STUDY ON SOIL MICROBIAL ACTIVITIES AND THEIR INTERRELATIONSHIPS IN ZHALONG WETLAND IN NORTHEAST CHINA

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Abstract. This study took Zhalong Wetland in northeast China as the experimental object, and conducted in-depth research on the distribution patterns of soil nutrients, soil microbial characteristics, and soil enzyme activity under different salinity levels. The results showing that in the soil microbial community structure of Zhalong Wetland, bacteria account for the main part, followed by actinomycetes, and the number of fungi is the least. Soil nutrients, microbial community quantity, microbial biomass carbon and nitrogen were distributed vertically in different degraded wetlands, showing obvious characteristics, and the distribution characteristics were different. The distribution of soil enzyme activity is complicated by many factors. There was a close relationship between soil nutrients and soil microbial characteristics during the degradation of wetland meadow. While, there was no significant relationship between total P and pH and soil microbial activity ($P > 0.05$). The correlation between soil nutrients and microorganisms in natural wetlands was analyzed by principal components, and the comprehensive evaluation table of soil total nutrients and the relationship table between soil nutrients and microorganisms were constructed.
Keywords: *soil ecosystem, microorganism, nutrients, enzyme, distribution*

Introduction

Wetlands, along with forests and oceans, are known as the three major global ecosystems, earning the nickname of “natural reservoirs” and “natural species reservoirs”, with wetland ecosystems being particularly important (Noyce et al., 2023; Zorai et al., 2023; Yu et al., 2023). Its number of enormous environmental functions and ecological effects are irreplaceable by any other ecosystem on Earth (Miller et al., 2023; Gonzalez-Flo et al., 2022). Wetlands not only provide a variety of material resources for human production and life, but also play an irreplaceable role in regulating and combating flood disasters, regulating climate, and preventing soil

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erosion, which other systems cannot replace (Gui et al., 2023; Filip et al., 2023). At the same time, wetlands have a strong self-purifying ability for various pollutants discharged into them, earning the nickname of “the kidney of the earth” (Yao et al., 2023). Therefore, the protection of wetlands is particularly important. In wetland ecosystems, soil plays an important role in maintaining regional ecological balance that no ecosystem can replace (Constanza et al., 1997). Soil microorganisms, as one of the important components of ecosystems, play an extremely important leading role in soil element cycling, nutrient mineralization and supply, as well as organic matter decomposition and transformation (Tian et al., 2003). Meanwhile, soil enzymes also play a positive regulatory role in soil biochemical processes (Zhao et al., 2008a). Wetland microbial biomass carbon (Wang et al., 2008) and nitrogen (Wu et al., 2008) are sensitive indicators of wetland changes (Marx et al., 2001). Although it only accounts for a small portion of the soil organic matter pool, it is the key to controlling the distribution of C, N, and other nutrients in the ecosystem. It can reflect the activity and quantity of wetland microorganisms, and thus reflect changes in wetland properties (Wu et al., 2006). There are many research studies on the community structure and metabolic characteristics of wetland soil microorganisms (Wang et al., 2009; Pei et al., 2009), while relatively few researches on the community structure, microbial quantity and soil enzymes of natural wetland soil.

Soil is a living and dynamic special habitat in the terrestrial ecosystem of the Earth, composed of minerals formed by weathering of rocks, organic matter produced by the decomposition of animal and plant residues, soil organisms (solid matter), as well as water (liquid matter), air (gaseous matter), humus, etc. (Kang and Freeman, 1999; Qiu et al., 2023). They are both interconnected and constrained, providing necessary living conditions for soil microorganisms, soil animals, and various types of plants (Zhao et al., 2006). Meanwhile, soil microorganisms (mainly soil bacteria, fungi and actinomyces) carry out various biochemical reactions and functions such as decomposition of organic matter and minerals (Zhao et al., 2008b), nutrient cycling and utilization, and plant availability, which play an extremely important and decisive role in improving soil physical and chemical properties, forming and maintaining soil aggregates and other soil structures, and improving soil fertility (Cohen et al., 2007). Therefore, soil microbial community is also one of the effective biological indicators that reflect changes in soil environmental quality (Wang et al., 2022).

Soil biological activity refers to the total intensity of various biological activities in the soil, which plays a driving role in the decomposition, transformation, absorption, utilization, and physical structure evolution and maintenance of exogenous organic matter and soil organic matter (Yang et al., 2022). It mainly includes soil microbial community, soil enzyme activity and various biochemical reactions they participate in to promote, which represents the degree of soil ripening and soil fertility, and plays a crucial role in promoting the virtuous cycle of soil ecosystem (Peng, 2003; Wu et al., 2006). The distribution of soil enzyme activity is influenced by multiple factors and is relatively complex (Yang et al., 2002; Ye et al., 2006). Soil enzymes are catalysts for all biochemical reactions in soil and are closely linked to organic matter decomposition, nutrient cycling and energy conversion (Liu et al., 2005). Soil enzyme activity reflects the process and direction of soil biochemistry (Acosta and Tabatabai, 2000), which is sensitive to the interference of external environment and human activities, and is one of the important indicators of soil environmental quality evaluation (Lu, 2004; Wan and Song, 2009). Besides, soil enzyme activity can also

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reflect the direction and degree of various biochemical processes going on in the soil (Haleem et al., 2000). The presence of a large number of soil microorganisms and various soil enzyme systems in the soil plays a positive role in promoting soil material transformation and energy flow. The soil microbial community, represented by soil enzyme activity, represents the intensity of material metabolism and energy cycling (Cui et al., 2022; Yu et al., 2022). Therefore, a systematic and in-depth study of soil microbial population structure, quantitative characteristics and its spatial and temporal dynamics can not only reveal the soil nutrient metabolism law and soil fertility characteristics, but also provide important theoretical references for further in-depth research on soil ecology and environment.

In the process of soil ecosystem degradation, community succession and human disturbance to soil ecosystem, soil enzyme activity is closely related to soil microbial quantity, microbial biomass and microbial diversity (Bamdad et al., 2022; Chen and Moorhead, 2022). This study conducted a systematic study on the soil microbial community and structure, microbial quantity, and spatiotemporal dynamics of the Zhalong Wetland Meadow. By analyzing the spatiotemporal variation patterns and interrelationships of soil microorganisms and soil enzyme activities, the relationship between soil microorganisms and soil enzyme activities was explored, providing scientific basis for further research on changes in soil fertility, nutrient transformation, and health evaluation of the Zhalong Wetland Meadow. In order to provide theoretical basis for the ecological restoration and protection of degraded natural wetlands in China, it is of great significance for the development of wetland microbial resources and the protection of wetland environment.

Materials and methods

Study area

Zhalong Wetland is located in the Songnen Plain and the lower reaches of Uyur River in the west of Heilongjiang Province in northeast China, 30 km north-west from Qiqihar City, with the geographic location of 46°52′-47°32′N, 123°47′-124°37′E, of which the area of reed swamp wetland is the first one in Asia and the fourth one in the world, with the total area of 2.13×10^5 hm^2. Belongs to the temperate continental monsoon climate, the annual average temperature is about 3.5℃, the coldest month is January, the hottest month is July. The annual sunshine is 2854.4 h, and the annual average accumulated temperature of $\geq$ 10℃ is 2620℃. The frost-free period is about 128 days, with an average annual precipitation of 420.7 mm and a maximum of 754 mm (in 1957).

