Phosphatase Activity Along Soil C and P Gradients in a Reed-Dominated Wetland of North China

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Received: 16 March 2009 / Accepted: 3 March 2010 / Published online: 12 May 2010
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Abstract Previous studies in oligotrophic wetlands showed that phosphatase activity (PHA) was positively related to soil total phosphorus (TP) content, and that PHA can serve as a predictor of wetland degradation. However, phosphatase dynamics are poorly known for eutrophic wetlands, where anthropogenic nutrient inputs elevate soil P content. We reported variations in PHA along a nutrient content gradient in soils of the eutrophic Baiyangdian wetland in North China. Soil PHA mainly responded to the soil organic carbon (C) content, and microbial biomass carbon. A positive correlation existed between PHA and C but not between PHA and TP in the wetland soils. The Baiyangdian wetland has high soil P content, averaging 598 mg kg^{-1} . The high TP content caused the lower mass ratios between sediment C and TP (C:TP) that are lower than those reported in many wetlands. Results suggest that there may be a TP threshold above which PHA has no relationship with TP. A laboratory incubation study showed that PHA increased with added C, suggesting that the soil C content was a limiting factor for P mineralization in the Baiyangdian wetland. PHA, together with its limiting factors, could reflect the state of eutrophication of the wetland.

Keywords Baiyangdian Lake · C:TP ratio · C-limited · Eutrophic wetlands · P threshold

Introduction

Enzyme activity plays an important role in maintaining the functioning of wetlands. It is sensitive to changes in environmental conditions such as soil water content, temperature, pH, and plant exudates (Shackle et al. 2000). Ectoenzymes respond rapidly to changes in nutrient and substrate availability. Spatial patterns in community ectoenzyme kinetics can elucidate potential organic matter sources and availability (Williams and Jochem 2006). Because of the importance of phosphorus (P) in plant nutrition and the complex nature of organic P in soils (Olander and Vitousek 2000), phosphatase activity (PHA) is often taken as an example of a suite of enzymes involved in organic matter degradation and nutrient cycling that can be affected by nutrient inputs (Newman and Reddy 1993; Marx et al. 2001).

PHA and other associated complementary indicators were found to act as an early warning indicator of wetland eutrophication (Newman et al. 2003). PHA was also recommended as a means of assessing the P status of aquatic systems (Healey and Hendzel 1980; Gage and Gorham 1985). Different researchers have found an inconsistent relationship between PHA and P in soils. Some reported a positive correlation (Amador et al. 1997; Chen 2003), but others found that high soil P concentrations reduced PHA (Tabatabai 1982). Nutrient assimilation by the microbial community is controlled not only by the nitrogen (N), P, and carbon (C) content, but also by the ratios of these components (Hill et al. 2006).

PHA provides a simple and rapid measure of P status that is consistent with other factors including C:P and N:P ratios, and surplus P content (Pettersson 1980). However, the relationship between C and P mineralization is very complex (Sinsabaugh and Foreman 2001) and little is

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known about the relationships between PHA and soil nutrient contents in eutrophic wetlands. In addition, C availability is important to PHA (Hoppe 2003) and organic matter decomposition, which is an important process controlling internal nutrient cycling and soil accumulation/loss (Newman et al. 2001).

In our study, a reed-dominated eutrophic wetland was selected to study variations of PHA along soil C and P gradients. The goals of this research were to (1) evaluate the response of soil PHA to soil C in a eutrophic wetland with surplus P supply, and (2) discuss the possibility of using PHA to assess wetland degradation.

Materials and Methods

Site Description

The field experimental site was located in the reed beds of the Baiyangdian wetland, at 366 km² the largest freshwater body in North China. There are more than 140 small lakes, which are divided by reed beds and a network of 3,700 intersecting ditches. Water levels in the wetland fluctuate depending on hydrologic conditions (Wang et al. 2002). The site has a temperate continental climate, with an annual mean temperature of 12.2°C, precipitation of 529.7 mm, and evaporation of 1,002 mm. Reeds are the dominant macrophyte.

