bc	1.01±0.11 Bb	7.91±0.18 Ab
	WR8	0-20	6.63±0.15 Ac	1.94±0.03 Bb	8.72±0.16 Ad	76.63±3.70Ba	1.97±0.05 Aba	8.31±0.19 Aa
		20-40	6.75±0.05 Bc	1.99±0.03 Bab	13.14±0.31 Ac	53.39±4.37 Bb	1.91±0.09 Aa	7.87±0.16 Bb
		40-60	7.20±0.06 Ab	2.12±0.03 Aa	14.18±0.27Ab	48.94±4.84 Bb	1.52±0.04 Bbc	7.83±0.26 Ab
		60-80	7.35±0.10 Aab	2.03±0.1 Bab	18.75±0.20 Aa	40.54±1.85 Bc	1.55±0.07 Ab	7.64±0.14 Cb
		80-100	7.43±0.07 Aa	2.09±0.04 Ba	19.14±0.36 Aa	34.11±2.10 Bc	1.38±0.09 Ac	7.52±0.20 Ab
Reed wetland	NW	0-20	5.66±0.12 Ad	2.06±0.06 Ac	19.03±0.20 Ac	88.00±4.58 Aa	3.60±0.41 Aa	8.13±0.21 Ca
		20-40	6.30±0.02 Bc	2.22±0.06 Ab	21.11±0.21Ab	83.22±3.57 Aa	2.54±0.02 Bb	7.45±0.14 Bb
		40-60	6.79±0.09 Bb	2.24±0.04 Aab	22.41±0.48 Aa	81.08±6.26 Aa	2.48±0.14 Ab	7.37±0.26 Bb
		60-80	7.26±0.06 Aa	2.23±0.03 Aab	22.80±0.28 Aa	77.12±9.46 Aa	2.38±0.44 Abc	7.16±0.22 Abc
		80-100	7.37±0.09 Ba	2.30±0.05 Aa	22.89±0.30 Aa	73.66±7.30 Aa	1.79±0.24 Ac	6.79±0.20 Bc
	WR1	0-20	6.32±0.10ABd	1.38±0.02 Cd	16.38±0.17 Cd	81.08±4.89 Aa	2.98±0.21 Aa	8.90±0.36 Ba
		20-40	7.24±0.07 Ac	1.44±0.05 Ccd	18.37±0.26 Cc	64.27±4.89 Bb	2.84±0.08 Aa	8.49±0.35 Aab
		40-60	7.33±0.09 Abc	1.57±0.06 Cb	20.98±0.26 Bb	55.37±4.59 Bbc	2.35±0.16 Ab	8.03±0.20 Ab
		60-80	7.5±0.08 Aab	1.53±0.01 Cbc	21.93±0.27 Ba	49.93±3.48 Bc	1.51±0.07 Bc	7.43±0.17 Ac
		80-100	7.66±0.08 Aa	1.72±0.02 Ba	22.19±0.18 Aa	34.61±1.24 Bd	1.50±0.18ABc	7.32±0.14 Ac
	WR8	0-20	5.85±0.21 Ad	1.92±0.04 Bb	17.94±0.25 Bd	48.95±1.72 Ba	1.84±0.08 Ba	9.74±0.27 Aa
		20-40	6.79±0.02 Ac	2.03±0.03 Bb	19.85±0.32 Bc	45.98±2.59 Ca	1.65±0.10 Ca	7.79±0.25 Bb
		40-60	7.12±0.02 Bb	2.05±0.07 Bb	19.93±0.18 Cc	45.15±4.65 Ba	1.33±0.13 Bb	6.72±0.18 Cc
		60-80	7.30±0.06 Aab	2.01±0.08 Bb	20.93±0.17 Cb	44.49±1.81 Ba	1.34±0.08 Bb	6.77±0.17 Bc
		80-100	7.41±0.04 Ba	2.27±0.04 Aa	22.68±0.33 Aa	32.63±1.44 Bb	1.16±0.22 Bb	6.66±0.15 Bc

Different capital letters indicated significant difference in restoration years of the same soil layer ($p < 0.05$); Different lowercase letters indicated significant difference between different soil layers in the same restoration years ($p < 0.05$)