Data sampling

Four research sampling sites were selected within the wetland, namely the Baohuqu, Tumutai, Yuweichang, and Yantongtun in May to November periods in 2011 (*Fig. 1; Table 1*). Three parallel 10 cm × 10 cm sample plots were randomly set up at each study site and soil samples were collected from the 0-10 cm, 10-20 cm and 20-30 cm soil horizons sequentially, and the samples were taken back to the laboratory in a sealed bag after picking out the visible plant debris and root fragments analyzed separately. All samples were passed through with the 2 mm soil sieve and stored in a refrigerator at 4°C for the determination of soil microbiological properties.

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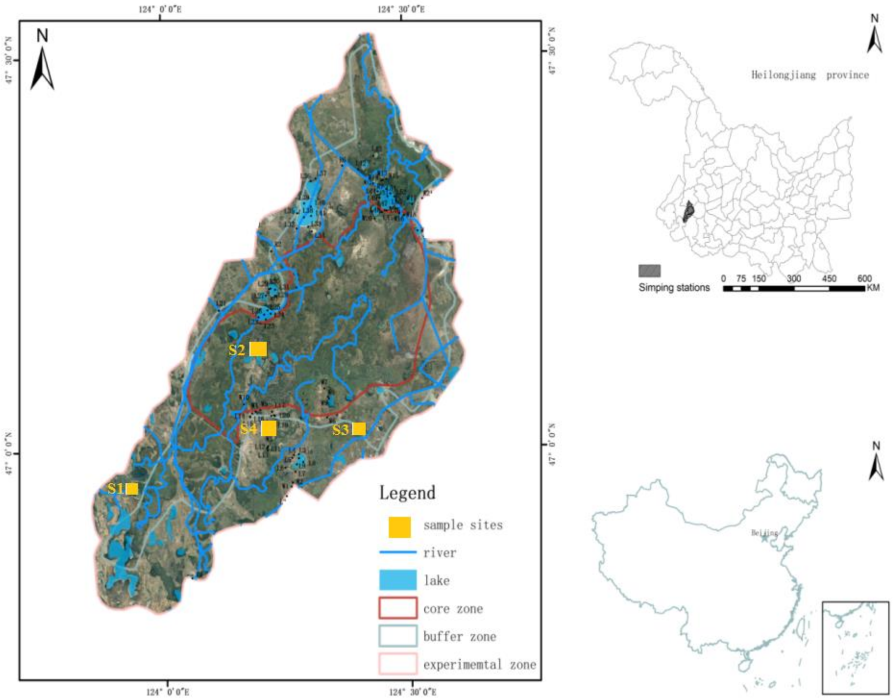

Figure 1. *Map of sampling sites*

Table 1. *Information of sampling sites*

Sampling sites	Longitude (E)	Latitude (N)	Altitude (m)	Interference
S1: Tumutai	123°57’	46°56’	138.60	Farmland, light grazing
S2: Baohuqu	124°14’	47°11’	142.00	Tourism
S3: Yuweichang	124°26’	47°04’	140.00	Grazing
S4: Yantongtun	124°10’	47°00’	137.20	Near the core area, mild interference

Experimental methods

Separation and identification of soil microbial population

Separation method: by dilution plate method (CAS, 1985).

All soil microbial community identification according to Buchanan (1984) and Krasilinikov (1958).

Determination of soil microbial population

Colonies number:

$$\text{number of bacteria per gram of dry soil} = \frac{\text{average number of colonies} \times \text{dilution}}{\text{dry soil weight}} \quad \text{(Eq.1)}$$

Determination of soil microbial biomass

Determination method: use chloroform fumigation K_2SO_4 extraction method for determination (Wu et al., 2006). The determination of microbial biomass carbon is carried out using the fumigation extraction instrument analysis method (TOC analyzer,

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Shimadzu Model TOC-500, JAPAN), and the microbial biomass nitrogen is determined using the fumigation extraction total nitrogen determination method.

Determination of soil enzyme activity

Determination methods: (1) Urease activity was determined by indophenol blue colorimetry (NH_4^+-N) (Li et al., 2008). (2) Acid and alkaline phosphatase activities were determined by a colorimetric method using disodium p-nitrophenylphosphate salt matrix (CAS, 1985). (3) Polyphenol oxidase activity was determined by the colorimetric method with o-phenyltriol (Guan, 1986). (4) Catalase activity was determined by potassium permanganate titration (Li et al., 2008). (5) β-Glucosidase activity measured by p-nitrophenyl-β-D-glucopyranoside (PNPG) matrix colorimetric assay (CAS, 1985).

Determination of soil physical and chemical properties

Determination methods (Bao, 2000; SSSA, 2000): (1) Moisture content is determined by the drying constant weight method (105°C). (2) pH value was determined by 1:2.5 water and soil ratio and potentiometric method. (3) Determination of organic carbon using potassium dichromate external heating method; Total nitrogen is measured using the Kjeldahl method. (4) Whole phosphorus was determined by the H_2SO_4-$HCLO_4$ decoction-molybdenum antimony colorimetric method. (5) Quick-acting potassium was determined by NH_4OAC leaching-flame photometry. (6) Hydrolyzed nitrogen was determined by alkaline hydrolysis diffusion method.

All the above measurement experiments were conducted in three parallel experiments were done by the lab analysis.

Data analysis

The experimental data were processed using SPSS 17.0 and Microsoft Excel 2003 software. The significance levels of microbial community, soil enzyme activity, microbial biomass and soil nutrients in the same soil layer in different study sites were measured by One-way ANOVA variance analysis with Duncan test ($P < 0.05$). Person correlation analysis is used to analyze the relationship between soil microbial biomass and soil environmental factors.

Standardized values of soil nutrient indicators and microbiological indicators for the test soil samples, the standardized index formula is:

$$x_{ij} = \frac{x_{ij} - \bar{X}_j}{\sqrt{Var(x_j)}} \qquad \text{(Eq.2)}$$

where $i = 1,2,3\ldots n$; $j = 1,2,3\ldots 12$, $\bar{X}_j$ and $\sqrt{Var(x_j)}$ represent the mean and standard deviation of the *j*th soil nutrient and soil microbial activity indicator, respectively.

Results and discussion

Distribution of soil microbial community populations

Statistical analysis of soil microbial communities of bacteria, fungi, and actinomycetes in different areas of Zhalong Wetland were shown in *Table 2*. Bacteria

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accounted for the largest proportion, accounting for an absolute advantage in all kinds of microorganisms, followed by actinomycetes (dominant bacteria, Zhang et al., 2013), the smallest number of fungi, accounting for the proportion of 90.38%, 9.50% and 0.12%. The study also found that the total number of soil microorganisms gradually decreases with the aggravation, which is consistent with the research results of Zhao and Zhou (2006).