The Baiyangdian wetland is the end of nine rivers and receives runoff from agricultural drainage and city wastewater. The Fuhe River is the largest tributary of the wetland and it has the highest concentration of nutrients of the nine.

During the period of our experiment, the average total N (TN) concentration in water in the Fuhe River mouth area was 25.9 ± 1.5 mg/L and the average total P (TP) concentration was 0.21 ± 0.07 mg/L.

Soil Sampling and Preparation

Eight sampling stations were selected from the Fuhe River mouth area (S1 to S5) to the central part (S6 to S8) of the Baiyangdian wetland along a nutrient gradient (Fig. 1). From November to February, the Baiyangdian wetland is frozen. Weather conditions in March and April are similar. In this period the wetland is minimally impacted by boat activity and the water level is kept stable, thus we chose this period for our enzyme study. From the Fuhe River mouth, a large nutrient load is discharged into the Baiyangdian wetland each year. Due to the interception by reed-bed/ditch systems in the mouth (Wang et al. 2002), we hypothesized that spatial distribution gradients of nutrients and related enzymes exist. The distance between adjacent sampling stations was generally about 500 m. However, a waterside village and an open water area existed between S2 and S3, and thus the distance between these two stations was about 1,000 m. Also, a farm was located between S4 and S5; to avoid this disturbance, the distance between S4 and S5 was also about 1,000 m. In the central part, three stations were selected far removed from the Fuhe River mouth.

At each station, three sampling locations were selected inside a reed stand. These locations were 0.25 m away from the surface water boundary, and the lateral distance between each location was 1 m. Three soil samples were collected at depths of 0.1 m, 0.3 m, and 0.5 m, and subsamples (3) from

Fig. 1 Locations of eight sampling stations in the Baiyangdian wetland from the river mouth to the central area of the wetland. S1 to S5 were located in the area of the Fuhe River mouth, and S6 to S8 were located in the interior

Table 1 Mean±S.E. values ($n \leq 15$) for soil organic carbon (C) (g/kg), soil total nitrogen (TN) (g/kg), soil total phosphorus (TP) (g/kg), C:TN (mass-mass), C:TP (mass-mass), TN : TP (mass-mass), and soil phosphatase activity (PHA)

		C	TN	TP	C:TN	C:TP	TN:TP	PHA
March	River mouth	13.6*±1.51	1.19*±0.17	0.69*±0.10	11.5*±1.01	20.4*±5.53	1.79*±0.50	78.0*±17.8
	Central part	24.0±4.66	2.01±0.19	0.53±0.04	12.0±2.76	44.6±5.97	3.78±0.48	102.8±4.20
April	River mouth	15.2*±4.69	1.26*±0.29	0.59±0.15	12.0*±1.78	26.3*±8.74	2.22*±0.76	63.9*±14.7
	Central part	31.0±2.55	2.28±0.35	0.53±0.07	13.7±1.31	58.7±2.78	4.30±0.26	129.7±47.7

*Indicates significant differences between River mouth and Lake center

each depth were mixed thoroughly in the field. After collection, samples were stored at 4°C and within 48 h transported to the State Key Laboratory of Environmental Aquatic Chemistry for analysis. Average values of total organic C (TOC), TN, and TP, and PHA values from the three depths were calculated. Microbial biomass C (MBC) was determined using the fumigation extraction method (Vance et al. 1987).

Laboratory Analyses and Experiments

We hypothesized that adding C to increase soil C:TP ratio would enhance P mineralization. To verify this, a laboratory incubation experiment was conducted. C availability can be important to PHA (Hoppe 2003), so we added glucose to soils to increase the C content. Station 1 had a relatively high TP content and low C:TP ratio, so we collected soil samples collected there in March and April for use in the incubation experiment. Eleven levels of glucose (0, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, and 0.20 g) were added to 1 g soil samples to create a gradient ($n=3$ per level). Soil samples were kept at 37°C for 24 h and then PHA was assayed.