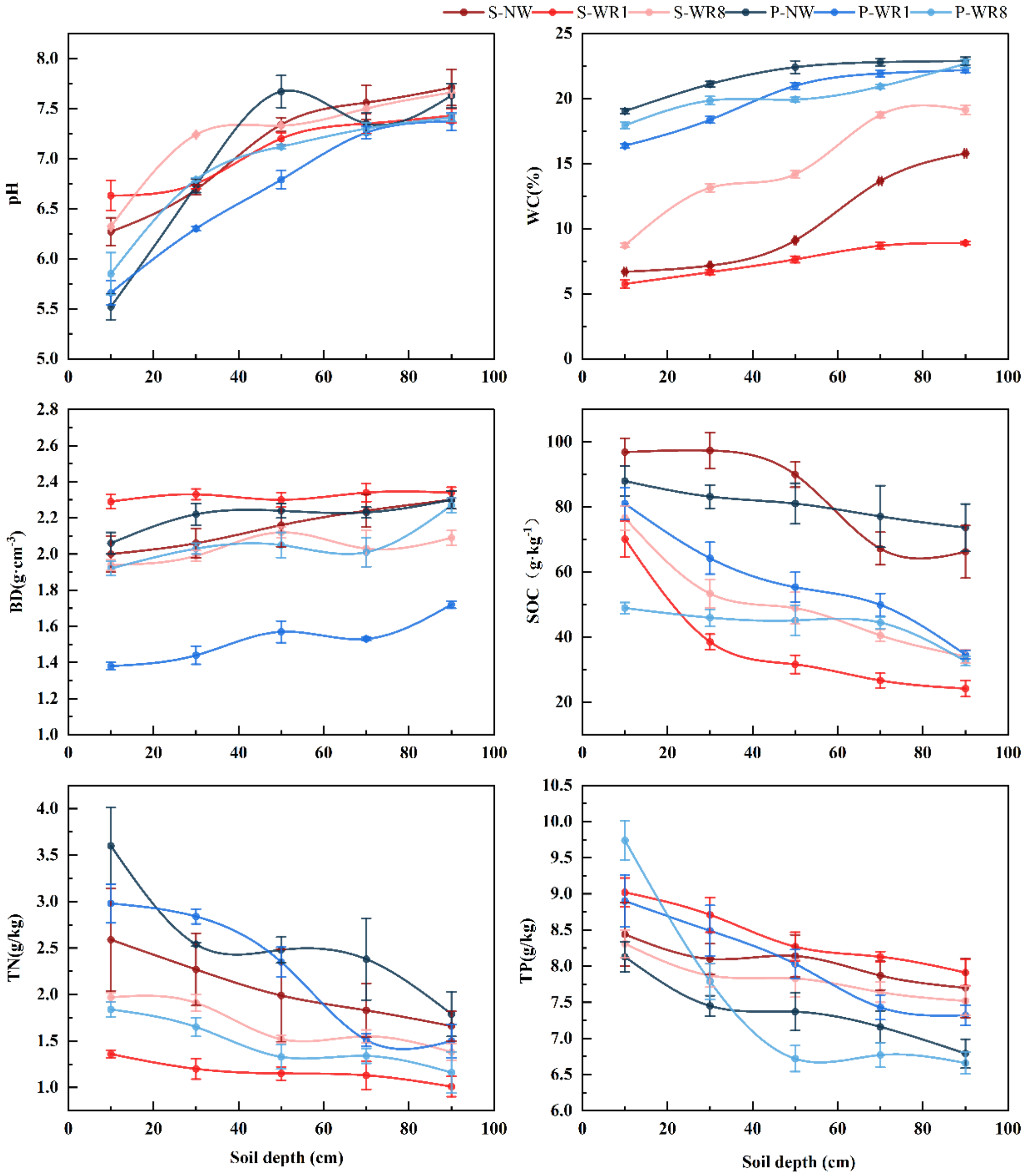

***Figure 2.** Soil physicochemical properties of samples under different remediation years in willow and reed wetlands. S-NW refers to native vegetation plot of willow wetland; S-WR1 refers to the 1-year restoration of willow wetlands; S-WR8 refers to the 8-year restoration of the willow wetland; P-NW refers to native vegetation plot of reed wetland; P-WR1 refers to 1-year reed wetland restoration; P-WR8 refers to the 8-year reed wetland restoration; same below*

The results of a three-factor analysis of variance (ANOVA) indicated (*Table 2*) that the influence of the soil layer × restoration years interaction on the soil BD was not significant ($p > 0.05$). However, the influences of vegetation, soil layer, and restoration years, as well as the interactions between the vegetation and soil layer, vegetation and restoration years, soil layer and restoration years, and all three factors on other physicochemical soil properties, were significant ($p < 0.05$).

***Table 2.** Three-factor ANOVA analysis of the effects of different vegetation, restoration years, and soil layers on wetland soil physicochemical factors*

Factor	**pH**		**WC**		**BD**		**SOC**		**TN**		**TP**	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Vegetation	109.226	0.000	19982.242	0.000	256.886	0.000	6.044	0.017	60.024	0.000	54.153	0.000
Soil layer	405.691	0.000	1072.509	0.000	32.588	0.000	92.947	0.000	34.071	0.000	70.181	0.000
Restoration years	19.157	0.000	1027.367	0.000	100.91	0.000	396.867	0.000	61.82	0.000	32.845	0.000
Vegetation × soil layer	18.629	0.000	92.513	0.000	2.574	0.047	8.532	0.000	4.463	0.003	12.368	0.000
Vegetation × restoration years	8.315	0.001	973.251	0.000	340.395	0.000	48.167	0.000	39.233	0.000	3.494	0.037
Soil layer × restoration years	10.593	0.000	29.292	0.000	1.324	0.249	6.102	0.000	2.197	0.040	4.952	0.000
The interaction of the three	4.857	0.000	78.711	0.000	2.544	0.019	3.651	0.002	2.413	0.025	4.704	0.000

Spatiotemporal variation of soil microbial biomass in willow and reed wetlands under different restoration years

Changes in the soil MBC and MBN contents across the different soil layers of the willow and reed wetlands are depicted in *Figure 3*. The MBC content of the 0-20 cm soil layer in the NW plot implied a higher concentration in the willow wetland compared with the reed wetland (a 42.87% increase). Furthermore, for the 0-100 cm soil layers, the MBC and MBN contents of each soil layer revealed that the reed wetland contained higher concentrations than the willow wetland in the NW and WR1 plots, with an increase rate that ranged from 1.81% to 736.37%. Conversely, in the WR8 plot, the willow wetland exhibited higher concentrations compared to the reed wetland, with an increase rate that ranged from 4.71% to 65.42%.

The MBC content of the willow and reed wetlands exhibited significant differences in the 0-20 cm soil layer ($p < 0.05$), but not in the 20-100 cm soil layer ($p > 0.05$). The MBN content showed no significant differences in the 0-20 cm and 80-100 cm soil layers ($p > 0.05$); however, there were significant variations in the 20-80 cm soil layer ($p < 0.05$). In the WR1 plot, significant differences in the MBC and MBN contents between the willow and reed wetlands were observed in all soil layers from 0-80 cm ($p < 0.05$), except for the 20-40 cm soil layer where no significant variance in MBC content existed between the two wetlands. No significant differences were found for the MBC and MBN content between the two wetlands in the 80-100 cm soil layer ($p > 0.05$). In the WR8 plot, significant variances in MBC and MBN content between the willow and reed wetlands were observed in the 40-60 cm soil layer ($p < 0.05$), but no significant differences were found in the other soil layers within the 0-100 cm range ($p > 0.05$).

Vertical profiles of the soil MBC and MBN contents in both the willow and reed wetlands exhibited significant differences in the 0-40 cm soil layer ($p < 0.05$), with insignificant variations being observed in the 40-100 cm soil layer ($p > 0.05$) (*Fig. 4*). The MBC and MBN contents in the subsoil of the NW and WR1, WR1 and WR8, and NW and WR8 differed significantly in the 0-20 cm soil layer for both the willow and reed wetlands ($p < 0.05$).

