Phosphatase activities in sediments of subtropical lakes with different trophic states

Isabela C. Torres, Benjamin L. Turner & K. Ramesh Reddy

Hydrobiologia
The International Journal of Aquatic Sciences

ISSN 0018-8158

Hydrobiologia
DOI 10.1007/s10750-016-3009-y

Springer

Hydrobiologia
DOI 10.1007/s10750-016-3009-y

PRIMARY RESEARCH PAPER

Phosphatase activities in sediments of subtropical lakes with different trophic states

Isabela C. Torres · Benjamin L. Turner · K. Ramesh Reddy

Received: 5 December 2015 / Revised: 16 August 2016 / Accepted: 3 October 2016

Abstract We characterized the vertical distribution of extracellular phosphatase enzymes; phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activities in sediments of three subtropical lakes were characterized by different trophic states. We then explored relationships between phosphatase activities, phosphorus (P) compounds, and microbial biomass and activity. Sediment P compounds had been characterized previously by two different methods: sequential fractionation and solution ^{31}P NMR spectroscopy. PMEase and PDEase activities declined with depth and were correlated strongly with microbial biomass and anaerobic respiration, indicating that bacterial phosphatase dominated in these sediments and is an important step in the anaerobic breakdown of organic matter. The oligo-mesotrophic lake had higher PMEase activity and the hypereutrophic lake had higher PDEase activity, while the eutrophic lake had the lowest activities of both enzymes. Principal component analyses showed that enzyme activities were related closely to concentrations of the P forms that they degrade: PMEase activity was correlated with phosphomonoesters, while PDEase activity was correlated with phosphodiesters (including nucleic acids and phospholipids). Enzyme activities were not related to the trophic state but with the concentration P forms found in the sediment. Overall, these results provide insight into the phosphorus cycle in subtropical lake sediments by demonstrating a link between phosphatase activity, P composition, and microbial activity.

Keywords Phosphomonoesterase · Phosphodiesterase · Microbial biomass · Microbial activity · Phosphorus forms

Handling editor: Zhengwen Liu

I. C. Torres · K. R. Reddy (✉)
Soil and Water Sciences Department, University of Florida, 2181 McCarty Hall A, Gainesville, FL 32611, USA
e-mail: krr@ufl.edu

I. C. Torres
Graduation Program in Geography, Department of Geography, Geoscience Institute, Federal University of Minas Gerais, Av. Antônio Carlos 6627, Belo Horizonte, MG CEP: 31270-901, Brazil

B. L. Turner
Smithsonian Tropical Research Institute, Apartado, Balboa, Ancon 0843-03092, Republic of Panama

Introduction

Sediment phosphorus (P) occurs in inorganic and organic forms. Organic P and cellular constituents of the biota represent 90% of the total P (TP) in freshwater ecosystems (Wetzel, 1999). Organic P is hydrolyzed by enzymes produced by microbial communities to release orthophosphate for biological

Published online: 27 October 2016

uptake (Gächter et al., 1988; Davelaar, 1993; Gächter & Meyer, 1993; Barik, et al., 2001). Consequently, the breakdown of organic P compounds through enzyme activity and the release of labile inorganic P are important components of P processing in sediments. Enzyme production can be induced by the presence of organic P and low levels of bioavailable inorganic P (Kuenzler, 1965; Aaronson & Patni, 1976). On the other hand, high levels of inorganic P inhibit the synthesis of enzymes (Elser & Kimmel, 1986; Jasson, et al., 1988; Barik et al., 2001).

Three main groups of hydrolytic enzymes responsible for organic P hydrolysis in the environment are non-specific and/or partially specific phosphoesterases (mono and diesterase), nucleotidases (mainly 5′-nucleotidase), and nucleases (exo and endonucleases) (Chrost & Siuda, 2002). Phosphomonoesterases (PMEase) are non-specific enzymes that hydrolyze simple phosphomonoesters, and are produced by many microorganisms (e.g., bacteria, algae, fungi, and protozoan) that are found in the water column and sediment of lakes. Non-specific PMEases are divided into two groups depending on the pH at which they exhibit maximum activity, alkaline (pH 7.6–10) and acid (pH 2.6–6.8) (Siuda, 1984). Both can be found inside or outside the cell, and the same cell can produce both alkaline and acid PMEase (Siuda, 1984).

Although both PMEase activities have been reported to be regulated by the availability of orthophosphate, acid PMEase is usually regarded as a constitutive enzyme (Siuda, 1984; Jasson et al., 1988). The production of constitutive enzymes is neither repressed nor stimulated by high or low orthophosphate availability in the environment. Instead, synthesis is related to P concentration and demand inside the cell (Siuda, 1984; Jasson et al., 1988). Jasson et al. (1981), however, suggested that in acidified lakes, acid PMEase may have a similar role to that of alkaline PMEase in neutral systems, as its production is also inhibited by orthophosphate. In aquatic systems, alkaline PMEase is by far the most studied enzyme, probably due to the high number of systems with neutral pH, that are inappropriate for the preservation of extracellular acid PMEase (Siuda, 1984). In contrast, PDEase hydrolyzes phosphodiesters and is known to degrade phospholipids and nucleic acids (Hino, 1989; Tabatai, 1994; Pant & Warman, 2000). It is the least studied enzyme in freshwater ecosystems. Few studies have reported on the occurrence and distribution of phosphatases or other organic P-hydrolyzing enzymes in sediments or their association with sediment bacteria (Wetzel, 1991; Chrost & Siuda, 2002). As sediment P is important in P cycling in lakes, and since it is well known that microorganisms can influence the organic P dynamics in sediments, the study of different P compounds and associated enzymes is important for understanding P cycling in sediments.