Enzyme activity was determined using the method of Tabatabai and Bremner (1969). For phosphatase assay, 1 g soil (wet weight) was mixed with 2 mL of Tris buffer (pH 7.4), 1 mL 0.1 M% 4-nitrophenyl phosphate disodium salt (substrate), and then placed in an incubator at 37°C for

0.5 h. We added 2 mL of 0.2 M NaOH to terminate the reaction, and then the reactants were filtered. The reaction product in each sample was measured at 410 nm using a spectrophotometer. TP content of soil samples was analyzed by the $\text{HClO}_4\text{-H}_2\text{SO}_4$ digestion method. TOC was determined using the methods of Nelson and Sommers (1982).

Statistical Data Analysis

SPSS 13.0 or SAS for Windows were used for statistical analyses and regression analysis. Spearman's correlation was used to assess relationships between environmental variable levels and PHA rates. A *t*-test was used to analyze differences between the river mouth and the central part of the wetland. The SAS software for Windows 9.0 was used for analysis of variance (ANOVA). Values are presented as the mean±standard error, and $\alpha=0.05$ for all tests.

Results

TN concentration in water from the Fuhe River mouth area averaged 25.9 ± 1.5 mg/L and TP concentration averaged 0.21 ± 0.07 mg/L. The average TP content in the wetland soil was 598 mg kg^{-1} , with the highest levels occurring near the river mouth and gradually decreased towards the central part of the Baiyangdian wetland (Table 1). In contrast, TOC and TN (Fig. 2) contents and PHA (Fig. 3)

Fig. 2 TP (Soil total phosphorus), C (soil organic carbon), TN (soil total nitrogen), levels in the Baiyangdian wetland soils during two sampling periods (mean±standard error). The upper graphs are from March 2007 samples and the lower graphs are from April 2007 samples

Fig. 3 Phosphatase activity (PHA) values (mean±standard error) at the eight sampling sites for two sampling periods (March and April 2007). Higher PHA values were found in the central part of the Baiyangdian wetland (levels indicated by the same letter are not significantly different, $P < 0.05$)

gradually increased from the river mouth area to the central part of the wetland. The highest C, TN, and PHA value was presented in central part of the wetland. PHA was positively correlated with soil TOC and C:TP ratios, but had no significant relationship with TP level (Table 2).

C:N:P ratios averaged 39:6.2:1 in the Baiyangdian wetland soils. C:TP ratios were higher in the central part of the wetland (Table 1) and gradually increase from river mouth area to the central part (Fig. 4). C:TP ratios were correlated with C levels, but had no relationship with P levels (Table 2). PHA, TOC, and TP levels differed between the sites at the river mouth and in the central part of the wetland (t -tests, $P < 0.05$), with exception of TP in April ($P = 0.553$) (Table 2). PHA was also positively correlated with MBC and C (Table 2) and MBC was correlated with TOC. MBC varied from 1.98 to 2.59% of C.

In the glucose addition experiment, C:P ratios of March soil samples ranged from 16.5 to 230.2, and ratios of April soil samples ranged from 21.8 to 321.2. Samples with higher soil C:P ratios had high PHA after one day of incubation and PHA was linear relation to C:P (Fig. 5). A positive correlation between PHA and C, but not between

Fig. 4 The ratio values among C (soil organic carbon), TN (soil total nitrogen), TP (soil total phosphorus) with mean±standard error for eight sampling sites during the two sampling periods

PHA and P, indicated that soil PHA responded mainly to soil C content (Table 2).

Discussion

The Relationship between PHA and TP

The Baiyangdian wetland had higher soil TP contents than those of other reported world wetlands (Table 3). P enrichment from external inputs results in great changes in wetland ecosystems (Corstanje and Reddy 2007). Phosphatase is an enzyme involved in P mineralization and it can be affected by P nutrient inputs (Jansson et al. 1988; Chróst and Rai 1993). Consequently, PHA has been recommended as a means of assessing the P status of aquatic systems. However, in different study areas the relationship between PHA and P concentrations was not consistent. In this study we found that PHA had no significant relationship with soil TP content.