In the willow wetland the MBC content in the WR1 plots was 10.28% of the NW, while in the WR8 plots it was 49.9%. The MBN content in the WR1 plots was 44.06% of the NW, whereas in the WR8 plots it was 81.45%. In the reed wetland the MBC content in the WR1 plots was 28.61% of the NW, while in the WR8 plots it was 46.3% of the NW. The MBN content in the WR1 plots was 57.71% of the NW, while in the WR8 plots it was 39.15% (*Table 3*).

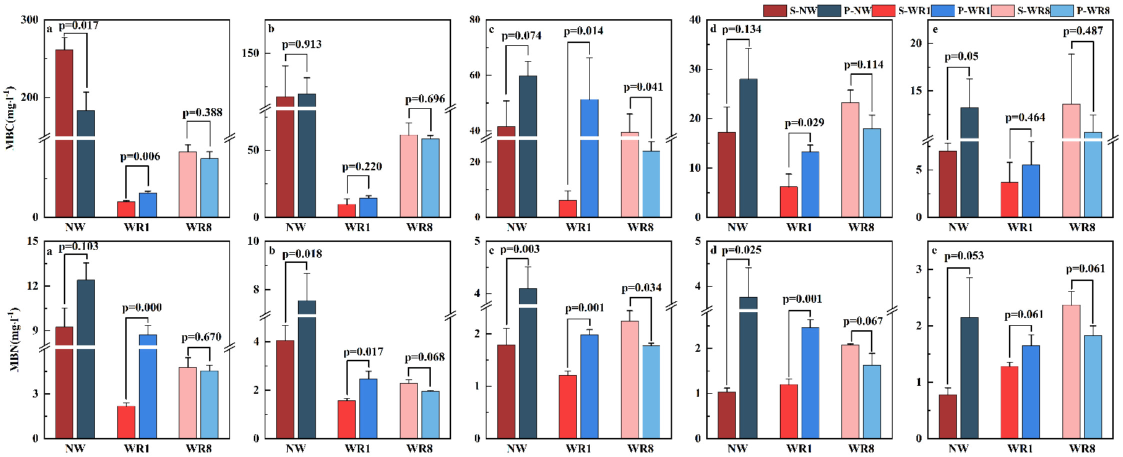

***Figure 3.** Changes in soil microbial biomass for willow and reed wetlands at different restoration years. (a) 0-20 cm; (b) 20-40 cm; (c) 40-60 cm; (d) 60-80 cm; (e)80-100 cm*

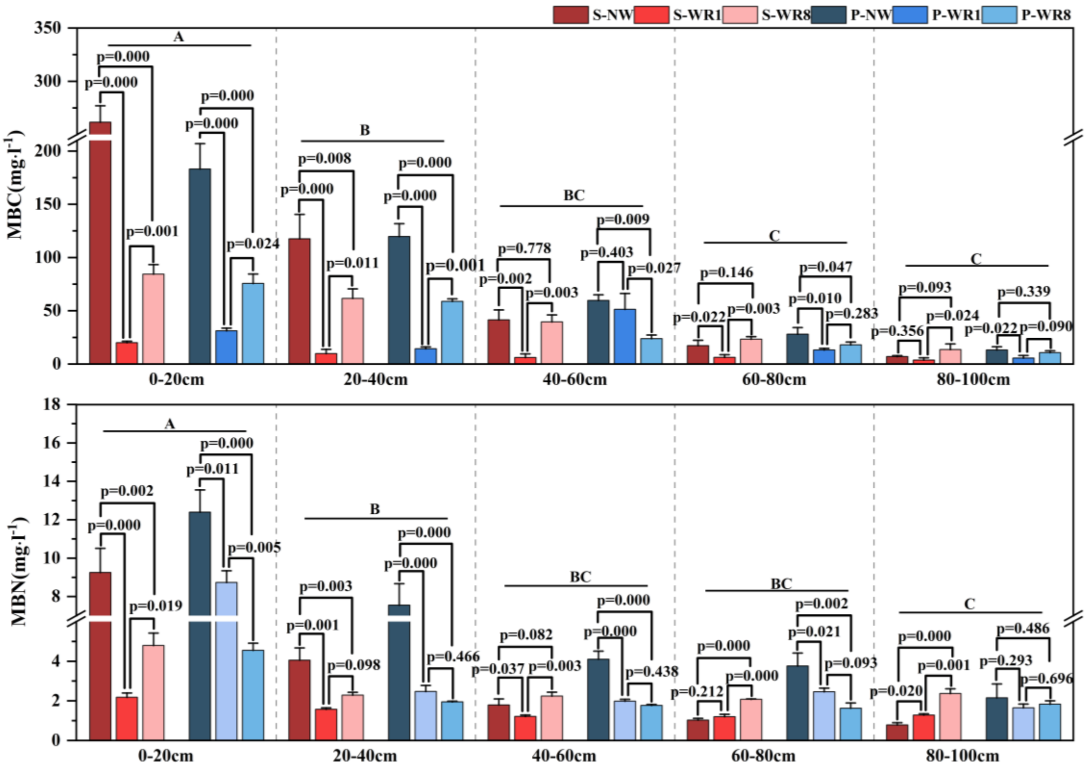

***Figure 4.** Spatial variations in soil microbial biomass for willow and reed wetlands. Different capital letters indicate significant differences among soil layers*

Table 3. *Three-factor ANOVA analysis of the effects of different vegetation, restoration years, and soil layers on the soil microbial biomass and enzyme activities in the wetlands*

Factor	MBC		MBN		Suc		Ure		Acp	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Vegetation	0.041	0.840	105.336	0.000	94.343	0.000	1.415	0.239	472.205	0.000
Soil layer	257.631	0.000	210.286	0.000	358.724	0.000	26.091	0.000	462.42	0.000
Restoration years	316.936	0.000	115.693	0.000	741.169	0.000	6.138	0.004	238.186	0.000
Vegetation× soil layer	8.997	0.000	11.573	0.000	67.344	0.000	1.903	0.122	123.475	0.000
Vegetation × restoration years	10.202	0.000	46.042	0.000	52.06	0.000	79.774	0.000	39.884	0.000
Soil layer × restoration years	95.717	0.000	31.339	0.000	115.093	0.000	3.355	0.003	42.629	0.000
The interaction of the three	8.066	0.000	7.865	0.000	25.89	0.000	1.959	0.067	26.325	0.000

Spatiotemporal variations in soil enzyme activities in willow and reed wetlands for different restoration years

Changes in the soil enzyme activities of both the willow and reed wetlands in different soil layers are depicted in *Figure 5* for different restoration year plots. In the NW, WR1, and WR8 plots, the Suc activities in the 0-60 cm soil layers consistently exhibited higher values in the willow wetland than in the reed wetland (with increments ranging from 0.77% to 77.97%), except for the 40-60 cm soil layer of the WR8 plots, where they were higher in the reed wetland than the willow wetland (with an increase of 4.4%). In the 60-100 cm soil layer the reed wetland consistently showed higher Suc activities than the willow wetland, with an increment ranging from 3.40% to 40.60%.