In this study, we investigated the stratigraphic distribution of two phosphatase activities, PMEase and PDEase, in sediments of subtrophical lakes characterized by different trophic states. We hypothesized that phosphatase activities would be higher in recently accreted sediments (surface) as compared to older sediments (sub-surface) and would be related to P forms and availability, as well as to microbial biomass and activity. The specific objectives of this study were (i) to measure vertical distribution of PMEase and PDEase activities and relate them to microbial biomass and activity in sediments, and (ii) to explore relationships between different phosphorus compounds and enzyme activity.

Materials and methods

Study sites

Three subtropical Florida lakes (USA) were selected for this study based on water quality variables and trophic status (Table 1; Fig. 1). Lake Annie (Fig. 1a), a small (0.37 km^2) oligo-mesotrophic lake, is located in South Central Florida (Highlands County) at the northern end of the Archbold Biological Station. Lake Annie is characterized by pristine water quality with little surface water input (mostly ground water), and low anthropogenic impact due to the absence of development around the lake (Layne, 1979). This lake has no natural surface streams, but two shallow man-made ditches allow surface water to flow into the lake and contribute to water and nutrient inputs during high-rainfall periods (Battoe, 1985). Benthic sediments vary from organic to sand in the littoral zone (Layne, 1979). Lake Okeechobee (Fig. 1b) is a large (1800 km^2) shallow lake located in south Florida. It is considered to be a eutrophic lake that has experienced cultural eutrophication over the last 50 years (Engstrom et al., 2006). Benthic sediments are

Table 1 Characteristics of sampled sites in the three different lakes with sampling date, location, sediment type, and water quality parameters (measured at 1 m)

Parameters	Lake		
	Annie[a] Central	Okeechobee[b] M9	Apopka[b] West
Sampling Date	June/2005	July/2005	May/2005
Sediment Type	Mud/Clay	Mud	Organic
Water Column Depth (m)	20	4.0	2.0
Secchi (m)	2.0	0.08	0.3
Latitude	27°12′27″	26°58′17.6″	28°38′01″
Longitude	81°21′44″	80°45′38.4″	81°39′36″
Temperature (°C)	30.2	29.5	26.6
Electrical Conductivity (μS cm^{-1})	41.9	385	443
pH	5.1	7.8	7.6
Dissolved Oxygen (mg l^1)	6.4	6.5	8.7
Dissolved Organic Carbon (μg l^1)*	13.8	14.5	31.1
Total Phosphorus (μg l^1)*	33.2	255.9	69.7
Soluble Reactive Phosphorus (μg l^1)*	7.4	90.4	11.1
Total Nitrogen (μg l^1)*	1807	3439	11149
Ammonium—NH_4-N (μg l^1)*	181.6	103.0	119.6

* Mean concentration in the water column

[a] Average depth: 0.5-1-2-5-10-20 (m)

[b] Average depth: 0.5-1-2 (m)

characterized as mud (representing 44% of the total lake surface area); sand and rock (28%); littoral (19%), dominated by macrophyte growth; and peat (9%) that refers to partially decomposed plant tissues (Fisher et al., 2001). Lake Apopka (Fig. 1c) is also a shallow lake with 125 km^2 surface area, located in central Florida. Once a clear-water macrophyte-dominated lake, Lake Apopka has changed to a turbid, algal-dominated lake since 1947 (Clugston, 1963). This shift may have been caused by nutrient input from several sources, including agricultural drainage from adjacent vegetable farms (Schelske et al., 2000). Even though these inputs were controlled and regulated to some degree, the eutrophication process continued and Lake Apopka is considered hypereutrophic. Benthic sediments are characterized by unconsolidated material, which mainly consists of algal deposits (Reddy & Graetz, 1991).

Field sampling

Sediment–water interface cores of variable lengths were collected using a piston corer (Fisher et al., 1992) or by SCUBA divers. One central site (80-cm core) was sampled in Lake Annie on June 25, 2005 (Fig. 1a; Table 1). Cores were collected at one site in the mud zone (M9 site, 70-cm core) of Lake Okeechobee on July 16, 2005 (Fig. 1b; Table 1). Sediments of the mud zone in Lake Okeechobee are characterized by having the highest concentration of P among all different sediment types of this lake (Fisher et al., 2001). A western site (98-cm core) was sampled in Lake Apopka on May 28, 2005 (Fig. 1c; Table 1). Cores were sectioned in the field at the following intervals: 0–5, 5–10, 10–15, 15–20, 20–30, 30–45, 45–60, 60–80, and 80–100 cm. Samples were placed in plastic bags, sealed, and kept on ice. Nine cores were collected from each site. Three cores were used to make a composite core to obtain sufficient material for all measurements. The nine cores yielded three replicates of composite sediments from each site. All measured sediment variables are reported on a dry weight basis (dw).

Water characteristics

Water parameters were measured to characterize the lakes in relation to their trophic status. Water transparency was determined using a Secchi disk. Water temperature (°C), electrical conductivity (EC), pH, and dissolved oxygen (DO) were measured with a YSI 556 Multi-Probe Sensor (YSI Environmental, Yellow Springs OH) at different depths (Table 1). Water samples were collected from various depths at each site using a Van Dorn bottle. Water column nutrient concentrations were measured using U.S. EPA