The relationship between PHA and P may be affected by high soil TP contents in the Baiyangdian wetland. Soil TP

Table 2 Spearman correlation coefficients for the relationship between phosphatase activity (PHA) and the soil chemical variables of soil cores ($n = 48$). * $P < 0.01$. C=soil organic carbon; MBC=microbial biomass carbon; TP=soil total phosphorus; TN=soil total nitrogen

	C	MBC	TN	TP	C:TP	C:TN	TN:TP	PHA
C								
MBC	0.977*							
TN	0.694*	0.713*						
TP	-0.135	-0.117	-0.221					
C:TP	0.624*	0.595*	0.004	0.031				
C:TN	0.906*	0.891*	0.707*	-0.503*	0.520*			
TN:TP	0.592*	0.602*	0.894*	-0.592*	-0.027	0.773*		
PHA	0.716*	0.676*	0.591*	-0.124	0.361*	0.682*	0.535*

Fig. 5 Changes in PHA with increasing C:TP ratios in a laboratory incubation experiment. After adding glucose, the samples with higher C:TP ratios had higher PHA

content was most likely to be the threshold (Qian et al. 2003). Richardson et al. (2007) found that three *Utricularia* species demonstrated a consistent-pronounced decline with increasing P concentrations. They also found that the composition, diversity, and population structure were all significantly altered above a TP threshold. It appears that enzyme based resource allocation significantly responds to changes only above a certain TP threshold value that induces resource allocation shifts within the microbial community (Penton and Newman 2007). These results indicate that there is an environmental threshold TP concentration above which changes in enzyme-based resource allocation will occur and the relationship between PHA and P will change.

The Relationship between PHA and C:TP

Microbial enzyme activity has been found to be controlled not only by N, P, and C, but also by the ratio of these components (Hill et al. 2006). PHA had a positive relationship with the C:

TP ratio in the Baiyangdian wetland where the average C:N:P value was 39:6.2:1. The C:TP ratio was less than those in most other reported wetlands (Table 3). Based on the Redfield ratio of 106C:16 N:1P (Redfield 1958), the Baiyangdian wetland is C-limited. The relationship between PHA and P concentrations may be affected by this. In P-limited wetlands, P added to the system is likely to be cycled rapidly, leading to limited or slow changes in nutrient contents (Newman et al. 2001). Zhang et al. (2007) found a positive relationship between PHA and TP in a P-limited wetland but C limitation resulted in constrained P mineralization (Penton and Newman 2007). Therefore, in P-limited wetlands, PHA would be positively correlated with P content and in C-limited wetlands this relationship would be less significant. PHA is a potentially valuable early warning indicator of wetland eutrophication when used in conjunction with other complementary indicators (Newman et al. 2003). The result of our and other studies showed that the limiting factors of wetlands may be important indicators of wetland eutrophication and that PHA associated with the limiting factors probably reflects the state of wetland eutrophication.

The Effect of High P Content to C and C:TP

It has been suggested that the distribution of C is governed not only by organic C availability but also by the availability of N and P (Hill et al. 2006). We found that, especially in the river mouth area, the anthropogenic P inputs and comparatively lower C caused the spatial gradient of P and PHA, resulting in very low C:TP ratios in the Baiyangdian wetland compared with other wetlands. From the river mouth to the central part of the wetland the soil C:TP ratio gradually increased and was significantly correlated with C but had no relationship with P. This result indicates that the high P content, affected by anthropogenic

Table 3 Mean values of soil organic carbon (C), soil total nitrogen (TN), soil total phosphorus (TP) content in various wetlands