In the WR8 plots, the Ure activities in all soil layers (0-100 cm) indicated that the willow wetland had higher activities than the reed wetland, with an increase ranging from 33.3% to 100%. In the WR1 plots, the Ure activities in all soil layers (0-100 cm) revealed that the reed wetland exhibited higher activity than the willow wetland, with an increase ranging from 66.67% to 300%. In the NW plot the Ure activities displayed variable change patterns in each soil layer (0-100 cm). For the 0-20 cm and 40-60 cm soil layers, the willow wetland showed higher activities than the reed wetland, with an increase rate ranging from 33.33% to 66.67%. There were no differences observed between the 20-40 cm and 80-100 cm soil layers. However, for the 60-80 cm soil layer, the reed wetland showed higher activities than the willow wetland, with an increase of 20%.

In the NW and WR1 plots, the Acp activity of the willow wetland was higher than that of the reed wetland in the 0-100 cm soil layer, with an increase that ranged from 2.32% to 132.88%. However, in the NW plots the reed wetland displayed higher Acp activities than the willow wetland at the 40-60 cm soil layer, with an increase of 1.20%. In the WR8 plots, the Acp activities of the willow wetland was higher than that of the reed wetland in the 0-60 cm soil layer, with an increase that ranged from 148.21% to 328.50%. Conversely, in the 60-100 cm soil layer, the Acp activities of the reed wetland surpassed that of the willow wetland, with an increase that ranged from 8.39% to 21.43%.

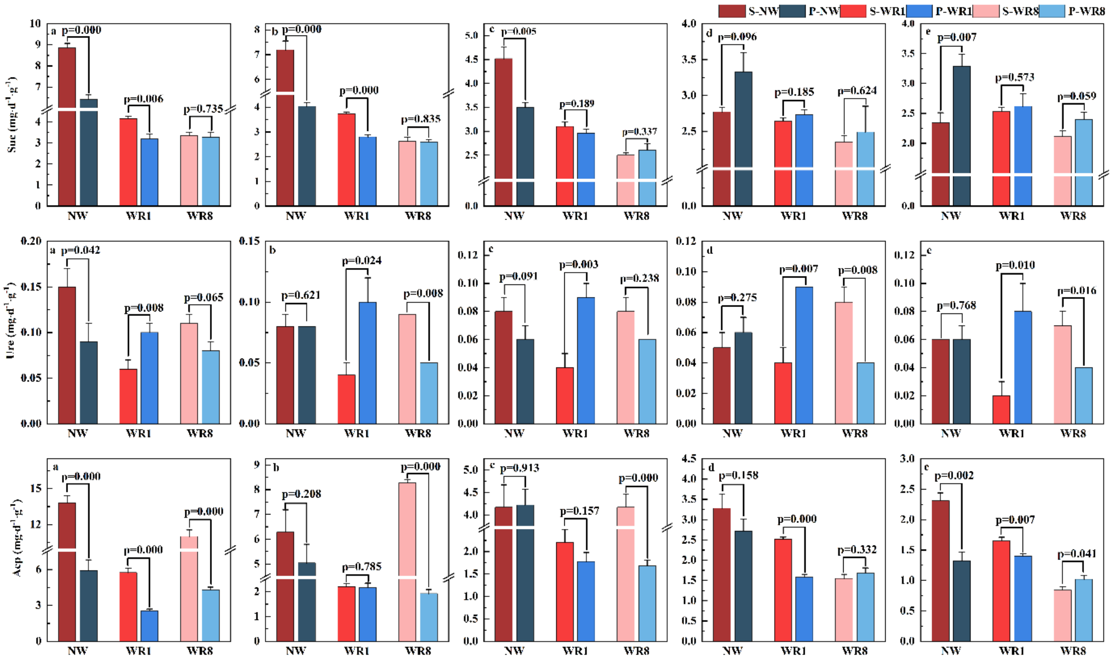

Figure 5. *Changes in soil enzyme activities in willow and reed wetlands for different remediation years*

In the vertical profile, the soil Suc, Ure, and Acp activities of the willow and the reed wetland aligned with the variations observed in the 0-40 cm soil layers (*Fig. 6*). In the willow wetland, the soil Suc, Ure, and Acp activities exhibited significant differences between the NW and WR1, NW and WR8, and WR1 and WR8 plots in the 0-20 cm soil layer ($p < 0.05$). In the reed wetland, no significant differences were observed in the soil Suc and Ure activities between the WR1 and WR8 plots in the 0-20 cm soil layer ($p > 0.05$), whereas a significant difference was found for the Acp activities in the 0-20 cm soil layer ($p < 0.05$).

In the willow wetland, the soil Suc activities were 62.95% that of the NW in the WR1 plots, and 50.37% that of the NW in the WR8 plots. The Ure activity was 47.61% that of the NW in the WR1 plots, and 102.38% that of the NW in the WR8 plots in the willow wetland. The Acp activity was 48.04% that of the NW in the WR1 plots and 86.57% that of the NW in the WR8 plots in the willow wetland. In the reed wetland, the Suc activity in the soil was 69.47% that of the NW plot in the WR1 plots and 65.05% that of the NW in the WR8 plots. The Ure activity was 131.43% that of the NW in the WR1 plots and 77.14% that of the NW in the WR8 plots in the reed wetland. The Acp activity was 49.19% that of the NW in the WR1 plots and 55.17% that of the NW in the WR8 plots in the reed wetland.

