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Research Article

Sedimentary Enzyme Kinetics of Land/Water Ecotones with Reed Domination

Land/water ecotones, the transitional boundary zone between terrestrial and aquatic ecosystems, can effectively trap and assimilate nutrients from external sources. The changes of nutrient cycling will cause a response in enzyme activity. In this paper, the enzyme kinetics of land/water ecotones in a shallow eutrophic wetland in China, dominated by a reed (*Phragmites australis*) community, are studied. The results indicate that land/water ecotones exhibit a strong filtration function and nutrient factor is an important feature affecting the distribution of enzymes in the transect of the littoral zone. The obvious spatial differences of enzyme activities and nutrients are present in the transect of the reed beds. The land area is the hot zone, where significant changes in enzyme activity occur. T-test results show that the landward spot, 1 m away from the water-land interface, is quite different from other spots, and the 1 m spot is the hot spot where great biochemical change happens. The principal component analysis of the environmental variables shows that nutrient factors have an important affect on enzyme distribution in the transect of the littoral zone. The nutrient factor explains about 54% of the observed variance. In particular, TOC (total organic carbon) correlates with the other five factors (TN; total nitrogen, TP; total phosphorus, phosphatase, β -glucosidase, and urease) with correlative coefficients of 0.932, 0.595, 0.488, 0.433, and -0.468 , respectively. The results indicate that TOC is a very important factor in Baiyangdian wetlands.

Keywords: Baiyangdian wetlands; Enzyme activity; Filtrating function; Total organic carbon

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1 Introduction

Land/water ecotones are the transitional boundary zone between terrestrial and aquatic ecosystems [1, 2]. Land/water ecotones can act as an instrument for the improvement of surface water and ground water quality [3]. Many researchers have reported the strong nutrient filtering effect of the littoral zones [4–6]. Land/water ecotones can not only significantly decrease the nitrogen and phosphorus concentrations in both overland flow and groundwater [7, 8], but also reduce upstream, non-point source, pollutants [2, 9]. Wang and Yin [10] pointed out that the purification functions of littoral zones could be summarized into two types: physical mechanisms and biological mechanisms.

Land/water ecotones, as a physical buffer, decrease the energy of flowing water, removing 80–90% of the sediment leaving agricultural fields [9, 11] and cause the deposition of nutrient substances. Substantial nutrients accumulated in littoral zones, as a result of a physical mechanism, thus leading to a spatial change in the nutrient distribution of the littoral zone. This change causes a response from the microbial environment in land/water ecotones.

Triska et al. [12] found that some important biogeochemical processes occur in littoral zones and Wang et al. [13] inferred that soil enzymes abound in littoral zones, where oxygen and carbon dioxide cause great change.

Enzymes are key biological means by which complex organic compounds degrade into small molecules that can be assimilated by soil microbes [14]. Enzymes respond rapidly and directly to changes in nutrient and substrate availability by manipulating enzyme kinetics to best capitalize on environmental conditions [15], and enzyme activities have the potential to reflect changes in nutrient cycling [16]. The activities of hydrolases, such as β -glucosidase, urease, and phosphatase, are the keys controlling the availabilities of C, N, and P in soil, respectively [17]. β -glucosidase is a good indicator for soil quality because it controls the mineralization of organic matter. Phosphatase is one example of a suite of enzymes involved in organic matter degradation and nutrient cycling that can be affected by nutrient loading [18, 19]. Urease hydrolyzes long chain peptides into polypeptides and ammonia [20]. Because of the close relationship between enzyme activities with soil microbes and nutrient cycling, it is possible to use the variations in enzyme activities in littoral sediments to reflect changes in both the physical and biological mechanisms. In this research, enzyme (β -glucosidase, phosphatase, urease) kinetics in the sediments of littoral zones in shallow eutrophic wetlands with reed domination in China were

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Figure 1. Distribution of sampling spots in the sampling stations.

studied. The goals of this research were to evaluate the purification mechanism of littoral zones and to investigate the relationship between nutrients and enzyme kinetics.