Table 2. *The number and proportion of microbial community in every study area. P-value from One-way ANOVA*

Site	Soil layer	Total number of microbial communities $10^6.g^{-1}$ dry soil	Number of fungi $10^3.g^{-1}$ dry soil	Proportion of fungal %	Number of actinomycetes $10^4.g^{-1}$ dry soil	Proportion of actinomycetes %	Number of bacteria $10^6.g^{-1}$ dry soil	Proportion of bacteria %
Tumutai	0~10 cm	62.26	89.78	0.14	3320.01	53.33	28.97	46.53
	10~20 cm	211.81	5.06	0.01	77.64	0.37	211.03	99.63
	20~30 cm	3.33	10.12	0.3	18.47	5.54	3.14	94.15
	P	0.000**	0.001**	0.043*	0.000**	0.062	0.004**	0.253
Baohuqu	0~10 cm	31.48	104.74	0.33	70.95	2.25	30.67	97.41
	10~20 cm	1.5	5.08	0.34	27.94	18.58	1.22	81.08
	20~30 cm	0.93	0.05	0.01	2.03	2.17	0.91	97.82
	P	0.352	0.000**	0.535	0.024*	0.425	0.019*	0.752
Yantongtun	0~10 cm	7.47	26.86	0.36	13.85	1.85	7.3	97.79
	10~20 cm	2.21	1.01	0.05	6.34	2.87	2.15	97.09
	20~30 cm	1.45	0.76	0.05	3.3	2.27	1.42	97.68
	P	0.042	0.025*	0.486	0.785	0.041*	0.231	0.942
Yuweichang	0~10 cm	60.75	9.08	0.01	123.55	2.03	59.5	97.95
	10~20 cm	3.52	5.1	0.14	32.28	9.16	3.2	90.69
	20~30 cm	3.75	5.61	0.15	35.7	9.51	3.39	90.34
	P	0.005**	0.017*	0.832	0.042*	0.352	0.542	0.758

**p < 0.01, *p < 0.05

Distribution of soil nutrients

Organic matter in wetland soil

From the distribution characteristics of soil organic matter content in different sites in Zhalong Wetland, it can be seen that the vertical distribution of soil organic matter in each study site is significant (*Fig. 2*). The maximum value appeared in the 0-10 cm soil layer of Tumutai, which was due to the organic matter left in the upper soil layer after the decay of grain straw left by people’s cultivation. The minimum value appears in the 20-30 cm soil layer of Yantongtun, which is due to severe human damage and overgrazing, which exacerbates the degradation and loss of organic matter in wetland soil.

Available K and tydrolytic nitrogen in wetland soils

Distribution of soil available K and tydrolytic nitrogen in Zhalong wetland were shown in *Figures 3* and *4*. The distributions of available K and tydrolytic nitrogen were regular and negatively correlated with soil depth. The relatively large gap between the 0-10 cm soil layer and the 20-30 cm soil layer in Tumutai. Besides, this phenomenon was due to the fact that people burned straw after the autumn harvest in this sample site to increase the amount of tydrolytic nitrogen and K in the surface layer.

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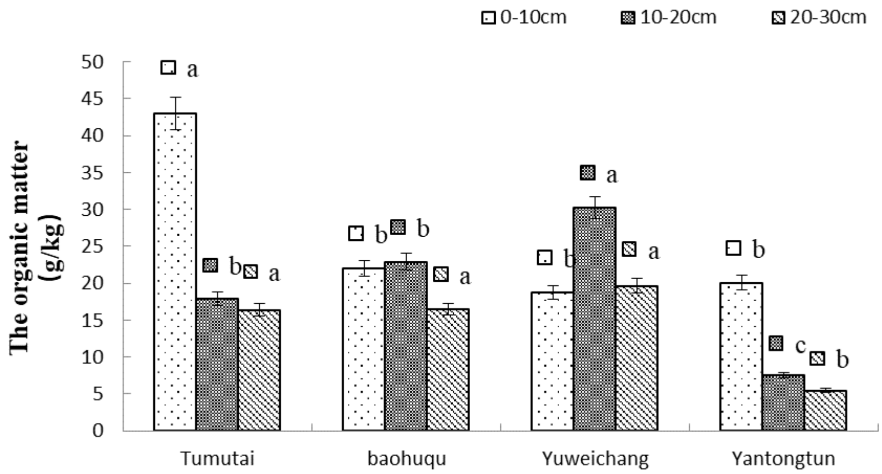

Figure 2. Distribution of the organic matter. Data are shown as mean ± SD. Different letters in the same column and soil layer presented significant difference at 0.05 level

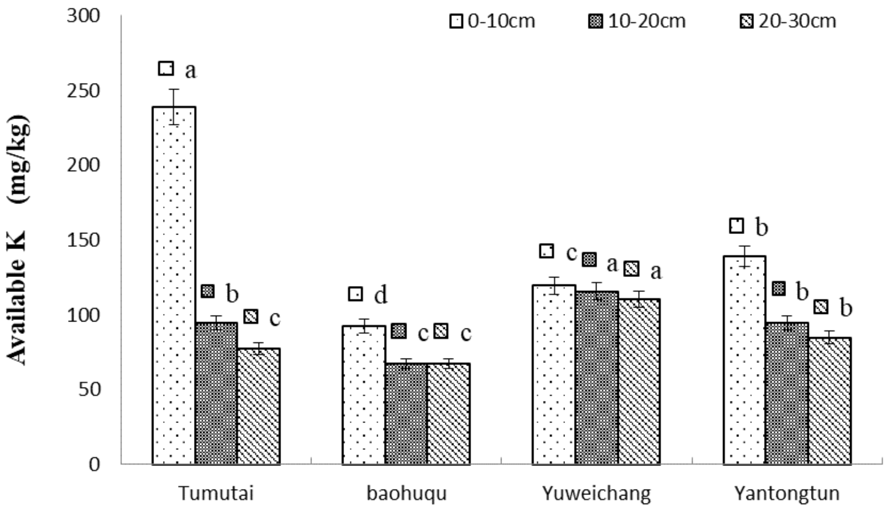

Figure 3. Distribution of the available K. Data are shown as mean ± SD. Different letters in the same column and soil layer presented significant difference at 0.05 level

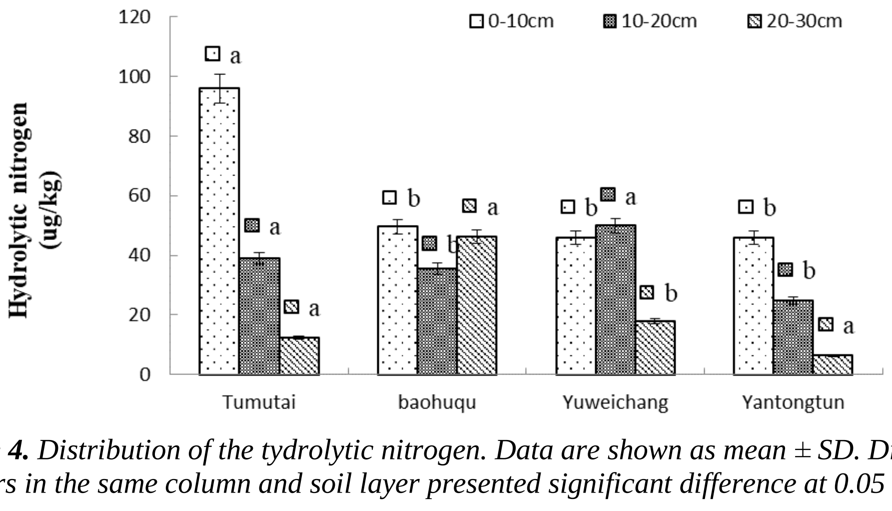

Figure 4. Distribution of the tydrolytic nitrogen. Data are shown as mean ± SD. Different letters in the same column and soil layer presented significant difference at 0.05 level

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Phosphorus in wetland soil

TP was vertically distributed, except Yuweichang, with its maximum value distributed in a soil layer of 20-30 cm (*Fig. 5*). The study site experiences multiple inundations with seasonal changes, and serious anthropogenic disturbances and salinization, which exacerbate the soil leaching process, change the original spatial distribution pattern of soil nutrients, and ultimately lead to further degradation and loss of soil nutrients.