Correlation analysis of soil physicochemical factors with microbial biomass and enzyme activities

The relationships between the soil microbial biomass, enzyme activities, and soil physicochemical properties in the willow and reed wetlands were different (*Fig. 7*). The TN, TP, MBC, MBN, Suc, Ure, and Acp in the soil of the willow and reed wetlands were significantly negatively correlated with the pH ($p < 0.01$). In the willow wetlands,

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the soil TP, Suc, and Acp were significantly negatively correlated with the WC ($p < 0.01$), while the MBC, MBN, and SOC were substantially negatively correlated with the WC ($p < 0.05$). In the reed wetlands, the WC was significantly negatively correlated with only the MBN, TN, TP, and Ure ($p < 0.01$). In the willow wetlands, the MBC, MBN, SOC, TN, Ure, and Acp of the soil were significantly negatively correlated with the BD ($p < 0.01$), while in the reed wetlands only the TP and Ure were significantly negatively correlated with the BD ($p < 0.01$).

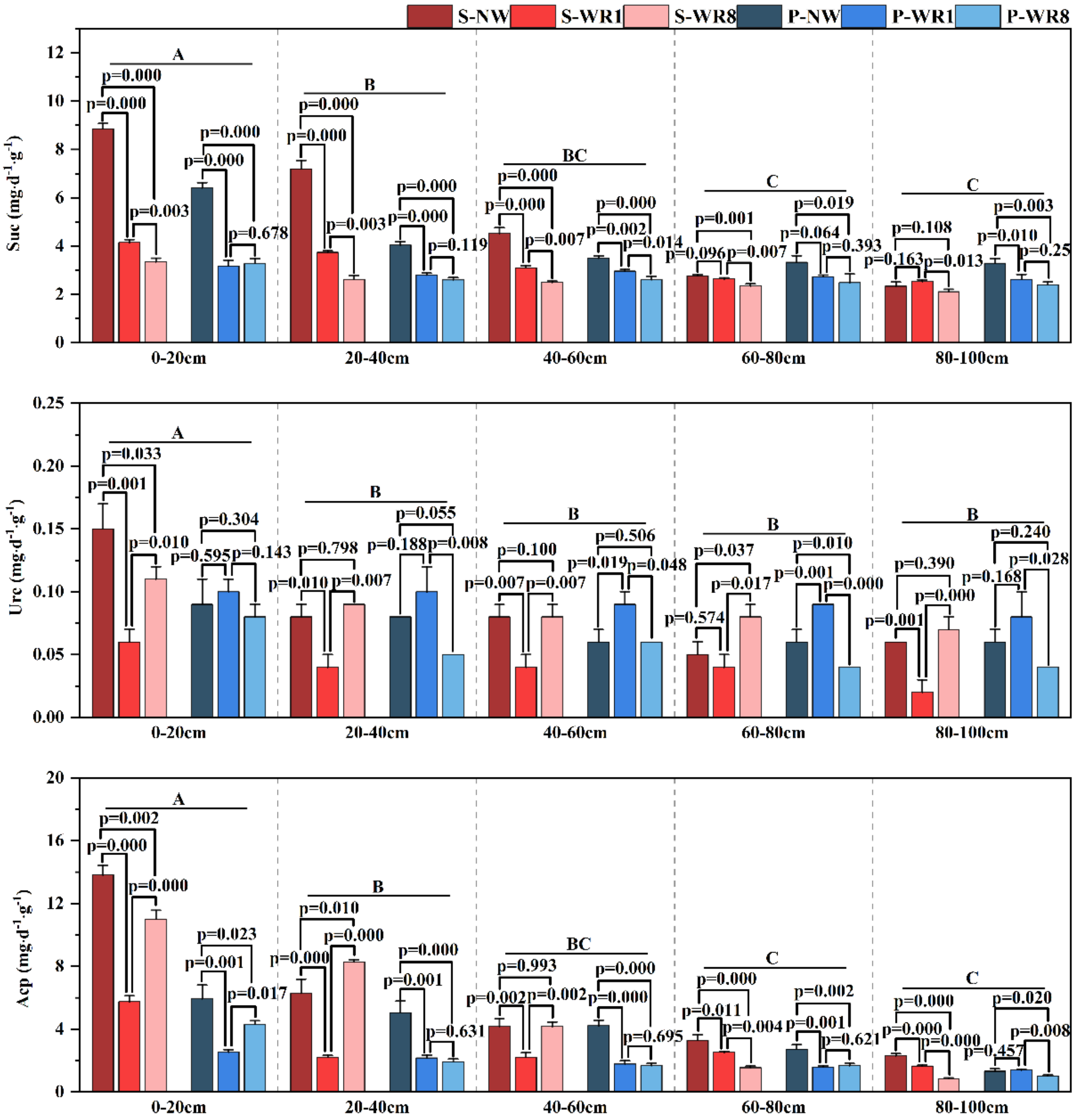

Figure 6. *Spatial variations in soil enzyme activities in willow and reed wetlands. Different capital letters indicate significant differences among soil layers*

In the soil of the willow wetland there were significant positive correlations between the Suc activities and the SOC, TN, TP, MBC, and MBN ($p < 0.01$). The Acp showed a significant correlation ($p < 0.05$) with the TP, while there was no significant correlation between the Ure and TP. In the soil of the reed wetland, there was a substantial negative correlation between the pH and SOC ($p < 0.05$), and a significant positive correlation between the pH and C:N ($p < 0.05$). The Suc, Ure, and Acp activities were significantly positively correlated with TN and MBN ($p < 0.01$), while the SOC also has significant positive correlations with the Suc and Acp ($p < 0.01$).