2 Materials and Methods

2.1 Site Description

The field experimental sites were located in the littoral wetlands of Baiyangdian Wetlands, the largest freshwater body in North China. Baiyangdian wetlands have a total surface area of 366 km² at a level of 10.5 m above sea water; fluctuations in the wetland water level depend on the hydrologic conditions. Baiyangdian wetlands consist of more than 140 small shallow wetlands, which are divided by reed beds and 3700 crisscrossed ditches. A local reed (*Phragmites australis* var. *Baiyangdiansis*) is the dominant hydrophyte in the Baiyangdian wetlands. The Baiyangdian wetlands have a temperate continental climate, with an annual mean temperature of 12.2°C, precipitation of 529.7 mm, and evaporation of 1002 mm. The average water content of the soil was 27.5%.

The Baiyangdian wetlands, as the mouth of 9 rivers, receives runoff, combined with municipal wastewater and non-point source pollutants. The river Fuhe is the largest tributary of the Baiyangdian wetlands. The area around the mouth of the Fuhe river has a higher concentration of nutrients in comparison with the other areas. During the experimental period, the average TN (total nitrogen) concentration in the water at the mouth of the river was 25.9 ± 1.5 mg/L and the average TP (total phosphorus) concentration was 0.21 ± 0.07 mg/L.

Eight sampling stations were established in the transaction, for sampling from the open water area to the land area, named A1–A8, respectively, these are detailed in Fig. 1. The interval distances between stations and the water table point were 0.8, 0.25, 0.25, 0.5, 1, 2, 4, and 6 m, respectively. Stations A3–A8 were distributed in the land area, A1 and A2 in the water area. Based on previous studies by this group it was hypothesized that the spatial patterns of nutrient

Figure 2. The seasonal hydrologic variability in the Baiyangdian wetlands in 2007.

and related enzymes would change greatly in the water table fluctuating zone, thus 4 spots from A2–A5 were distributed in the water table fluctuating zone.

The water table of the Baiyangdian wetlands undergoes yearly fluctuations, because of upstream flow, agricultural irrigation, and evaporation. From March–July the water table level keeps decreasing because of evaporation. The rainy season in the Baiyangdian wetlands begins in July. Upstream rainwater ensures that the Baiyangdian wetlands water table keeps increasing until the end of rainy season. The water table reaches its highest level during spring, due melting ice. The yearly fluctuation is about 1 m, Fig. 2, which shows the seasonal hydrologic variability in the Baiyangdian wetlands in 2007. This fluctuation takes water into the land area, based on the geomorphic structure of the reed beds. Soil sampling was carried out on the 27th of July and on the 27th of August 2007, when the Baiyangdian wetlands water table was at its lowest, to study the effect of fluctuations in the water table.

Table 1. The soil nutrient contents and enzyme activities.

	Water area				Land area			
	Mean	Median	SD	CV	Mean	Median	SD	CV
TOC	1.43	1.35	0.20	0.06	1.02	1.09	0.34	0.08
TN	1.41	1.44	0.29	0.08	0.97	1.01	0.29	0.07
TP	0.58	0.58	0.06	0.04	0.59	0.61	0.08	0.03
Phosphatase	32.88	38.05	11.41	0.14	64.97	68.13	35.70	0.13
β -Glucosidase	32.62	44.62	41.85	0.52	86.06	91.09	42.12	0.12
Urease	5.93	5.44	2.68	0.18	7.11	8.67	5.83	0.19

2.2 Soil Sampling

A soil auger ($\varnothing = 6.5$ cm) was used to collect sediments. At each spot three soil samples were selected in the transect and the horizontal distance between two samples was about 1 m. Three soil samples were collected below the land surface (at depths of 0.1 m, 0.3 m, and 0.5 m). In the field, three subsamples from each depth were thoroughly mixed, stones and visible roots removed, then sieved (<2 mm) and stored at field moisture content at 4°C. The samples were analyzed at the State Key Laboratory of Environmental Aquatic Chemistry within 48 h.