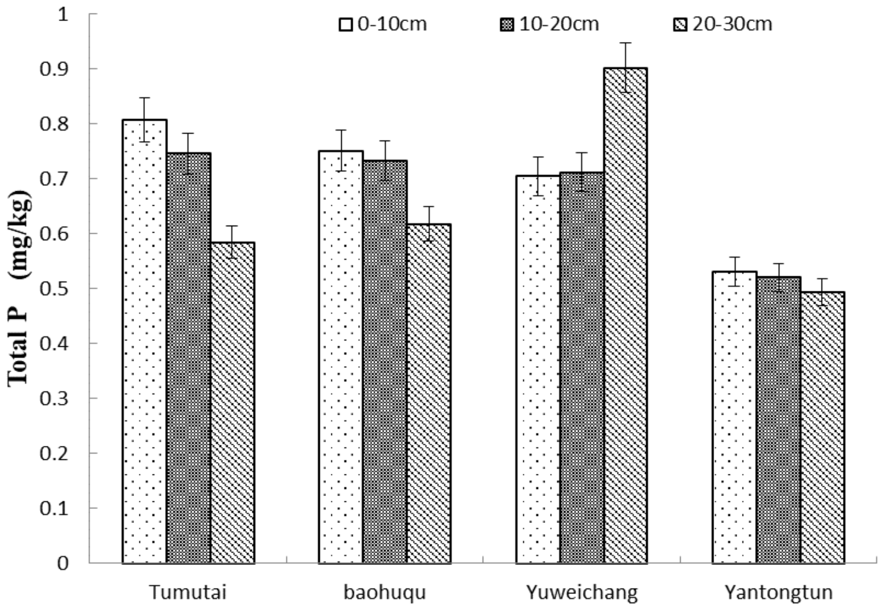

Figure 5. *Distribution of the TP. Data are shown as mean ± SD*

Distribution of soil physical and chemical properties

The physico-chemical properties of the soil have a direct bearing on the survival of the various microorganisms within the soil and the basic physico-chemical properties of the sample plots (*Table 3*). The pH value decreased gradually with the increase of soil depth, and soil total N increased first and then decreased with the deepening of soil layer. The amount of organic carbon in Yantongtun was the lowest, and the average organic carbon in the three layers of soil samples in Tumutai was 2.31 times higher than that in Yantongtun. The average amount of organic carbon in the three layers of soil samples in the reserve was 1.84 times higher than that in Yantongtun, and the average amount of organic carbon in the three layers of soil samples in the reed farm was 2.05 times higher than that in Yantongtun.

Distribution of soil microbial biomass C and N

The distribution of soil microbial biomass C and N in the Zhalong wetland is shown in *Figures 6* and *7*. The carbon and nitrogen contents of soil microbiomass in each research sample site were characterized by obvious variations, which were negatively correlated with the depth of the soil layer, but there were some differences in the spatial distribution among different soil layers in each research site. In addition, there are also great differences between the same soil layer in various places. It was also found that

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the spatial distribution difference of soil MBN content in different soil layers was significantly smaller than that of MBC, which was the same as the results of previous studies on forest ecosystem (Liu et al., 2010), grassland ecosystem (Wu et al., 2010), wetland ecosystem (Wang et al., 2009; Yang et al., 2009).

Soil moisture content

It is a piece of evidence that the moisture content of fresh soil samples is much higher than that of air-dried soil samples, and the moisture content of each layer of fresh soil samples in various fields can basically remain level (*Figs. 8* and *9*). The water content of the protected area also increases with the increase of the soil layer, because the protected area is often flooded with water, and the water was very sufficient. Meanwhile, the moisture content of air-dried soil is very stable and increases with the increase of soil layer (Yang et al., 2009; He et al., 2009a, b).

Table 3. *Basic physical and chemical properties of studied soils*

Site	Soil layer	Total N (g/kg)	OrganicC (g/kg)	C/N	pH
Tumutai	0-10 cm	1.46 ± 0.04Aa	24.93 + 3.63Aa	17.11	9.74 ± 0.02Cc
	10-20 cm	2.4 ± 0.04Aa	10.37 + 1.59Bb	4.33	9.24 ± 0.04Cc
	20-30 cm	1.54 ± 0.16Aa	9.51 + 1.02Aa	6.18	8.79 ± 0.02Cc
Baohuqu	0-10 cm	1.63 ± 0.05Aa	12.77 + 2.46Bb	7.86	9.99 ± 0.07Aa
	10-20 cm	1.14 ± 0.08Cc	13.27 + 1.14Bb	11.65	9.31 ± 0.04Cc
	20-30 cm	1.46 ± 0.04Aa	9.56 + 0.85Aa	6.56	9.13 ± 0.04Bb
Yuweichang	0-10 cm	1.39 ± 0.46Aa	10.87 + 1.68Aa	7.8	10.0 ± 0.02Aa
	10-20 cm	1.93 ± 0.23Bb	17.54 + 1.42Bb	9.08	9.99 ± 0.03Aa
	20-30 cm	1.15 ± 0.15Bb	11.39 + 0.96Bb	9.9	9.91 ± 0.05Aa
Yantongtun	0-10 cm	1.02 ± 0.19Aa	11.62 + 0.89Aa	11.42	9.92 ± 0.04Bb
	10-20 cm	1.29 ± 0.08Cc	4.39 + 2.90Bb	3.41	9.42 ± 0.02Bb
	20-30 cm	0.53 ± 0.01Cc	3.17 + 0.88Aa	5.96	9.21 ± 0.04Bb

The data showed as mean ± SD. Different letters in the same column and soil layer presented significant difference at 0.05 level

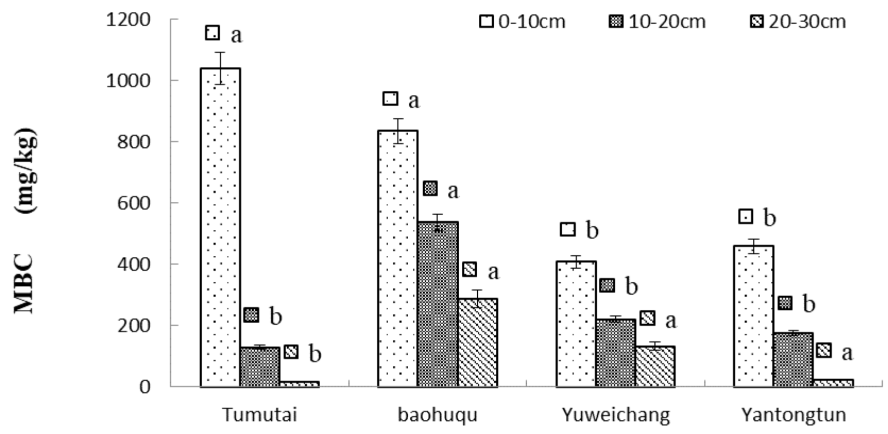

Figure 6. *Vertical changes in soil microbial biomass carbon (MBC). Data are shown as mean ± SD. Different letters in the same column and soil layer presented significant difference at 0.05 level*