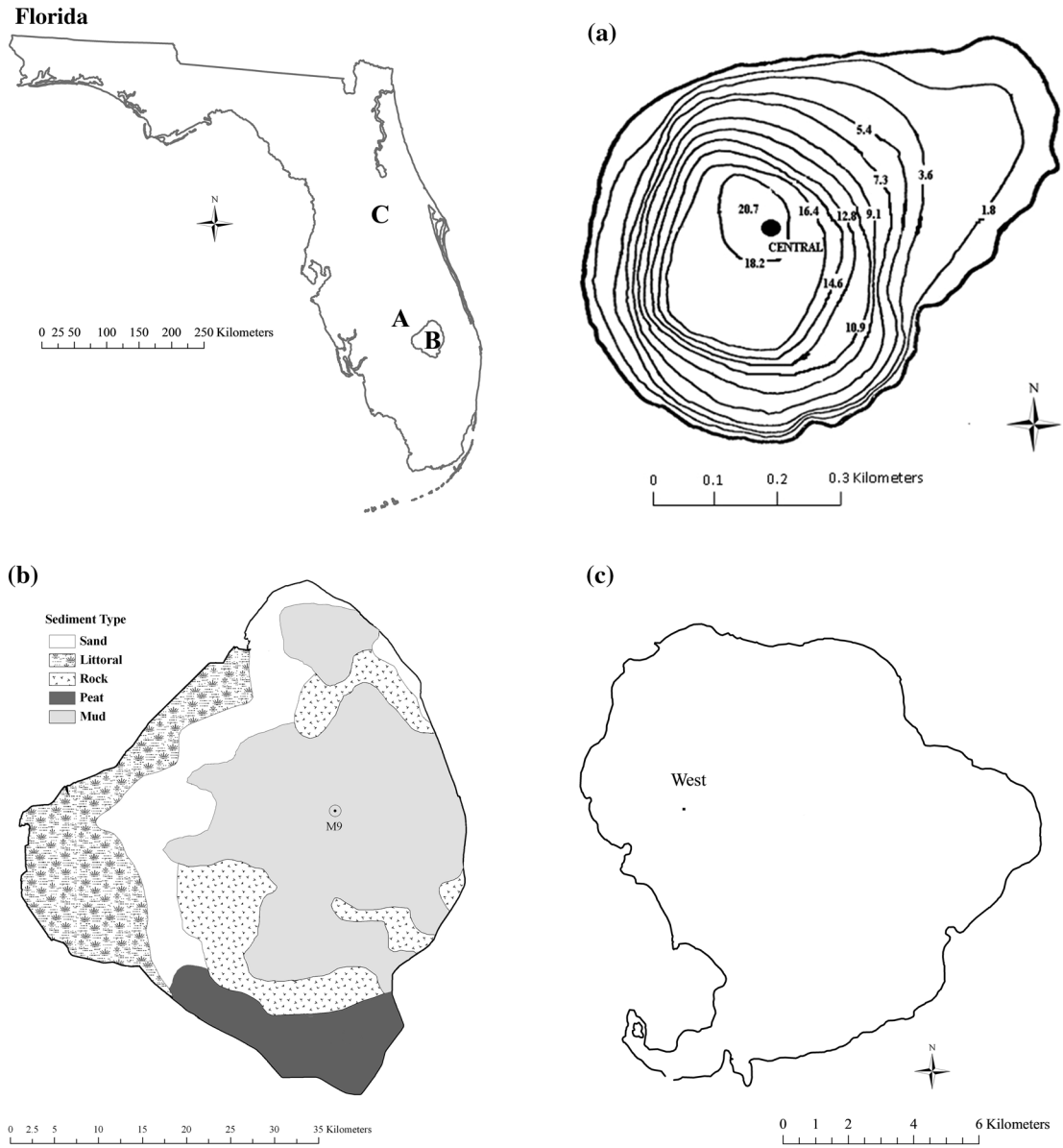

Fig. 1 Map of the three subtropical lakes with sampled sites and their location in Florida State: **a** Lake Annie (with water column depth in meters, modified from Layne, 1979), **b** Lake Okeechobee with different sediment types, and **c** Lake Apopka

methods (EPA, 1993). Total Kjeldahl nitrogen (TN) was measured by digestion with concentrated sulfuric acid (H_2SO_4) and Kjeldahl salt catalyst, and determined colorimetrically (Method—351.2). Total P was digested with 11 N H_2SO_4 and potassium persulfate (Method—365.1). Water samples were filtered through a 0.45-µm membrane filter, and the filtrate was analyzed for dissolved reactive phosphorus (DRP) (Method—365.1), ammonium-N (NH_4–N) (Method—351.2), and dissolved organic carbon (DOC) (automated Shimadzu TOC 5050 analyzer, Method—415.1).

Sediment properties

Samples were transported on ice and stored in the dark at 4°C. Before each analysis, samples were homogenized and sub-samples taken. Sediment bulk density

(BD) was determined on a dry weight basis (i.e., g of dry/cc wet) at 70°C for 72 h, and pH was determined on wet sediments (1:2 sediment-to-water ratio). Organic matter content (LOI-loss on ignition) was determined by weight loss at 550°C.

Lake Annie and Lake Apopka sediments were characterized by having high water content (>95%) and sediment pore water was extracted (centrifuged at 10,000×*g* for 10 min) and DRP and DOC were measured as described for water samples. Both DRP and DOC concentration present in these sediments were used to compare with enzyme activities.

Sediment P compounds were characterized by two different methods: sequential fractionation scheme and solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy; data and methods were reported previously (Torres et al., 2014). Sequential fractionation identified the following operationally defined P forms: (1) labile inorganic and organic P; (2) microbial biomass P (MBP); (3) inorganic P bound to Ca, Mg, Fe, and Al; (4) organic P associated with fulvic (FAP) and humic (HAP) fractions (moderately and highly resistant organic P, respectively). Phosphorus-31 NMR spectroscopy identified different P compounds based on their functional groups, including orthophosphate, pyrophosphate (pyro-P), polyphosphate (poly-P), phosphomonoesters, phosphodiesters (e.g., DNA and lipids), and phosphonates. Data from ^{31}P NMR represent combined samples from triplicate cores from each site.

Sediment microbial biomass carbon

Microbial biomass carbon (MBC) in sediments was used as a proxy of microbial biomass and measured with the chloroform fumigation–extraction method (Hedley & Stewart, 1982; Vance et al., 1987). Briefly, sediment samples were split in two: one sample was treated with alcohol-free chloroform (0.5 mL) to lyse microbial cells, placed in a vacuum desiccator, and incubated for 24 h. The duplicate sample was left untreated. Both sets were extracted with 0.5 M K_2SO_4 centrifuged at 10,000×*g* for 10 min and filtered through Whatman # 42 filter paper. Carbon extracts were acidified (pH < 2) and analyzed in an automated Shimadzu TOC 5050 analyzer (Method—415.1, EPA, 1993). Microbial biomass C was calculated by the difference between treated and non-treated samples.