2.3 Laboratory Analyses

Enzyme activity was determined using literature methods [21]. For the phosphatase assay, 1 g of soil (wet weight) was mixed with 2 mL of Tris buffer (pH 7.4), 1 mL of 0.1% 4-nitrophenylphosphate, and disodium salt (substrate). It was then placed in an incubator at 37.0°C for 0.5 h. To terminate the phosphatase reaction, 2 mL of 0.2 mol L⁻¹ NaOH was added, and the reactants were then filtered. The reaction product in each sample was measured using spectrophotometry at 410 nm.

The activity of β -glucosidase was measured according to the method described in [22]: 1 g of soil was placed into a 50 mL flask, and then 0.25 mL of toluene, 4 mL of pH 6.0 modified universal buffer (MUB), and 1 mL of 0.5 mol L⁻¹ *p*-nitrophenyl- β -D-glucoside (PNG) solution were added. The flask was swirled to fully mix the contents, stoppered, and incubated at 37°C for 1 h. Then, 1 mL of 0.5 mol L⁻¹ CaCl₂ and 4 mL of 0.1 mol L⁻¹ pH 12 Tris (hydroxymethyl) aminomethane (THAM) buffer were added to stop the reaction. The soil suspension was allowed to develop a yellow color and the color intensity was determined using a spectrophotometer at 410 nm.

Assays for urease activity were as described by García-Gil et al. [23]. Urease was determined in 0.1 mol L⁻¹ phosphate buffer at pH 7. 1 mol L⁻¹ urea and 0.03 mol L⁻¹ BAA (benzoylargininamide) were used as substrates. 2 mL of buffer and 0.5 mL of substrate were added to 0.5 g of the prepared sample and it was then incubated at 30°C for urease or 37°C for protease for 90 min. Both activities were determined by the NH₄-N released. Enzyme assays were corrected to a dry weight basis.

Total phosphorus (TP) of the soil samples was analyzed by the HClO₄-H₂SO₄ digestion method. Total organic carbon (TOC) was measured by digestion of the soil samples with K₂Cr₂O₇ and subsequent titration with H₂SO₄ [24]. All enzyme (phosphatase, β -glucosidase, urease) assays were corrected to a dry weight basis.

2.4 Statistical Data Analysis

The SPSS 13.0 System for Windows was adopted to analyze the experimental data. Spearman's correlation ($p = 0.05$) was used to assess the relationships among the environmental variables and enzymes. One sample t-test ($p = 0.05$) was used to analyze the difference between land area and water area. The SAS System for Windows 9.0 was adopted for analyses of variance (ANOVA) and the principal component analysis for environmental variables. The Origin-Pro 7.5 System was used to plot the experimental data of both the field and indoor experiments.

3 Results

3.1 Horizontal Distributions of Enzyme Activities and Nutrient Contents

The distribution of soil TOC (total organic carbon), TN, TP, phosphatase, β -glucosidase, and urease was studied in the transect of reed beds through the littoral zone of the Baiyangdian wetlands. The mean contents of TOC, TN, and TP were 11.5 g kg⁻¹, 1.12 g kg⁻¹, and 0.60 g kg⁻¹, respectively, and are listed in Tab. 1. An obvious spatial difference of nutrient content was found in the transect of the reed beds. The mean sedimentary TOC and TN contents in the water area were greater than those in the land area, and the mean TP contents in the water area and land area were the same. All maximum nutrient contents were found in the land area. In particular, the highest TOC and TP contents were found in the 1 m area. The coefficient of variation (CV) of TOC in the land area was greater than that in the water area. The mean TN in the land area was less than that in the water area but they had the same CV. The CV of TP in both areas was very small. The greatest change in nutrient content showed that the land area was the hot zone. The 1 m spot was the hot point because the highest content of TOC and TP were apparent there.

The enzyme activities pointed to obvious spatial differences in the transect of the reed beds. Both phosphatase and β -glucosidase increased from the water to the land area at the 1 m spot and gradually decreased inside the land area, Fig. 3. The mean enzyme activities of phosphatase, β -glucosidase, and urease, in the transect, were 60.01, 79.48, and 7.86 (μ g/g/h), respectively (Tab. 1). The mean enzyme activities of phosphatase, β -glucosidase, and urease in the water area were lower than those in the land area. All the highest enzyme activities were found in the land area. In particular, the highest activities of both phosphatase and β -glucosidase were detected at the 1 m spot inside the land area. The CV of the three

Figure 3. Horizontal distributions of TOC (%), TN (g kg^{-1}), TP (g kg^{-1}), β -glucosidase, urease and phosphatase ($\mu\text{g g}^{-1} \text{h}$) in the red reed.