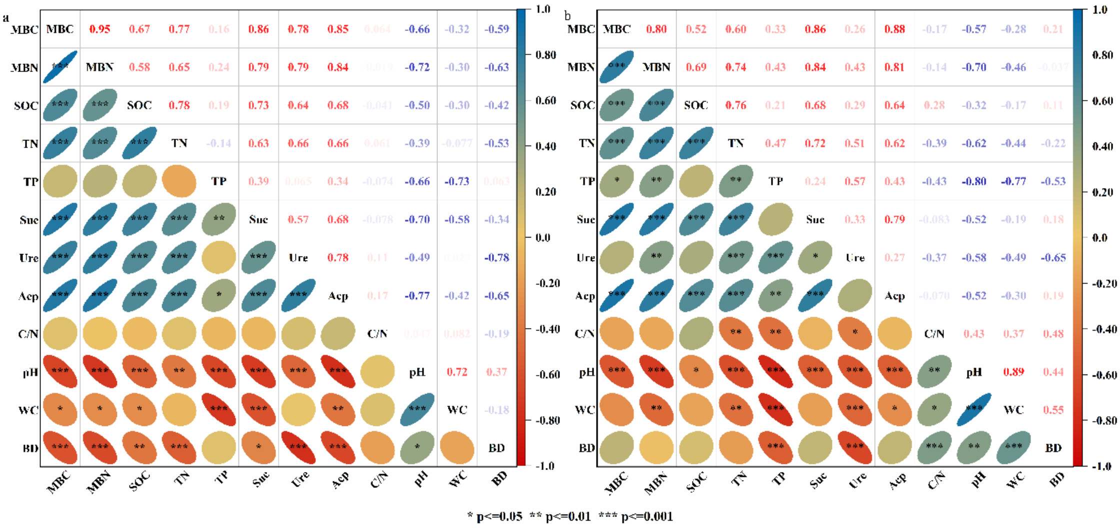

Figure 7. *Correlation analysis between the soil physicochemical factors, microbial biomass, and enzyme activities for different restoration years in the willow and reed wetlands. (a) Willow wetland. (b) Reed wetland*

Redundancy analysis of soil physicochemical factors with microbial biomass and enzyme activities

Redundancy analysis was performed on the soil enzyme activities and microbial biomass in the wetlands of different restoration years with two types of vegetation, along with soil physicochemical properties (*Fig. 8a, b*). Seven physicochemical factors, including the SOC, TN, TP, pH, and others were selected as environmental variables (*Table 4*).

In the willow wetland the combined explanatory power of the first two axes of soil physicochemical factors for enzyme activities, microbial C and N accumulation reached 89.98%. The correlation coefficients for environmental factors were 0.9488 and 0.7589, respectively, which indicated a strong relationship between the soil enzyme activities, microbial biomass, and soil physicochemical properties. The smallest acute angles between the MBC, MBN, Suc, and the SOC and TN indicated that the SOC and TN were the primary factors that influenced the MBC, MBN, and Suc. The smallest acute angle between the Ure and SOC indicated that the SOC was the main factor that influenced the Ure. The smallest acute angle between the Acp and C/N indicated that C/N was the primary factor that influenced the Acp.

In the reed wetland the combined explanatory power of the first two axes of soil physicochemical factors for enzyme activities, microbial C, and N accumulation reached 83.98%. Specifically, the first axis explained 83.91% of the variability, while the second axis accounted for a marginal 0.07%. The correlation coefficients with environmental factors were 0.9168 and 0.6544, respectively, which indicated a significant correlation between the soil enzyme activities, microbial biomass, and soil

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physicochemical properties. Furthermore, the smallest acute angles were observed between the MBC, MBN, Suc, Acp, Ure, and SOC, TN, and TP, which indicated that the SOC, TN, and TP were the primary factors that influenced the MBC, MBN, Suc, Acp, and Ure.

The ranking of environmental factors was determined through Monte Carlo tests (*Table 5*). In the willow wetland the explanatory power for soil enzyme activities and microbial biomass followed a descending order: TN, pH, C/N, BD, WC, SOC, and TP. Similarly, in the reed wetland, the relative ranking for soil enzyme activities and microbial biomass was TN, BD, pH, WC, C/N, SOC, and TP.