Microbial activity

Anaerobic microbial respiration (CO_2) was used as a measure of sediment microbial activity, and quantified by incubating a known amount of wet sediment equivalent to 0.5 g of dry weight, in 5 mL of DI water at 30°C. Samples were placed in a glass vial and closed with rubber stoppers and aluminum crimp seals and purged with N_2 gas to achieve anaerobic conditions. Gas samples were obtained after 2, 4, 7, and 10 days, and CO_2 released was measured by gas chromatography using a Shimadzu 8A GC-TCD equipped with Poropak N column (Supelco Inc., Bellefonte, PA), using He as a carrier gas. Prior to CO_2 measurements, headspace pressure was determined with a digital pressure indicator (DPI 705, Druck, New Fairfield, CT). Concentrations of CO_2 were determined by comparison with standard concentrations, and production rates were calculated by linear regression ($r^2 > 0.95$).

Enzyme activity

Enzyme activities, including PMEase and PDEase, were determined colorimetrically using as substrate *p*-nitrophenyl phosphate and bis-*p*-nitrophenyl phosphate, respectively (Tabatai, 1994; Alef et al., 1995), both from Sigma Chemical Co (St Louis, MO). Assays were conducted using three replicates and a control for each sample to account for non-enzymatic color development. As PMEase activity depends on pH (Tabatai, 1994; Alef et al., 1995), alkaline phosphatase activity was measured in Lake Okeechobee and Lake Apopka, while acid phosphatase activity was measured in Lake Annie (Table 1).

A known amount of wet sample, 0.5 g for high organic sediment, and 1 g for mineral sediment, was added to polypropylene centrifuge bottles with the artificial substrate (1 ml of 0.05 M *p*-nitrophenyl phosphate for PMEase, and bis-*p*-nitrophenyl phosphate for PDEase), toluene (to inhibit microbial growth during measurement), and a pH buffer (pH = 11 for alkaline, pH = 6.5 for acid phosphatase, and pH = 8 for PDEase), and incubated at 37°C for 1 h. Enzymatic activity was stopped after incubation by the addition of 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH (for PMEase), and 0.1/0.5 M THAM/NaOH (THAM: tris-hydroxymethyl-aminomethane) extractant solution (for PDEase). Samples were

centrifuged and filtered through a Whatman #1 paper filter and analyzed at 420 ηm using a UV–VIS spectrophotometer (Shimadzu Model UV—160) (Tabatai, 1994; Alef et al., 1995). Absorbance was compared with standards. Control values were subtracted from sample values to account for non-enzymatic substrate hydrolysis.

Statistical analysis

Standard deviation values were calculated from triplicate cores. Sediment P compound methods and data reported in another study (Torres et al., 2014) and combined data were used to explore relationships between these different variables and enzyme activity. A Pearson correlation analysis was performed to determine relations among P forms and enzyme activity, as well as among enzyme activity and microbial biomass. Regression analyses were conducted to compare sediment P forms and activities of enzymes and microbes. A principal component analysis (PCA) was performed to address relations among variables, and how they relate to each lake and sediment depth. All statistical analyses and figures were done with Statistica 7.1 (StatSoft 2006, Tulsa, OK, USA) software.

Results

Sediment properties

Acidic pH conditions were observed in Lake Annie sediments and neutral to alkaline values in Lake Okeechobee and Lake Apopka deposits (Table 2). Sediment bulk density values were lowest in Lake Apopka, followed by Lake Annie reflecting their high fluid content relative to Lake Okeechobee sediments. Bulk density increased with depth in lakes Apopka, Annie, and Okeechobee. Organic matter content was highest at Lake Apopka, followed by Lake Annie and Lake Okeechobee sediments (Table 2).

Sediment microbial biomass carbon and activity

Lake Apopka had the highest concentration of MBC and anaerobic respiration, followed by lakes Annie and Okeechobee mud sediments. There was a general decrease in microbial biomass and anaerobic respiration with depth in all sediments (Table 3).

Enzyme activity

Lake Okeechobee sediments had very low enzyme activities for both PMEase (1.1–5.5 mg *p*-nitrophenol g^{-1} dw h^{-1}) and PDEase (1.0–5.5 mg bis-*p*-nitrophenol g^{-1} dw h^{-1}) (Table 3). Phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activities decreased with sediment depth at all sites (Table 3). Lake Annie sediments had the highest PMEase activity compared with sediments of the other lakes, while PDEase was higher in Lake Apopka sediments. Lake Okeechobee sediments had similar values for both enzymes activity, while Lake Annie and Lake Apopka had higher PMEase activity. Phosphomonoesterase activity was strongly correlated ($r > 0.7$) with phosphomonoesters, labile organic P, FAP, and HAP, and increased linearly with phosphomonoesters (Fig. 2). Phosphodiesterase activity was correlated strongly ($r > 0.7$) with MBP, lipid-P, DNA-P, and pyrophosphate, and increased linearly with phosphodiester (i.e., Lipid-P, DNA-P) concentration (Fig. 3).

Microbial biomass (MBC) was correlated strongly and positively with enzyme activity. Person correlation coefficients were as follows: for Lake Annie sediments PMEase $r = 0.78$ and PDEase $r = 0.83$; for Lake Okeechobee mud sediments PMEase $r = 0.91$ and PDEase $r = 0.94$; and for Lake Apopka sediments PMEase $r = 0.87$ and PDEase $r = 0.95$. Anaerobic respiration (CO_2 production rates) was correlated significantly with both PMEase and PDEase activities in all lake sediments (Fig. 4a–c).