Figure 4. Vertical distributions of TOC (%), TN (g kg^{-1}), TP (g kg^{-1}), β -glucosidase, urease and phosphatase ($\mu\text{g g}^{-1} \text{h}$) in the red reed.

enzymes in the land area was greater than that in the water area. This result was consistent with the spatial distribution regularity of the nutrients, whereby the land area was the hot zone and the 1 m spot the hot point.

3.2 Vertical Distributions of Enzyme Activities and Nutrient Contents

The vertical distributions of the enzyme activities and nutrient contents in the sediment cores are shown in Fig. 4. Enzyme and nutrient content profiles approximately followed a similar decrease-

ing trend from the upper to the lower sediments, with the exception of urease. The CV of the enzyme and nutrient contents of the upper sediments were greater than those of the lower sediments.

3.3 Relationships between Enzymes and Physical and Chemical Variables

A hierarchical cluster analysis (HCA) was conducted to statistically examine the distributions of enzyme activities and nutrient contents in the transect of the reed beds. Fig. 5 shows the results in a HCA dendrogram. The samples were classified into two cluster

*****HIERARCHICAL CLUSTER ANALYSIS*****

Dendrogram using Average Linkage (Between Groups)

Figure 5. The cluster analysis of different sampling spots, using all of the nutrient factors and enzyme factors. The results demonstrate that A4 (0.5m spot) and A5 (1 m spot) are different from the others spots.

Table 2. The results of discriminant analysis.

		Predicted Group Membership ^a			Total
		group	1.00	2.00	
Original	Count	1.00	2	0	2
		2.00	0	6	6
	%	1.00	100.0	0	100.0
		2.00	0	100.0	100.0

^a 100% of original grouped cases correctly classified.

groups, G1 and G2. G1 included A4 and A5, the other points belonged to G2. Discriminant analysis was used to examine the accuracy of the cluster analysis results. The classification results showed that the original grouped cases were correctly classified, Tab. 2. Both A4 and A5 were located in the land area near the 1 m point, indicating that this place is quite different from the other locations. The same spatial distribution pattern of enzyme activities and nutrient content provided further evidence that the 1 m point is the hot point, where soil enzymes abound and high biochemical action occurs.

The relationship between the enzymes and nutrients was analyzed using the Spearman correlation and the results are listed in Tab. 3. Complex relationships were found between the enzymes and nutrients. Phosphatase was positively related to β -glucosidase ($r = 0.603$) and TP ($r = 0.443$). Positive relationships were found between β -glucosidase and TOC ($r = 0.433$), TN ($r = 0.507$), and TP ($r = 0.506$). In particular, TOC was correlated to the other five factors (TN, TP, phosphatase, β -glucosidase, and urease) and the correlative coefficients are 0.932, 0.595, 0.488, 0.433, and -0.468 , respectively. The results of the correlation analysis showed that TOC is a very important factor in the Baiyangdian wetlands.

Mutual correlations were common among the environmental variables in the sediments. Therefore, multiple linear regression analyses of the enzymes could not be performed directly and principal components were derived in order to analyze the relationships among these variables in the transect of the reed beds. The principal component analysis on the environmental variables resulted in three components, which explained 89% of the total variance, as detailed in Tab. 4. The first component (C1) explained about 54% of

Table 3. Spearman correlation coefficients for the relationships between enzymes and nutrients of sediment cores ($n = 21$).