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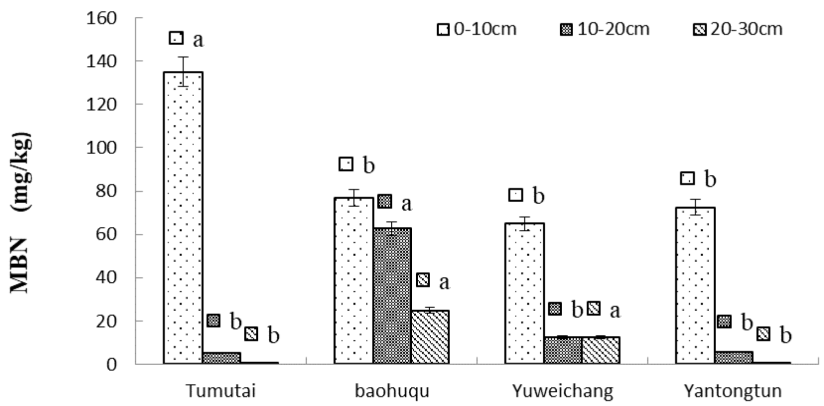

Figure 7. *Vertical changes in soil microbial biomass nitrogen (MBN). Data are shown as mean ± SD. Different letters in the same column and soil layer presented significant difference at 0.05 level*

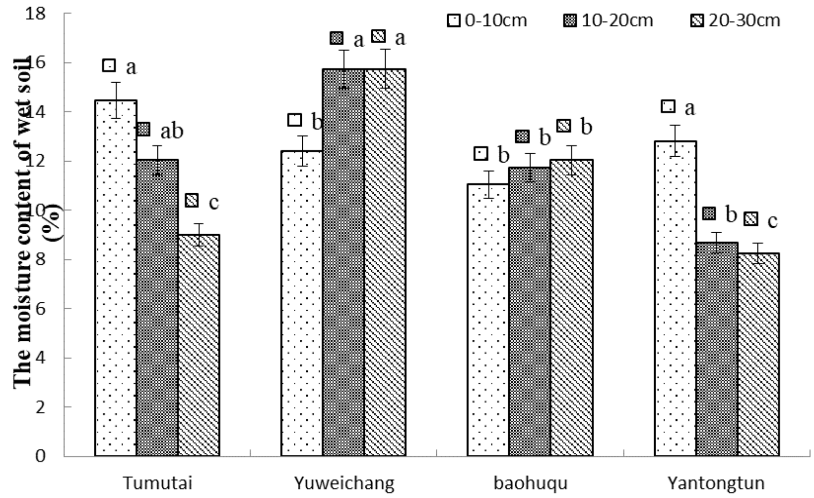

Figure 8. *Moisture content of fresh soil. Data are shown as mean ± SD. Different letters in the same column and soil layer presented significant difference at 0.05 level*

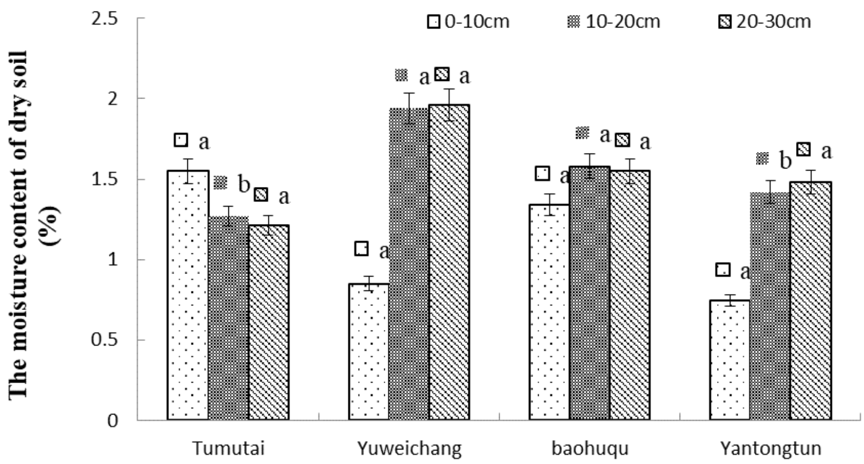

Figure 9. *Moisture content of dry soil. Notes: Data are shown as mean ± SD. Different letters in the same column and soil layer presented significant difference at 0.05 level*

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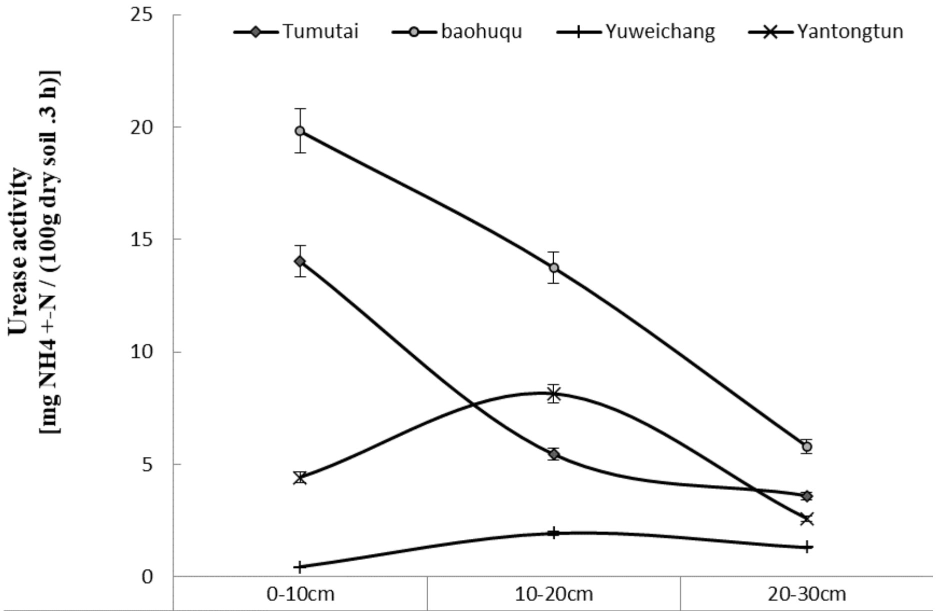
Spatial distribution of soil enzyme activities

Distribution of urease in soil

In *Figure 10,* urease activity in the protected areas and Tumu platform showed a decreasing trend with the increase of soil layer. However, in strong saline and alkaline soil areas such as Yantongtun and Yuweichang, the urease activity shows a trend of first increasing and then decreasing with the increase of soil layer, but the increase and decrease was very small, and the urease activity was not high, indicating that the intensification of salinity will affect the urease activity.



	0-10cm	10-20cm	20-30cm
Tumutai	14.03	5.44	3.59
baohuqu	19.82	13.75	5.79
Yuweichang	0.44	1.95	1.31
Yantongtun	4.42	8.16	2.58

Soil layer

Figure 10. *Distribution of urease activity. Data are shown as mean ± SD*

Distribution of phosphatase activity in soil

Phosphatase is mainly from soil fungi, and its activity can not only characterize the status of wetland soil phosphorus, but also reflect the pollution status of wetland organic phosphorus to a certain extent. The distribution pattern of phosphatase activity in each study site can be seen clearly (*Fig. 11*), and the maximum activity appeared in the 0-10 cm soil layer of Yantongtun, which is due to the fact that the strong salinization in this sample site can activate the phosphatase activity to a certain extent.