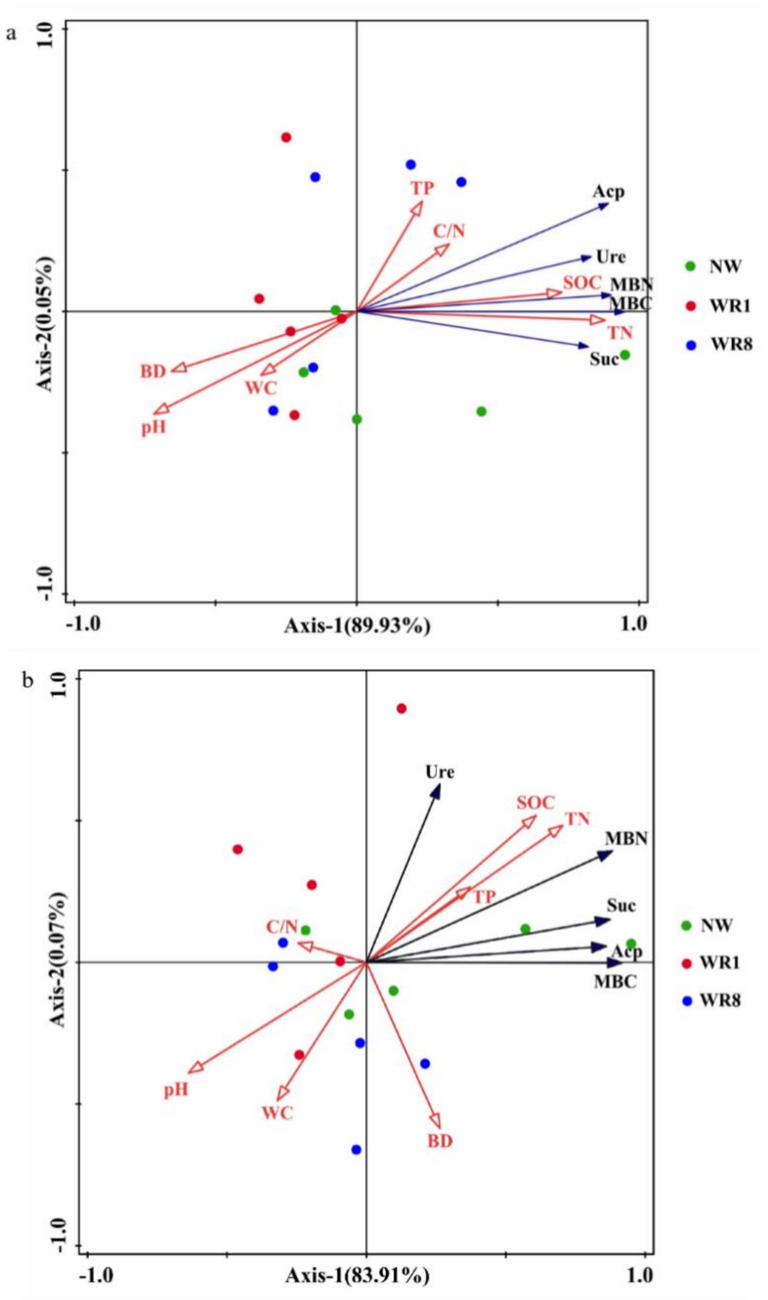

Figure 8. *RDA ranking of soil microbial biomass, enzyme activities, and soil physicochemical properties for different restoration years in willow and reed wetlands. (a) Willow wetland. (b) Reed wetland*

Table 4. *Characteristic values of RDA sequences of soil microbial mass and enzyme activities and their cumulative interpretations*

	Statistic	Eigenvalues	Explained variation (cumulative)%	Pseudo-canonical correlation	Explained fitted variation (cumulative)%
Willow wetland	Axis 1	0.8993	89.93	0.9488	99.93
	Axis 2	0.0005	89.98	0.7589	99.98
	Axis 3	0.0002	90.00	0.9480	99.99
	Axis 4	0.0000	90.00	0.8867	100.00
Reed wetland	Axis 1	0.8391	83.91	0.9168	99.90
	Axis 2	0.0007	83.98	0.6544	99.98
	Axis 3	0.0001	83.99	0.7795	100.00
	Axis 4	0.0000	83.99	0.8463	100.00

Table 5. *Ranking and significance testing of soil environmental factor variables and their interpretation*

	Name	Order of importance	Explains %	Contribution %	pseudo-F	P
Willow wetland	TN	1	69.6	77.3	29.7	0.002
	pH	2	11.3	12.5	7.1	0.020
	C/N	3	6.7	7.5	5.9	0.034
	BD	4	2.3	2.5	2.2	0.166
	WC	5	<0.1	0.1	<0.1	0.750
	SOC	6	<0.1	<0.1	<0.1	0.940
	TP	7	<0.1	<0.1	<0.1	0.878
Reed wetland	TN	1	41.7	49.6	9.3	0.006
	BD	2	15.0	17.8	4.1	0.086
	pH	3	16.9	20.1	7.0	0.016
	WC	4	5.8	6.9	2.8	0.110
	C/N	5	0.5	0.6	0.2	0.660
	SOC	6	1.9	2.3	0.9	0.412
	TP	7	2.2	2.6	1.0	0.348

Discussion

Changes in soil enzyme activities, microbial C, and N biomass with restoration years in willow and reed wetlands

Acid phosphatase, sucrose, and urease play critical roles in facilitating the cycling of C, N, and P in wetland ecosystems (Chae et al., 2017). Variations in the soil microbial biomass and enzyme activities were distinct between vegetation communities in the same wetland ecosystem (He et al., 2020). Moreover, with longer recovery times the abundance of soil microorganisms and organic matter in both wetlands increased (Abbott et al., 2022). In this study, under identical site conditions the soil acid phosphatase (Acp) activity in the willow (WR1) wetland was 36% higher than in the reed wetland. For the WR8 plots the difference was even greater, with a 61% higher Acp activity. These findings indicated that both the vegetation type and restoration duration significantly affected the soil Acp levels. With longer restoration years the roots of woody plants extend deeper into the soil, which promotes the cycling of nutrients through root exudates. Furthermore, through the decomposition of fallen branches and leaves, woody plants gradually enrich the soil with organic matter, which contribute to the recovery and improvement of soil enzyme activities (Fu et al., 2023).

The soil sucrose (Suc) activity was 13% higher in the WR1 plots of the willow wetland compared to the reed wetland. However, in the WR8 plots the soil Suc activities in both wetlands were comparable. This suggested that the reed vegetation progressively recovered and expanded, which led to deeper root soil penetration and increased the supply of organic matter as substrates for sucrose enzymes, resulting in their enhanced activity (Song et al., 2023). In the WR1 plots, the activity of soil urease (Ure) was higher in the reed wetland compared with the willow wetland; however, in the WR8 plots the converse was true. These findings indicated that different vegetation types exhibited variations in utilization and transformation of soil N at different restoration stages. In the initial phases of restoration, the increased soil total N content stimulated the reproduction and metabolic activities of wetland soil microorganisms, which consequently boosted urease activities (Wang et al., 2021). This study found that in the WR1 plots, the reed wetland had a higher TN (total nitrogen) content than did the willow wetland, while in the WR8 plots the willow wetland exhibited a higher TN content than did the reed wetland, which confirmed this pattern.

The soil microbial C and N contents are essential for the participation of organic matter for biogeochemical processes and the cycling of C and N (Arunachalam et al., 1999). There were variations in the soil MBC and MBN contents between the willow and reed wetlands at the WR1 and WR8 sites. In the WR1 sites, the MBC and MBN contents were higher in the reed wetland in contrast to the willow wetland, while in the WR8 site the MBC and MBN contents were higher in the willow wetland compared with the reed wetland. Perennial herbaceous plants can have positive impacts on the soil microbial C and N levels during the early stages of restoration (Stefanowicz et al., 2022), whereas woody plants require additional time to adapt to the restoration environment and supply organic matter (Zhang et al., 2019). Wang et al. found that (based on their study of changes in soil enzyme activities in Chinese fir plantations of varying ages) both young and old stands exhibited higher soil nutrient contents and enzyme activities compared with intermediate aged stands (Wang et al., 2018). This study observed increased soil enzyme activities and microbial C and N content in both the willow and reed wetlands with the progress of wetland restoration over time. This might have been attributed to the gradual improvement in soil functionality and quality that ensued from the rejuvenation of vegetation, accompanied by an increased organic matter content, improved soil aeration, and water holding capacity, all of which created favorable conditions for microbial growth and reproduction (Li et al., 2023). However, during the early stages of wetland restoration, the soil was relatively unstable and significant different compared with the natural wetlands (Berkowitz et al., 2018). In this study, after eight years of restoration the soil enzyme activities, as well as microbial C and N content in the willow and reed wetlands were still lower compared with the natural wetland (NW) site.