Variables	TOC	TN	TP	PHO ^a	GLU ^a	URE ^a
TOC	1.000	–	–	–	–	–
TN	0.932 ^c	1.000	–	–	–	–
TP	0.595 ^c	0.651 ^c	1.000	–	–	–
PHO	0.488 ^b	0.434	0.443 ^b	1.000	–	–
GLU	0.433 ^b	0.507 ^b	0.506 ^b	0.603 ^c	1.000	–
URE	-0.468^b	-0.618^c	-0.259	0.018	-0.245	1.000

^a PHO: phosphatase; GLU: β -glucosidase; URE: urease; ^b Correlation is significant at the 0.05 level (2-tailed); ^c Correlation is significant at the 0.01 level (2-tailed).

the observed variance. Several variables were significantly correlated to this first component, namely TOC, TN, and TP. C1 could be seen to be influenced by the nutrient factor. The second component (C2) explained about 19% of the observed variance and the variable of distance was strongly correlated to this component. The third component (C3) explained about 16% of the observed variance and the variable of depth was strongly correlated to this component. Results of principal component analysis indicate that the nutrient factor is the determining factor affecting the distribution of enzyme activities, and both the distance factor and depth factor are important factors. A stepwise multiple linear regression analysis between the PCA components and enzyme activities showed that the three enzymes could be described as:

$$\text{PHA} = 58.2 + 16.92 \text{ C1} + 18.26 \text{ C2} - 2.45 \text{ C3} \quad (r^2 = 0.558, p < 0.0001)$$

$$\text{GLU} = 79.5 + 19.79 \text{ C1} + 8.58 \text{ C2} - 13.03 \text{ C3} \quad (r^2 = 0.338, p < 0.0001)$$

$$\text{URE} = 7.57 - 2.74 \text{ C1} + 2.31 \text{ C2} - 1.17 \text{ C3} \quad (r^2 = 0.528, p = 0.0005)$$

4 Discussion

4.1 The Purification Mechanisms of Littoral Zones

Nutrients entered wetland systems via surface runoff and subsurface flow from two pollution source types, namely, conspicuous point sources, such as a factory or village and non-point pollution sources, such as agriculture [25]. Several researchers have shown that littoral zones can effectively attenuate nutrients in ground and surface water [26] and that littoral wetlands can effectively trap and digest nutrients from external sources [27–29]. The spatial change of nutrients showed that surface runoff was the source of nutrients coming into Baiyangdian wetlands. The dynamics of nutrient changes in reed belts were varied and complicated [30, 31]. Water level fluctuations were assumed to be an important factor for the dynamics of reed belts [32]. The Baiyangdian wetlands have slight fluctuations in daily water levels, wave fluctuations due to wind may reach 0.05 m, and boat traffic may cause water levels to fluctuate by 0.1 m [10]. The yearly fluctuation change, caused by upstream flow, agricultural irrigation, and evaporation, was about 1 m. This fluctuation takes water into the land area, based on the geomorphic structure of the Baiyangdian wetlands. In this study the highest TOC and TP contents appeared at 1 m in the land area, although the highest mean nutrient content was found in the water area. This result indicated that water fluctuation will take nutrients into the inner land area, through surface water and subsurface water flow. The high CV of the nutrient content in the land area proved that

Table 4. Results of Principal Component Analysis (PCA) for physical and chemical indexes in a transect of reed bed through the riparian zone of Baiyangdian wetlands.

Variables	Component 1	Component 2	Component 3	Component 4	Component 5
Distance	−0.291	0.860	−0.104	0.280	0.295
Depth	0.339	0.303	0.872	−0.155	0.096
TOC	0.533	0.197	−0.252	0.495	0.607
TN	0.569	−0.170	−0.024	0.337	0.730
TP	0.438	0.319	−0.405	−0.734	0.058
Eigenvalue	2.69	0.97	0.78	0.48	0.09
Variance Explained, (%)	0.54	0.19	0.16	0.10	0.02
Cumulative, (%)	0.54	0.73	0.89	0.98	1.00

Figure 6. The partial pressure of CO₂ and dissolved oxygen in the ditch water and subsurface water.

this area was the hot zone, where significant changes in nutrient content occurred and the 1 m spot was the hot point.