Distribution of polyphenol oxidase activity in soil

The activity of soil polyphenol oxidase provides an insight into the humification process of the soil and the characteristics of polyphenol oxidase at each study site (*Fig. 12*). The activity of polyphenol oxidase gradually decreases with the increase of soil depth, due to the frequent seasonal flooding, except Yantongtun.

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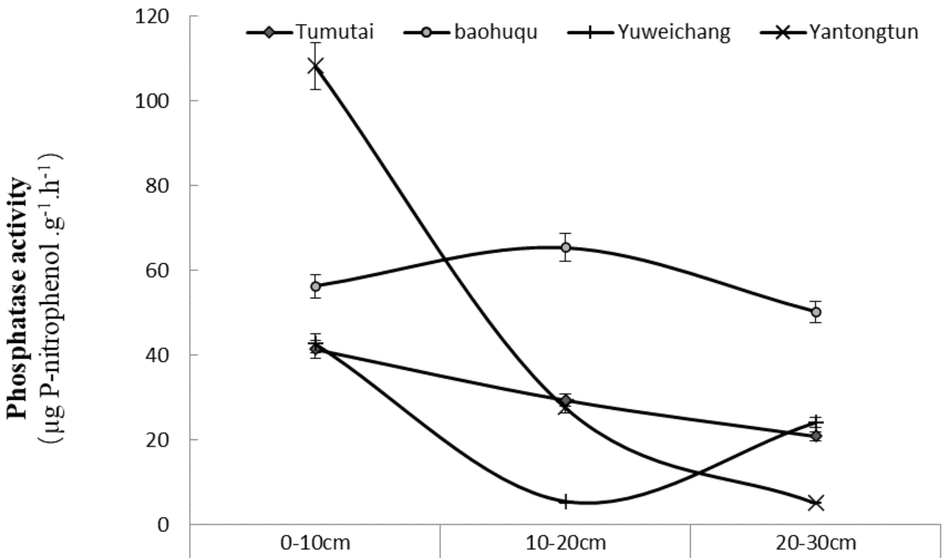
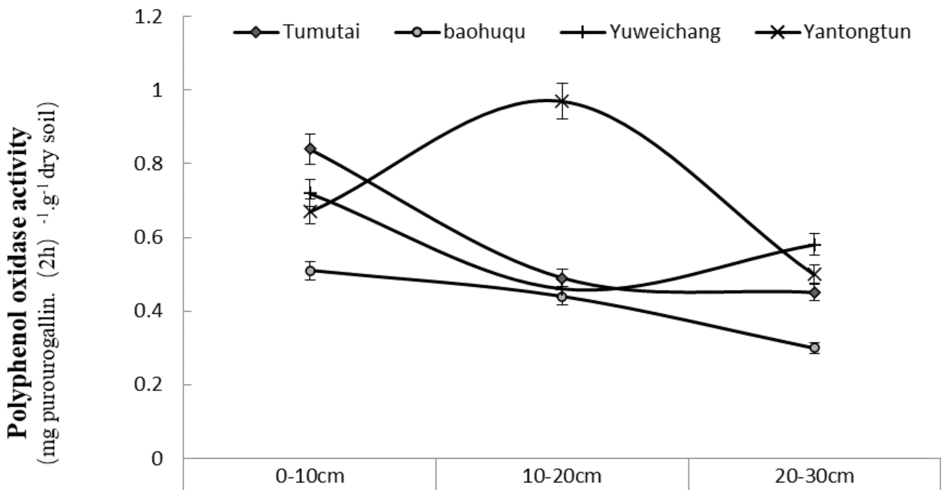


	0-10cm	10-20cm	20-30cm
Tumutai	41.35	29.37	20.86
baohuqu	56.3	65.42	50.18
Yuweichang	42.79	5.39	24.09
Yantongtun	108.23	27.69	5.14

Soil layer

Figure 11. *Distribution of phosphatase activity. Data are shown as mean ± SD*



	0-10cm	10-20cm	20-30cm
Tumutai	0.84	0.49	0.45
baohuqu	0.51	0.44	0.3
Yuweichang	0.72	0.46	0.58
Yantongtun	0.67	0.97	0.5

Soil layer

Figure 12. *Distribution of polyphenol oxidase activity. Data are shown as mean ± SD*

Distribution of catalase activity in soils

Catalase enzymatically facilitates the decomposition of hydrogen peroxide, and it is also involved in the conversion of materials and energy in the soil. From *Figure 13*, it

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can be seen that the difference in catalase activity was not significant in each sample site, and the study also showed that the difference was not significant in different soil layers of the same sample site, with the minimum value occurring in the 20-30 cm soil layer of the Yantongtun.

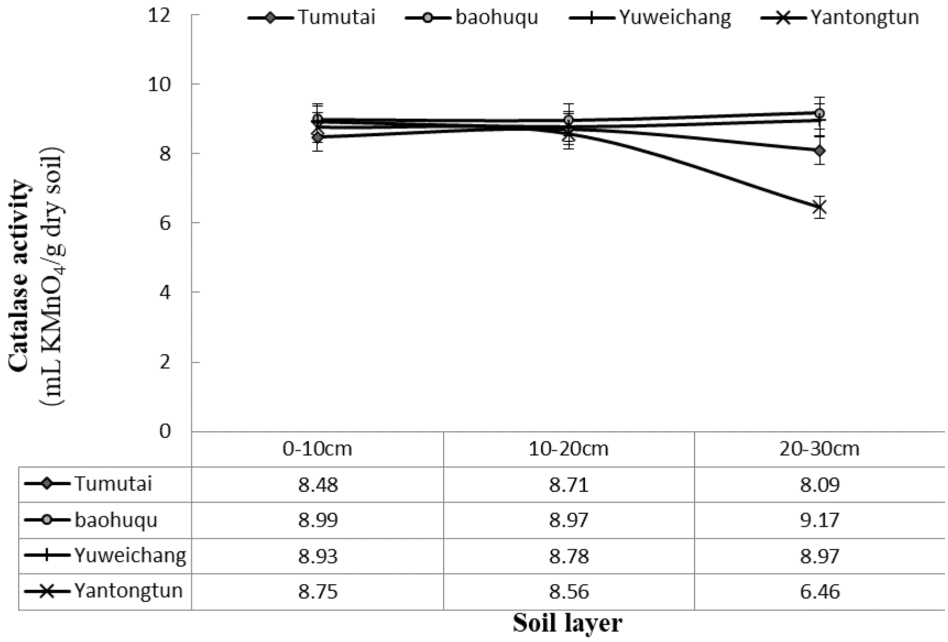

Figure 13. *Distribution of Catalase activity. Data are shown as mean ± SD*

Distribution of β-glucosidase activity in soils

The β-Glucosidase is the limiting enzyme in the microbial breakdown of cellulose to glucose, and the distribution of β-glucosidase activity is extremely remarkable (*Fig. 14*). There was a significant negative correlation between β-glucosidase activity and soil depth, and β-glucosidase activity in surface soil was significantly higher than that in other soil layers. This is due to the large distribution of plant litter in the surface soil and the high content of substances such as cellulose and polysaccharides.