Pore water DOC and DRP concentrations in Lake Annie and Apopka data were used to verify if there was a relation with enzyme production. In Lake Annie, there was no relationship between either acid PMEase or PDEase activities and pore water DOC and DRP. For Lake Apopka, however, there was an inverse relationship between DRP pore water concentration and both PMEase and PDEase (Fig. 5a). Enzyme activities in Lake Apopka showed a strong linear relationship with pore water DOC (Fig. 5b).

Principal Component Analyses (PCA) were conducted. One analysis used data from ^{31}P NMR (Torres et al., 2014), and microbial and enzyme activities ($n = 25$) (PCA-1, Fig. 6). The other used data from chemical P fractionation (Torres et al., 2014), and

Table 2 pH, bulk density (BD), organic matter content (LOI—loss on ignition), and pore water dissolved organic carbon (DOC) and dissolved reactive phosphorus (DRP) in sediment profiles of the three lakes

Lake	Site	Depth (cm)	pH	BD (g dry cm^{-3} of wet)	LOI (%)	Pore water (mg kg^{-1})	
						DOC	DRP
Annie	Central	5	5.4 ± 0.15	0.04 ± 0.006	58 ± 0.4	268 ± 163	0.4 ± 0.1
		10	5.2 ± 0.05	0.04 ± 0.003	57 ± 1.2	96 ± 11	0.1 ± 0.03
		15	5.3 ± 0.09	0.06 ± 0.003	55 ± 1.0	73 ± 24	0.1 ± 0.05
		20	5.3 ± 0.11	0.07 ± 0.003	54 ± 1.6	73 ± 4	0.1 ± 0.03
		30	5.4 ± 0.03	0.07 ± 0.003	52 ± 0.8	209 ± 168	0.2 ± 0.1
		45	5.5 ± 0.09	0.08 ± 0.002	52 ± 0.1	161 ± 44	0.3 ± 0.1
		60	5.5 ± 0.06	0.10 ± 0.006	50 ± 0.9	242 ± 135	0.7 ± 0.1
		80	5.7 ± 0.06	0.11 ± 0.007	50 ± 1.0	232 ± 163	0.8 ± 0.1
Okeechobee	M9	5	7.7 ± 0.16	0.11 ± 0.005	36 ± 1.7	ND	ND
		10	7.7 ± 0.03	0.16 ± 0.003	37 ± 1.2	ND	ND
		15	7.8 ± 0.04	0.20 ± 0.020	21 ± 1.3	ND	ND
		20	7.8 ± 0.04	0.23 ± 0.020	26 ± 6.9	ND	ND
		30	7.9 ± 0.03	0.26 ± 0.056	16 ± 3.8	ND	ND
		45	7.9 ± 0.05	0.33 ± 0.040	25 ± 6.5	ND	ND
		60	8.0 ± 0.01	0.30 ± 0.009	29 ± 4.7	ND	ND
		70	8.0 ± 0.08	0.33 ± 0.043	35 ± 3.5	ND	ND
Apopka	West	5	7.5 ± 0.07	0.01 ± 0.001	69 ± 0.7	1586 ± 189	0.7 ± 0.1
		10	7.3 ± 0.06	0.02 ± 0.001	67 ± 3.2	1160 ± 201	0.5 ± 0.2
		15	7.2 ± 0.02	0.02 ± 0.004	67 ± 1.1	892 ± 96	0.4 ± 0.1
		20	7.2 ± 0.06	0.03 ± 0.006	65 ± 2.7	842 ± 124	0.3 ± 0.1
		30	7.3 ± 0.04	0.03 ± 0.009	64 ± 1.0	658 ± 149	4 ± 2
		45	7.2 ± 0.05	0.04 ± 0.014	66 ± 1.9	557 ± 88	8 ± 3
		60	7.1 ± 0.10	0.06 ± 0.010	68 ± 1.3	478 ± 45	11 ± 5
		80	7.0 ± 0.06	0.07 ± 0.005	69 ± 0.8	479 ± 35	13 ± 1
		98	7.0 ± **	0.07 ± **	71 ± **	432 ± **	5 ± **

Mean ± standard deviation (SD)

** No replicates for SD calculation

ND not determined

microbial and enzyme activities ($n = 107$) (PCA-2, Fig. 7). The PCA-1 had 47.1% of the data variability explained by Axis 1. Axis 2 explained 31.8% of the data variability (Fig. 6a). Lipid-P, DNA-P, anaerobic respiration, MBP, MBC, and PDEase activity were the variables selected by Axis 1, while orthophosphate, phosphomonoesters, labile organic P, fulvic acid P (FAP), humic acid P (HAP), and PMEase activity were selected by Axis 2. Principal component analysis separated each enzyme activity with P forms that they degrade into different clusters. The position of the sites and sediment depth in relation to the variable loadings in the PCA showed that the three lakes are separated into different groups (Fig. 6b). Lake Apopka placed in the PDEase cluster and Lake Annie in the PMEase cluster. Lake Okeechobee was further from these clusters (Fig. 6b).

In the second PCA (PCA-2), using sequential P fractionation, Axis 1 explained 53.3% of the data variability and the variables selected were organic matter content (LOI), anaerobic respiration, MBP, MBC, and PMEase activity. Axis 2, with 27.7% of the data variability explained, selected FAP and HAP (Fig. 7a). The PCA-2 also separated enzyme and the P forms that they degrade into different clusters. The position of the sites and sediment depth in relation to

Table 3 Microbial biomass carbon (MBC), anaerobic respiration, phosphomonoesterase activity (PMEase), and phosphodiesterase activity (PDEase) in sediment profiles of the three lakes