Location	n	C	TN	TP	C:N: P
This study					
Baiyangdian	48	12.6 (mg cm ⁻³)	2.34 (mg cm ⁻³)	0.84 (mg cm ⁻³)	39 : 6.2 : 1
Other wetlands					
Belize ^a	166	49.3 (mg cm ⁻³)	3.2 (mg cm ⁻³)	0.09 (mg cm ⁻³)	1415: 79 : 1
Tahoe Basin ^a	48	48.9 (mg cm ⁻³)	3.5 (mg cm ⁻³)	0.46 (mg cm ⁻³)	247: 17 : 1
Central Valley ^a	101	15.7 (mg cm ⁻³)	1.2 (mg cm ⁻³)	0.50 (mg cm ⁻³)	81: 5 : 1
New Caledonia ^a	29	31.8 (mg cm ⁻³)	2.16 (mg cm ⁻³)	0.07 (mg cm ⁻³)	1097:17:1
Fort Benning ^b	186	29.25 (g kg ⁻¹)	1.58 (g kg ⁻¹)	166.7 (mg kg ⁻¹)	455:21:1
Florida Everglades ^c	27	466.73 (g kg ⁻¹)	35.47 (g kg ⁻¹)	0.34 (g kg ⁻¹)	3581:89:1
Paludi di Ostiglia ^d	50	—	—	—	119:9:1
Pichavaram ^e	—	34.1 (mg cm ⁻³)	9.3 (mg cm ⁻³)	0.57 (mg cm ⁻³)	154:4:1

^a From Rejmankova (2005), ^b Silveira et al. (2009), ^c Penton and Newman (2007), ^d Longhi et al. (2008), ^e Krishna Prasad and Ramanathan (2008)

P inputs, caused the low C:TP ratios in the Baiyangdian wetland. It has been hypothesized that higher concentrations of C will be found in plants in nutrient-poor environments than in nutrient-rich environments (Waterman et al. 1984; Coley et al. 1985). Our results were consistent with this. It has also been suggested that nutrient loading would result in a greater energy allocation for C mineralization (Penton and Newman 2007). In the river mouth area, high soil P content would increase C mineralization causing a low soil C content.

Human activities have caused great changes in the nutrient ratios in the Baiyangdian wetland. With the large inputs of P, the C:TP ratio has decreased, leading to an enhanced C mineralization rate and a constrained P mineralization rate. This causes a further decrease in the C:TP ratio and the wetland system falls into a vicious spiral. When a wetland system is in such an unbalanced state, the system is degraded and the ecosystem's health is impaired. The results of both field and incubation experiments showed that in the eutrophic Baiyangdian wetland, C and the C:TP ratio were the limiting factors for PHA, rather than P content. Based on the results of other research about relationships between PHA, C, and P under different nutrient conditions, we inferred that PHA and its associated limiting factors probably reflect the state of wetland eutrophication, and that PHA may be used as an indicator of wetland degradation.

Acknowledgments This research was funded by the National Basic Research Program of China (No. 2006CB403306), the National Natural Sciences Foundation of China (40701086), and the National Water project (2008ZX07209-006).