Relationship between the components of soil

Relationship between soil microbial biomass, soil biological activity, and soil nutrient indicators

In soil, microbial load, biological activity and nutrient indicators are closely related. In *Table 4,* soil MBC was significantly correlated with the contents of β-glucosidase, urease, alkaline phosphatase and organic carbon ($P < 0.05$). Soil MBN was significantly correlated with β-glucosidase, urease, acid phosphatase and C/N ratio ($P < 0.05$), which they controlled various nutrients and trace elements required for microbial growth and reproduction (Liu et al., 2010).

Relationship between soil nutrients and soil microbial activity

Soil nutrients and microbial activity are non-linearly related (*Table 5*), and the magnitude of the partial correlation coefficient reflects the close relationship between soil

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nutrients and soil microbial activity (*Table 6*). There was significant correlation between organic carbon and actinomyces number and catalase activity ($P < 0.05$). Available K, total N and hydrolyzed nitrogen were significantly correlated with actinomycetes count, bacteria count and β-glucosidase activity, respectively ($P < 0.05$). C/N, on the other hand, correlated significantly ($P < 0.05$) with MBN, and soil C/N ultimately influenced the magnitude of soil microbial nitrogen (Liao et al., 2000). The relationship between soil TP, pH and soil microbial activity was not significant ($P > 0.05$).

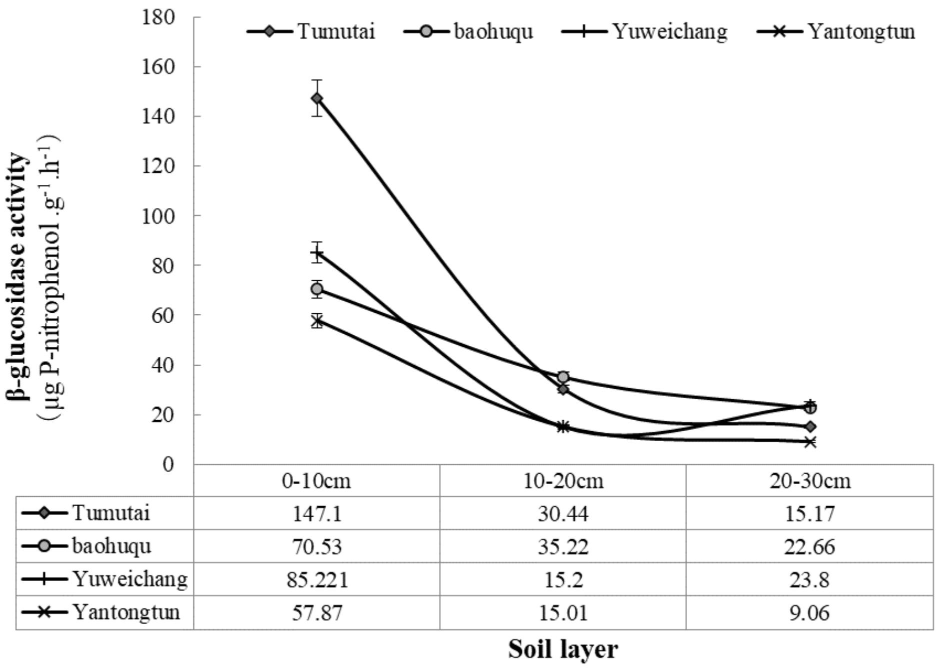

Figure 14. *Distribution of beta glucosidase activity. Data are shown as mean ± SD*

Table 4. *Partial correlation coefficients among soil biological activity and soil nutrient content*

	MBC	MBN
Urease	0.985**	0.76*
Acid phosphatase	0.04	0.815**
Alkaline phosphatase	0.946**	0.63
Polyphenol oxidase	-0.534	-0.069
Catalase	-0.331	-0.154
β-glucosidase	0.977**	0.953**
Bacteria	0.118	-0.545
Fungi	0.216	-0.2
Actinomycete	-0.371	-0.458
Total number of bacteria	0.088	-0.574
Organic C	0.91**	-0.228
Total N	0.109	-0.416
Total P	-0.451	-0.4
Hydrolyzed N	0.327	0.06
Available K	-0.32	-0.416
C/N	-0.214	0.796*
pH	0.07	0.259

**$p < 0.01$, *$p < 0.05$

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Table 5. *Collinearity diagnostics of every soil nutrient models*

	Total number of bacteria	Actinomycete	MBN	Catalase	β-glucosidase	Constant
Organic C model	-	1.627	-	16.921	-	1.000
Total N model	1.634	-	-	-	-	1.000
Hydrolyzed N model	-	-	-	-	2.643	1.000
Available K model	-	1.401	-	-	-	1.000
C/N model	-	-	2.365	-	-	1.000

Values in the table are the condition indexes for the selected indicators in each model; "-" indicates unselected

Table 6. *Partial correlation coefficients among soil nutrient content and soil microbial activity*

	Organic C	Total N	Hydrolyzed N	Available K	C/N
Urease	-0.054	0.153	0.17	-0.413	-0.342
Acid phosphatase	-0.1	-0.169	0.044	0.27	-0.185
Alkaline phosphatase	-0.152	-0.266	0.023	0.346	-0.042
Polyphenol oxidase	-0.508	-0.2	-0.251	0.501	-0.202
Catalase	0.647*	0.576	0.464	0.142	-0.087
β-glucosidase	0.002	-0.023	0.845**	0.395	-0.196
MBC	0.161	0.119	0.405	0.075	-0.292
MBN	0.152	-0.031	0.217	0.257	0.816**
Bacteria	-0.095	0.688*	0.083	0.023	-0.365
Fungi	0.175	0.171	0.065	0.072	-0.257
Actinomycete	0.834**	0.049	0.268	0.889**	0.348
Total number of bacteria	-0.095	0.05	0.107	0.23	-0.322

**$p < 0.01$, *$p < 0.05$

Weight coefficients and eigenvectors of soil total nutrient load factors

Each soil nutrient index of soil samples with different degradation levels was analyzed and, according to the principle of principal component analysis, when the cumulative variance contribution is greater than 85%, it can be used to represent the overall variability of the study samples (Li et al., 2000). The variable load factor weight coefficient of each soil nutrient (*Table 7*). For load factor weights, organic matter, organic C and Hydrolyzed N were the first three main Factor 1, while available K and C/N were the negatively correlated nutrient element of Factor 2. For eigenvectors, all Factor 1 soil nutrients were positive correlation, and available K and C/N were negative correlation of Factor 2.

Weight coefficients and eigenvectors of total microbial activity load factors in soil

Similarly, principal component analysis was carried out for each microbial activity index of all soil samples, followed by variable load factor weight coefficient of each soil microbial activity index (*Table 8*). For load factor weights, all total soil microbial activity were positive of Factor 1, while urease, MBC and fungi were the negatively

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correlated nutrient element of Factor 4. For eigenvectors, all Factor 1 soil nutrients were positive correlation, and urease, MBC and fungi were negative correlation of Factor 4.