The transition zones between the reed beds and ditches were particularly active areas, as a result of a physical mechanism [13], and caused changes in the nutrient spatial distribution. That simultaneously led to the microbial response. In previous studies it was shown that the partial pressure of CO₂ in the subsurface water progressively increased with distance and the CO₂ pressure was 23–704 times larger than the atmospheric pressure of CO₂ (as seen in Fig. 6 of Wang et al. [13]). A tendency for O₂ to increase occurred from 0.5 to 8 m. This result indicated that littoral zones had well developed microorganisms and abounded in soil enzymes. In this study the obvious spatial difference in enzyme activity was observed in the transect of the reed beds. The phosphatase and β -glucosidase increased and gradually decreased from the water area to inside the land area. The mean values of the three enzymes in the land area were higher than those in the water area and all the highest enzyme activities were found in the land area. Consistent with the spatial distribution trend of the nutrients, the highest phosphatase and β -glucosidase presented 1 m inside the land area and the T-test result showed that enzyme activity at the 1 m point was significantly different from the other points. This result indicates that land/water ecotones have a strong filtration effect on nutrients [4, 6, 12, 33] and that there is a hot zone where significant biogeochemical processes occur.

4.2 Relationships between Enzymes and Nutrient Content

The evaluation of soil quality was quite complex and required the consideration of physical, chemical, and biological variables [34].

Enzymatic activity in soil is controlled by physical and chemical conditions, although the major limiting factors for microorganisms are temperature and water content [35]. Enzyme activity immediately responded to changes in the soil environment, due to the close correlation between soil nutrients and enzyme activity [36, 37]. Complex relationships were found between the soil nutrients and soil enzymes in the littoral zones of the Baiyangdian wetlands. In the transect considered, three principal components could be found, namely, nutrient factor, distance factor, and depth factor. Sinsabaugh [38] pointed out that the extracellular soil enzyme activity might be directly affected by physico-chemical conditions. In the Baiyangdian wetlands nutrient factors could explain more than half of the observed variance. This result indicated that nutrient factors were the most important feature affecting the distribution of enzymes in the transect of the littoral zone. The relationships between enzymes and nutrients were fairly complex and the mutual interactions existed among biochemical indexes. Both the geographic location and climate conditions affect the PCA component coefficients. The stepwise multiple linear regression analysis between the PCA components and enzymes activities was an attempt to explore the relationship between the enzymes and nutrients.

In the Baiyangdian wetlands significant correlations were found between total organic carbon (TOC) and five other factors. Total organic carbon is usually considered as one of the most important properties of soils because of its impact on ecosystem sustainability, affecting other physical, chemical, and biological characteristics of the soil [39]. Enzyme activity can provide an indication of quantitative changes in the organic matter of soil [40]. Among the hydrolytic enzymes, phosphatase and β -glucosidase activities have been fre-

quently used as indicators of changes in the quantity and quality of a soils organic matter [41]. Melero et al. [35] and Monokrousos et al. [42] found that the activities of enzymes involved in the N and P cycles were enhanced through organic management. The results of the correlation analysis showed that TOC is a very important factor in the Baiyangdian wetlands. Hill et al. [43] pointed out that the microbial enzyme activity was controlled, not only by the N, P, and C availability, but also by the ratio of these components. In the transect, the average C:N:P values were 57:4.7:1. The Baiyangdian wetlands were C-limited wetlands, based on the Redfield ratio of 106C:16N:1P [44]. Therefore TOC, as the limiting factor of the Baiyangdian wetlands, greatly affected soil enzyme activity.

5 Conclusions

The results of this study showed that littoral zones exhibited a strong chemical filtrating function and that nutrient factor was the most important factor affecting the distribution of enzymes in the transect of the littoral zone. The obvious spatial differences in the enzyme activities and nutrients were presented in the transect of the reed beds. Water fluctuation took nutrient elements into the inner land area through surface water and subsurface water flow. Enzyme kinetics in the littoral zones presented high mineralization, especially in the 1 m area, as a result of nutrient content and oxygen distribution. The land area was the hot zone, where significant change in enzyme activity occurred. The principal component analysis on the environmental variables showed that nutrient factors were the most important factors affecting the distribution of enzymes in the transect of the littoral zone. The significant correlations between total organic carbon (TOC) and the other five factors indicated that TOC is a very important factor in the Baiyangdian wetlands.

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