Lake	Site	Depth (cm)	MBC (mg kg^{-1})	Anaerobic Respiration ($mgCO_2$-C kg^{-1} d^{-1})	PMEase (mg *p*-nitrophenol g^{-1} h^{-1})	PDEase (mg bis-*p*-nitrophenol g^{-1} h^{-1})
Annie	Central	5	5419 ± 195	179.1 ± 20.1	131.1 ± 16.7	14.7 ± 2.6
		10	5089 ± 166	142.3 ± 25.4	94.3 ± 8.9	10.6 ± 3.6
		15	4387 ± 218	87.2 ± 31.0	65.5 ± 17.5	7.5 ± 3.1
		20	4205 ± 177	76.5 ± 31.9	48.4 ± 10.2	5.5 ± 1.2
		30	3919 ± 405	55.5 ± 11.5	47.5 ± 6.9	5.3 ± 0.8
		45	3652 ± 692	41.8 ± 6.8	42.9 ± 14.9	4.3 ± 0.7
		60	3401 ± 624	36.2 ± 4.1	27.6 ± 5.6	2.2 ± 0.3
		80	2740 ± 422	28.6 ± 8.0	26.4 ± 5.9	1.6 ± 0.6
Okeechobee	M9	5	3821 ± 479	20. ± 0.5	5.5 ± 0.4	5.5 ± 0.1
		10	3672 ± 187	16.2 ± 1.3	4.4 ± 0.5	4.5 ± 0.3
		15	3465 ± 231	15.6 ± 0.9	3.6 ± 1.0	4.0 ± 0.7
		20	2773 ± 205	9.8 ± 0.2	3.1 ± 0.5	2.1 ± 0.8
		30	2616 ± 283	8.2 ± 0.7	2.2 ± 0.3	1.3 ± 0.5
		45	2361 ± 164	7.9 ± 1.4	2.0 ± 0.5	1.3 ± 0.3
		60	2177 ± 92	7.2 ± 0.6	1.7 ± 0.3	1.2 ± 0.2
		70	1983 ± 437	9.7 ± 1.6	1.1 ± 0.5	1.0 ± 0.3
Apopka	West	5	36617 ± 3193	284.0 ± 71.2	70.4 ± 10.1	28.0 ± 1.9
		10	32926 ± 5437	238.5 ± 74.4	44.3 ± 10.0	24.0 ± 3.7
		15	30486 ± 3924	196.2 ± 59.9	26.7 ± 11.2	17.3 ± 3.2
		20	22265 ± 5640	178.8 ± 68.1	26.7 ± 17.0	17.4 ± 4.9
		30	19355 ± 4608	170.6 ± 65.6	17.5 ± 15.7	10.4 ± 4.2
		45	14725 ± 4586	152.9 ± 59.3	15.4 ± 14.3	6.3 ± 3.8
		60	11037 ± 4291	117.1 ± 20.4	9.7 ± 6.4	3.2 ± 0.9
		80	9584 ± 1273	100.7 ± 7.9	9.1 ± 5.8	1.7 ± 0.3
		98	8011 ± **	83.4 ± **	10.9 ± **	1.7 ± **

Mean ± standard deviation (SD)

** No replicates for SD calculation

the variables loadings in PCA-2 showed results similar to the first PCA-1 (Fig. 7b). Samples from the three lakes were separated into different groups, and Lake Apopka placed in the PDEase cluster and Lake Annie in the PMEase cluster.

Discussion

There are few studies reporting on P-related enzyme activity in freshwater sediments, and they focus on PMEase activity. The PMEase activities reported here for Lake Annie and Lake Apopka are much greater compared to those observed in other freshwater systems. Lake Okeechobee sediment PMEase activities, although much smaller compared to the other lakes of this study, were similar or greater compared to those reported for other freshwater sediments. For example, in shallow eutrophic Lake Donghu (China), PMEase activity in surface sediments was much lower compared to the values detected in surface sediments of Lake Annie and Lake Apopka, but higher compared to the values detected in Lake Okeechobee sediments (17.6–44.05 mg *p*-nitrophenol g^{-1} dw h^{-1}) (Yiyong et al., 2001). Wobus et al. (2003), in a study of sediments of reservoirs of different trophic states in Germany, reported higher values for PMEase in oligotrophic Muldenberg, (17.2 mg *p*-NP g^{-1} dw h^{-1}) compared to mesotrophic Saidenbach (0.8 mg *p*-NP g^{-1} dw h^{-1}) and eutrophic

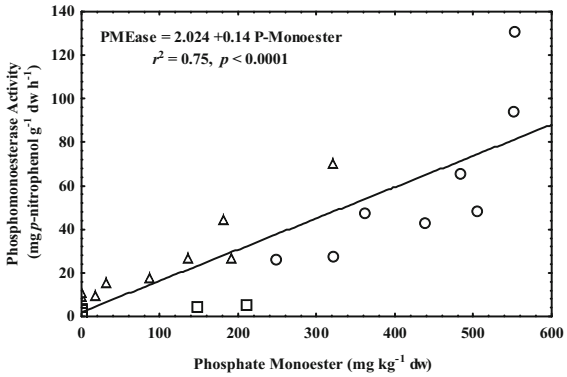

Fig. 2 Relationship between phosphomonoester concentration and phosphomonoesterase activity in sediments from Lake Annie (*circles*), Lake Okeechobee (*squares*), and Lake Apopka (*triangles*)

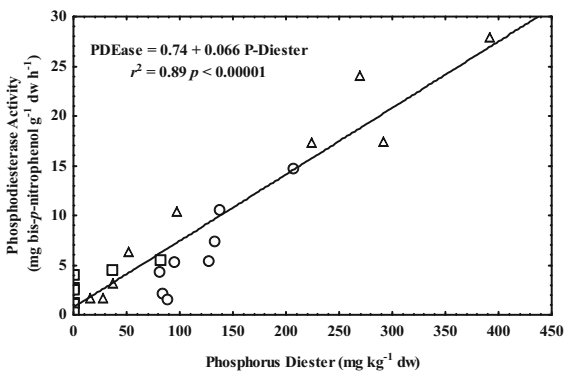

Fig. 3 Relationship between phosphodiester concentration and phosphodiesterase activity in sediments from Lake Annie (*circles*), Lake Okeechobee (*squares*), and Lake Apopka (*triangles*)

Quitzdorf (0.17 mg *p*-NP g^{-1} dw h^{-1}). In a study of shallow, nutrient-rich, freshwater sediments, Boon and Sorrell (1991) reported values of PMEase that ranged from 1.35 to 1.75 mg *p*-NP g^{-1} dw h^{-1}. Small values of PMEase were reported by Barik et al. (2001) for 12 different nutrient-rich fishpond sediments (25–59 µg *p*-NP g^{-1} dw h^{-1}). Wright & Reddy (2001) reported PMEase values in soils of a freshwater wetland (Florida Everglades) ranging from ≤5.0 mg *p*-NP g^{-1} dw h^{-1} in P-impacted sites to 25 mg *p*-NP g^{-1} dw h^{-1} in non-impacted sites.