Table 7. *Component matrix and characteristic vector of total soil nutrient*

		Load factor weights		Eigenvectors	
		Factor 1	**Factor 2**	**Factor 1**	**Factor 2**
Total soil nutrient	Organic matter	0.983	0.051	0.455	0.045
	Total N	0.312	0.909	0.144	0.8
	Total P	0.655	0.393	0.303	0.346
	Hydrolyzed N	0.894	0.007	0.414	0.006
	Available K	0.823	-0.325	0.381	-0.286
	Organic C	0.983	0.051	0.455	0.045
	C/N	0.854	-0.445	0.395	-0.392

Table 8. *Component matrix and characteristic vector of total soil microorganism*

		Load factor weights				Eigenvectors			
		Factor 1	**Factor 2**	**Factor 3**	**Factor 4**	**Factor 1**	**Factor 2**	**Factor 3**	**Factor 4**
Total soil microbial activity	Urease	0.721	-0.031	0.062	-0.513	0.317	-0.019	0.049	-0.501
	Acid phosphatase	0.527	-0.407	0.554	0.131	0.232	-0.251	0.437	0.128
	Alkaline phosphatase	0.166	-0.855	0.255	0.242	0.073	-0.528	0.201	0.236
	Polyphenol oxidase	0.414	0.036	-0.338	0.713	0.182	0.022	-0.267	0.696
	Catalase	0.344	-0.156	0.72	0.102	0.151	-0.096	0.568	0.1
	Glucosidase	0.924	0.172	-0.105	0.195	0.406	0.106	-0.083	0.19
	MBC	0.981	-0.071	0.023	-0.125	0.431	-0.044	0.018	-0.122
	MBN	0.965	-0.086	-0.006	0.06	0.424	-0.053	-0.005	0.056
	Bacteria	0.003	0.865	0.459	0.105	0.0013	0.534	0.362	0.103
	Fungi	0.865	0.094	-0.129	-0.315	0.38	0.058	-0.102	-0.308
	Actinomycetes	0.74	0.303	-0.459	0.098	0.325	0.187	-0.362	0.096
	Total bacteria	0.116	0.898	0.382	0.118	0.051	0.555	0.302	0.115

Standardized values of soil nutrient and microbial indicators for the test soil samples

The standardized values of soil fertility index and microbial activity index in each degradation stage of wetland can be obtained by statistical calculation (*Table 9*). *Table 8* clearly shows various soil fertility indicators and microbial activity indicators.

Table 9. *Standardized value of every soil nutrient, soil microorganism index*

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Total soil nutrient	x_1	0.205	0.293	-0.364	2.358	-0.221	-0.373	0.001	-1.28	-1.495	-0.133	1.049	-0.039
	x_2	0.46	-0.582	0.101	0.101	2.115	0.276	-0.844	-0.268	-1.882	-0.038	1.116	-0.556
	x_3	0.603	0.462	-0.457	1.046	0.56	-0.722	-1.141	-1.232	-1.444	0.236	0.29	1.799
	x_4	0.448	-0.152	0.301	2.424	-0.006	-1.139	0.285	-0.609	-1.395	0.287	0.461	-0.907
	x_5	-0.35	-0.888	-0.889	2.809	-0.297	-0.677	0.662	-0.294	-0.51	0.234	0.154	0.045
	x_6	0.205	0.293	-0.364	2.358	-0.221	-0.373	0.001	-1.28	-1.495	-0.133	1.049	-0.039
	x_7	-0.154	0.857	-0.501	2.312	-1.096	-0.602	0.796	-1.342	-0.661	-0.17	0.171	0.39

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		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Total soil microbial activity	x_1	2.156	1.154	-0.163	1.199	-0.221	-0.526	-0.39	0.229	-0.693	-1.047	-0.797	-0.902
	x_2	0.58	0.899	0.366	0.057	-0.363	-0.661	2.398	-0.422	-1.211	0.107	-1.202	-0.548
	x_3	0.47	0.476	0.862	-0.791	-1.873	-1.685	1.416	0.413	-0.196	0.545	-0.18	0.542
	x_4	-0.351	-0.714	-1.474	1.393	-0.451	-0.679	0.494	2.051	-0.41	0.762	-0.626	0.005
	x_5	0.573	0.548	0.825	-0.132	0.197	-0.668	0.248	-0.018	-2.914	0.499	0.295	0.545
	x_6	0.656	-0.215	-0.525	2.545	-0.333	-0.71	0.344	-0.714	-0.861	1.018	-0.709	-0.497
	x_7	1.499	0.566	-0.212	2.14	-0.708	-1.057	0.324	-0.558	-1.041	0.164	-0.421	-0.696
	x_8	0.879	0.546	-0.344	2.252	-0.804	-0.912	0.778	-0.801	-0.915	0.599	-0.64	-0.637
	x_9	0.027	-0.470	-0.474	-0.007	3.03	-0.439	-0.371	-0.456	-0.467	0.492	-0.434	-0.432
	x_{10}	2.253	-0.445	-0.628	1.937	-0.438	-0.31	0.117	-0.6	-0.609	-0.374	-0.432	-0.469
	x_{11}	-0.255	-0.301	-0.327	3.173	-0.229	-0.31	-0.315	-0.323	-0.326	-0.203	-0.294	-0.289
	x_{12}	-0.011	-0.508	-0.517	0.477	2.948	-0.48	-0.413	-0.498	-0.51	0.454	-0.473	-0.47

“1,2,3…12” in table indicate 0-10 cm, 10-20 cm,20-30 cm soil horizon in Baohuqu, Tumutai, Yantongtun, Yuweichang respectively; “$x_1,x_2…x_7$” in total soil nutrient indicate the same as in *Table 6*; “$x_1,x_2…x_{12}$” in total soil microbial activity indicate the same as in *Table 7*

By substituting the standardized data of different soil samples and their indicators in *Table 8*, the corresponding total nutrient values and total microbial activity values of each soil sample can be obtained (*Table 10*). The results of correlation analysis showed that there was a significant linear correlation between soil total nutrients and total microbial activity in wet meadows with different degradation degrees:

$$Y = 1.06428x + 4E - 16 \qquad R^2 = 0.6512$$

Table 10. *Total soil microorganism activity value and total soil nutrient value of every soil samples*

	Total soil nutrient value	**Total soil microbial activity value**
1	0.425	1.104
2	0.107	0.053
3	-0.515	-0.576
4	3.451	2.36
5	0.256	0.661
6	-0.924	-0.963
7	-0.145	0.361
8	-1.675	-0.505
9	-2.34	-1.438
10	0.065	0.475
11	1.194	-0.833
12	0.151	-0.699

Conclusions

(1) In the microbial community structure of wet meadow soil in Zhalong Wetland, bacteria were dominant, followed by actinomycetes, and fungi were the least in number, with the proportions of 90.38%, 9.50% and 0.12%, respectively.

(2) All components of Zhalong wetland have obvious distribution characteristics, but there are some differences in their distribution. Soil nutrients and microbial activities decrease significantly with the aggravation.

(3) There is a close relationship between soil nutrients and soil microorganisms in Zhalong Wetland, while only P and pH have no significant relationship with soil microbial activity ($P > 0.05$).

(4) Soil microbial biomass carbon and nitrogen had obvious trends with the deepening of soil layer. There were also great differences in soil microbial biomass in the same soil layer. In addition, the spatial variation of soil MBN among different soil layers in each study site was significantly smaller than that of MBC.

(5) Correlation analyses showed that the distribution of soil microbial carbon and nitrogen in the wetland was significantly ($P < 0.05$) positively correlated with soil organic carbon and effective phosphorus content, and positively correlated with soil water content ($P < 0.01$). Soil organic carbon content, soil moisture content and litter input were significantly correlated ($P < 0.05$). Meanwhile, human destruction has a significant impact on soil microbial biomass, mainly indirectly affecting microbial distribution by altering the original hydrological characteristics, wetland types, vegetation communities, and other factors of the wetland.

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