References

- Amador JA, Glucksman AM, Lyons JB et al (1997) Spatial distribution of soil phosphatase activity within a riparian forest. *Soil Science* 162:808–825
- Chen H (2003) Phosphatase activity and P fractions in soils of an 18-year-old Chinese fir (*Cunninghamia lanceolata*) plantation. *Forest Ecology and Management* 178:301–310
- Chróst RJ, Rai H (1993) Ecto-enzyme activity and bacterial secondary production in nutrient-impooverished and nutrient-enriched freshwater mesocosms. *Microbial Ecology* 25:131–150
- Coley P, Bryant J, Chapin F (1985) Resource availability and plant antiherbivore defense. *Science* 230:895–899
- Corstanje R, Reddy R (2007) Soil microbial ecophysiology of a wetland recovering from phosphorus eutrophication. *Wetlands* 27:1046–1055
- Gage MA, Gorham E (1985) Alkaline phosphatase activity and cellular phosphorus as an index of the phosphorus status of phytoplankton in Minnesota lakes. *Freshwater Biology* 15:227–233
- Healey FP, Hendzel LL (1980) Physiological indicators of nutrient deficiency in lake phytoplankton. *Canadian Journal of Fisheries and Aquatic Sciences* 37:442–453
- Hill BH, Elonen CM, Jicha TM, Cotter AM, Trebitz AS, Danz NP (2006) Sediment microbial enzyme activity as an indicator of nutrient limitation in Great Lakes coastal wetlands. *Freshwater Biology* 51:1670–1683
- Hoppe HG (2003) Phosphatase activity in the sea. *Hydrobiologia* 493:187–200
- Jansson M, Olsson H, Pettersson K (1988) Phosphatases; origin, characteristics and function in lakes. *Hydrobiologia* 170:157–175
- Krishna Prasad MB, Ramanathan AL (2008) Sedimentary nutrient dynamics in a tropical estuarine mangrove ecosystem. *Estuarine, Coastal and Shelf Science* 80:60–66
- Longhi D, Bartoli M, Viaroli P (2008) Decomposition of four macrophytes in wetland sediments: organic matter and nutrient decay and associated benthic processes. *Aquatic Botany* 89:303–310
- Marx MC, Wood M, Jarvis SC (2001) A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology and Biochemistry* 33:1633–1640
- Nelson DW, Sommers LE (1982) Total carbon, organic carbon and organic matter. In: Page AL (ed) *Methods of soil analysis. chemical and microbiological properties*, 2nd edn. American Society of Agronomy, Madison
- Newman S, Reddy KR (1993) Alkaline phosphatase activity in the sediment–water column of a hypereutrophic lake. *Journal of Environmental Quality* 22:832–838
- Newman S, Kumpf H, Laing JA, Kennedy WC (2001) Decomposition responses to phosphorus enrichment in an Everglades (USA) slough. *Biogeochemistry* 54:229–250
- Newman S, McCormick PV, Backus JG (2003) Phosphatase activity as an early warning indicator of wetland eutrophication: problems and prospects. *Journal of Applied Phycology* 15:45–59
- Olander LP, Vitousek PM (2000) Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry* 49:175–191
- Penton CR, Newman S (2007) Enzyme activity responses to nutrient loading in subtropical wetlands. *Biogeochemistry* 84:83–98
- Pettersson K (1980) Alkaline Phosphatase Activity and Algal Surplus Phosphorus as Phosphorus–Deficiency Indicators in Lake Erken. *Archiv für Hydrobiologie* 89:54–87
- Qian S, King RS, Richardson CJ (2003) Two statistical methods for the detection of environmental thresholds. *Ecological Modelling* 166:87–97
- Redfield AC (1958) The biological control of chemical factors in the environment. *American Scientist* 64:205–221
- Rejmankova E (2005) Nutrient resorption in wetland macrophytes: comparison across several regions of different nutrient status. *The New Phytologist* 167:471–482
- Richardson C, King RS, Qian P, Vaithyanathan R et al (2007) Estimating ecological thresholds for phosphorus in the Everglades. *Environmental Science and Technology* 41:8084–8091
- Shackle VJ, Freeman C, Reynolds B (2000) Carbon supply and the regulation of enzyme activity in constructed wetlands. *Soil Biology and Biochemistry* 32:1935–1940
- Silveira ML, Comerford NB, Reddy KR, Prenger J, DeBusk WF (2009) Soil properties as indicators of disturbance in forest ecosystems of Georgia. USA. *Ecological Indicators* 9:740–747
- Sinsabaugh RL, Foreman CM (2001) Activity profiles of bacterioplankton in a eutrophic river. *Freshwater Biology* 46:1239–1249
- Tabatabai MA (1982) Soil enzymes. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil analysis, Part 2, Chemical and microbiological properties*, 2nd edn. American Society of Agronomy, Madison, pp 903–948
- Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry* 1:301–307

- Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass Carbon. *Soil Biology and Biochemistry* 19:703–707
- Wang WD, Wang DL, Yin CQ (2002) A field study on the hydrochemistry land/inland water ecotones with reed domination. *Acta Hydrochimica et Hydrobiologica* 30:117–127
- Waterman P, Ross J, Mckey D (1984) Factors affecting levels of some phenolic compounds, digestibility, and nitrogen content of the mature leaves of *Barteria fistulosa* (Passifloraceae). *Journal of Chemical Ecology* 10:387–401
- Williams CJ, Jochem FJ (2006) Ectoenzyme kinetics in Florida Bay: implications for bacterial carbon source and nutrient status. *Hydrobiologia* 569:113–127
- Zhang T, Wang X, Jin X (2007) Variations of alkaline phosphatase activity and P fractions in sediments of a shallow Chinese eutrophic lake (Lake Taihu). *Environmental Pollution* 150:288–294