Spatial variations in soil microbial C and N content and enzyme activities in the willow and reed wetlands

With greater soil depth the organic matter content of the soil decreased, translating to reduced soil aeration and water utilization capacities, which can negatively affect microbial growth and metabolism (Dai et al., 2020). In this study, overall variations in the soil MBC and MBN contents in the NW, WR8, and WR1 subterranean domains of willow and reed wetlands decreased with greater soil depth. Preceding studies also indicated that the presence of litter in the upper layer of the wetlands, a high concentration of organic matter in the soil, and active plant root systems contributed to the greater availability of energy substrates. This facilitated the growth and reproduction of soil microorganisms that translated to an overall increase in soil microbial biomass (Zhang et al., 2016). However, deeper soil retains a higher microbial C and N content. In the WR1 plots of the reed wetland, the microbial biomass carbon (MBC) content in the 40-60 cm soil layer was higher and surpassed that of the 0-40 cm soil layer. This result indicated that in the early stages of wetland restoration, the soil environment is unstable, along with the distribution of microbial biomass and decomposition of organic matter (Zhao et al., 2021).

Across the vertical profile, the overall variations in the NW, WR8, and WR1 subsurface soil enzyme activities in each vegetation wetland demonstrated an overall decreasing trend with greater soil depth. This decline may have been attributed to the predominant distribution of plant roots at the soil surface in the study area. Furthermore, the significant accumulation of litter enriched with organic matter occurred on the soil surface, which provided microorganisms with ample energy and nutrients. Consequently, the enzyme activities in the topsoil were typically higher than those in the lower layers (Liu et al., 2021). In this study, significant variations in the soil microbial biomass and enzyme activities were observed primarily within the 0-40 cm soil layer in both the reed and the willow wetlands. Conversely, no significant changes were observed in the 40-100 cm soil layer. These findings suggested that the influence of vegetation growth on the soil surface microbial biomass and enzyme activities were more pronounced in contrast to the deeper soil layers (Mao et al., 2016).

Effects of soil physicochemical factors on soil microbial mass, C, N, and enzyme activities

The activities of soil enzymes were significantly influenced by environmental factors, which indirectly impacted them through their effects on the growth and activities of soil microorganisms, as well as variations in the structures of microbial populations. These regulatory aspects had the capacity to modulate the rate of nutrient transformation in the soil, thereby exerting an influence on the overall health of the soil ecosystem (Li et al., 2022). Research has indicated that the soil microbial biomass and enzyme activities can be influenced by multiple factors, including pH, organic C, temperature, and moisture (Lu et al., 2023). Significantly positive correlations were observed in the willow wetland between the soil MBC, MBN, Suc, Ure, Acp, and SOC, TN ($p < 0.01$), which was also the case in the reed wetland, except for an insignificant correlation between the soil Ure and SOC. These findings implied that Ure activities in the reed wetland were less influenced by the SOC, which was potentially due to the soil pH and salt content (Sauze et al., 2018); however, further experimental verification is required. Both types of vegetation wetlands exhibited a significantly negative correlation between the soil MBC, MBN, Suc, Ure, Acp, and pH ($p < 0.01$). This might have been attributed to the adaptation of soil microorganisms to slightly acidic environments, which allowed them to better adapt. Additionally, a higher pH can impact the transformation of organic matter, thereby affecting the participation of soil enzymes in the material metabolism cycle (Puissant et al., 2019).

Based on redundancy analysis, the TN content was the primary factor that influenced the soil microbial biomass and enzyme activities in both the willow and reed wetlands. Yan et al. conducted a study to examine the effects of modified soil nutrients (C and N) on soil enzyme activities in both bare and vegetated soil. The investigation found that the addition of both C and N enhanced the soil enzyme activities. Further, the soil treated with a combination of C and N exhibited higher enzyme activities in contrast to the soil treated with C alone, which suggested that N was a vital nutrient involved in the synthesis of soil enzymes (Yan et al., 2020).

Conclusion

Significant disparities in soil microbial C and N content, as well as enzyme activities, were observed between rejuvenated willow and reed wetlands under varied restoration times in the Zhegao River Wetland. The results indicated that the MBC content in the 0-100 cm soil layer of the willow wetlands in WR1 and WR8 accounted for 10.3% and 49.9%, respectively, of that of the NW, while the MBN content was 44.1% and 81.4%, respectively. In the reed wetlands, the MBC content in the 0-100 cm soil layer in WR1 and WR8 was 28.6% and 46.3%, respectively, of that of the NW, whereas the MBN content was 57.8% and 39.1%, respectively. The soil enzyme activities related to carbon (C), nitrogen (N), and phosphorus (P) cycling varied with the duration of vegetation restoration. In the willow and reed wetlands, the sucrase activity pattern was NW > WR1 > WR8, and the acid phosphatase activity pattern was NW > WR8 > WR1. The urease activity in the willow wetland was WR8 (0.086 $mg \cdot d^{-1} \cdot g^{-1}$) > NW (0.084 $mg \cdot d^{-1} \cdot g^{-1}$) > WR1(0.04 $mg \cdot d^{-1} \cdot g^{-1}$), while the urease activity in reed wetland was WR1(0.092 $mg \cdot d^{-1} \cdot g^{-1}$) > NW (0.07 $mg \cdot d^{-1} \cdot g^{-1}$) > WR8(0.054 $mg \cdot d^{-1} \cdot g^{-1}$). The activities of MBC, MBN, Suc, Ure, and Acp in the reed and willow wetland soils for different years of vegetation restoration decreased with greater soil depth. Wetland restoration leads to improvements in the soil environment, including an increased soil nutrient content, which is essential for energy conversion, water utilization, and the overall stability of wetland ecosystems. To enhance our understanding of the responses of wetland soil to restoration processes, additional investigations will be conducted on soil microbial diversity, composition, and their interactions with environmental factors, such as temperature and humidity. These findings will serve as a scientific foundation for wetland conservation and management.

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