Phosphatase activity decreased with depth in all lakes as reported elsewhere (Sinke et al., 1991; Wobus et al., 2003). The decrease of phosphatase activity with depth reflects lower microbial biomass (MBC), and is accompanied by decreased anaerobic respiration. A

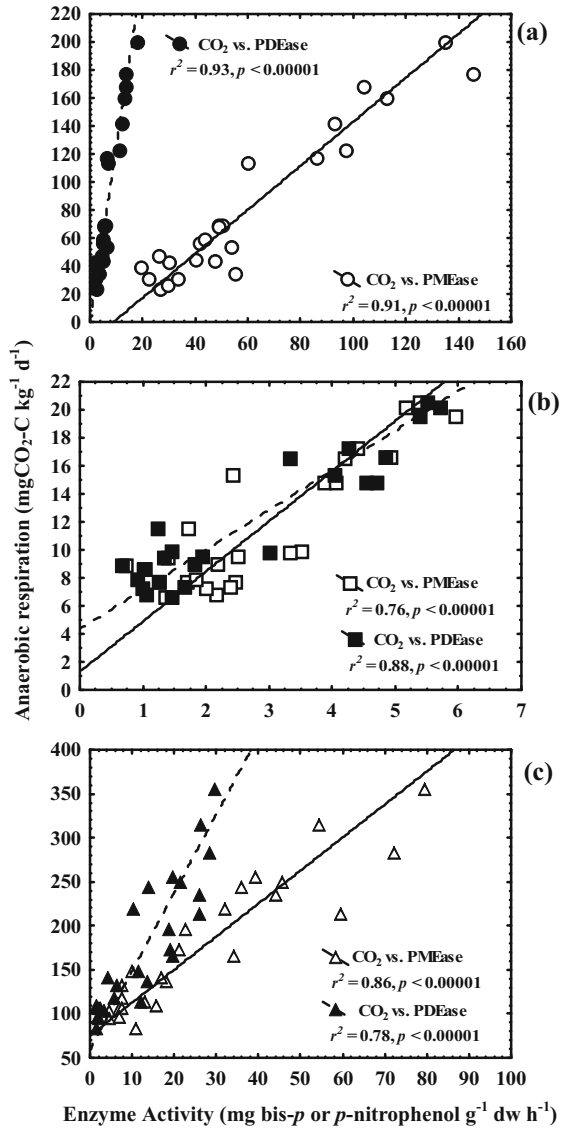

Fig. 4 Relationship between anaerobic respiration and phosphomonoesterase (PMEase) activity (closed symbols); and anaerobic respiration and phosphodiesterase (PDEase) activity (open symbols) in sediments from: **a** Lake Annie (*circles*), **b** Lake Okeechobee (*squares*), and **c** Lake Apopka (*triangles*)

positive correlation between PMEase activity and microbial biomass was found in several ecosystems (Kobori & Taga, 1979; Davis & Goulder, 1993; Massik & Cotello, 1995; Barik et al., 2001). The same results were found in our study with high Pearson's correlations coefficients between MBC and both enzyme activities. Enzymes can be produced by several organisms. Enzymes produced by algae predominate in the water column, while in sediments

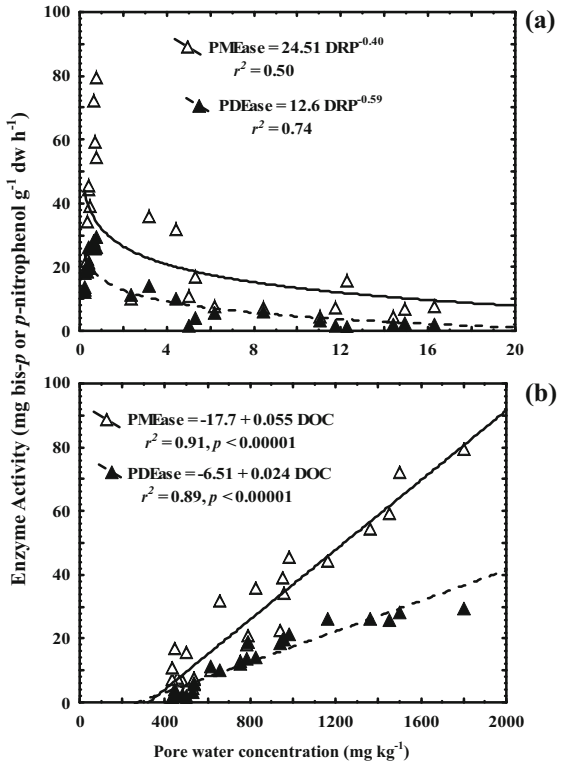

Fig. 5 Relationship between enzyme activity, phosphomonoesterase (PMEase) and phosphodiesterase (PDEase), and **a** pore water dissolved reactive phosphorus (DRP) and **b** dissolved organic carbon (DOC) concentrations in sediments from Lake Apopka

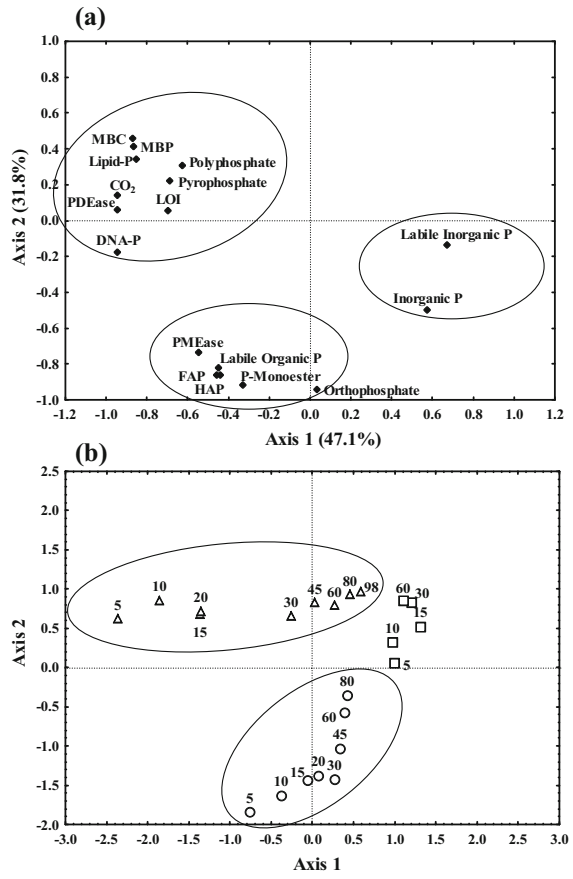

Fig. 6 Results of the principal component analysis 1 (PCA-1): **a** loadings of different phosphorus compounds measured by ^{31}P NMR and P fractionation, enzymes, and microbial activities ($n = 25$); and **b** the plot of the scores of the sites and sediment depth (numbers cm) from Lake Annie (*circles*), Lake Okeechobee (*squares*), and Lake Apopka (*triangles*). MBC: microbial biomass carbon; *LOI* organic matter content; *CO_2* anaerobic respiration; *PMEase* phosphomonoesterase activity; *PDEase* phosphodiesterase activity; P forms measured by chemical fractionation (*FAP* fulvic acid bond-P; *HAP* humic acid bond P; labile inorganic P; labile organic P; inorganic P; MBP: microbial biomass phosphorus); P forms measured by ^{31}P NMR (lipid-P, DNA-P, polyphosphate, pyrophosphate, P-monoester: phosphomonoester; and orthophosphate)

bacterial enzymes are usually dominant (Siuda, 1984). Strong correlations (PMEase $r = 0.65$, PDEase $r = 0.91$, all data combined) as well as significant linear relationships (Fig. 4) between enzyme activities and anaerobic respiration indicate that bacterial enzymes dominate in these sediments.

The strong relationship between anaerobic respiration and enzyme activities in sediments is a significant finding of this study (Figs. 4a–c). There is a lack of studies comparing anaerobic respiration and PMEase and PDEase activities in lake sediments in the literature. However, it has been reported the production of PMEase in anaerobic systems as in anaerobic bioreactors (Anupama et al., 2008). Moreover, it is well known that during the degradation of organic substrates in anaerobic systems, the first step is the breakdown of polymers to monomers by the production of extracellular enzymes (Megonigal et al., 2004). As the microbial community produces enzymes for hydrolyzing organic P to inorganic P, these use remaining organic compounds to produce energy for the cell. Consequently, it is not surprising to find such a close relationship.

In addition to reflecting microbial biomass and activity, phosphatase activities were also influenced by P availability and composition in these lakes. Wobus et al. (2003) reported higher activities of PMEase in an oligotrophic reservoir compared to meso and eutrophic reservoirs. In our study, the highest PMEase activity

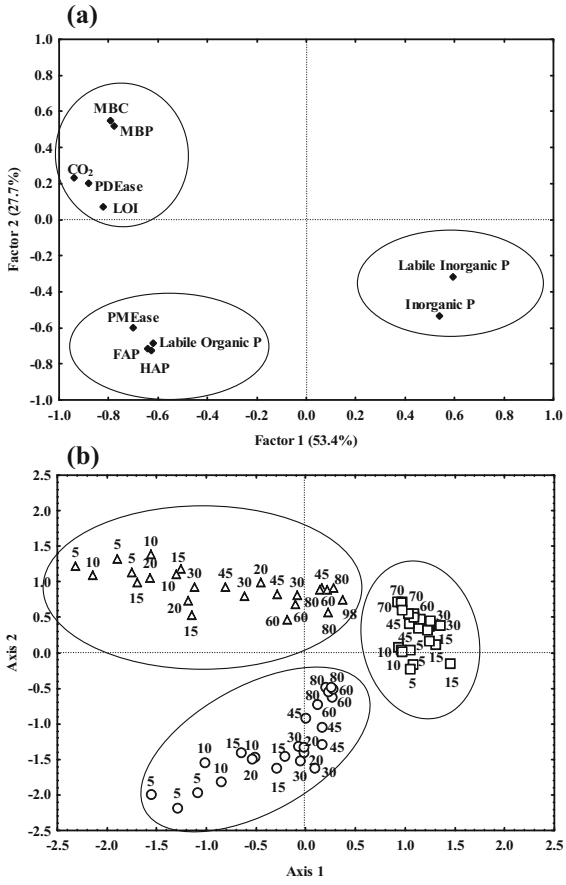

Fig. 7 Results of the principal component analysis 2 (PCA-2): different phosphorus compounds measured by P fractionation, enzymes, and microbial activities ($n = 107$); **a** loadings of variables; and **b** the plot of the scores of the sites and sediment depth (numbers cm) from Lake Annie (*circles*), Lake Okeechobee (*squares*), and Lake Apopka (*triangles*). *MBC* microbial biomass carbon, *LOI* organic matter content, CO_2 anaerobic respiration, *PMEase* phosphomonoesterase activity, *PDEase* phosphodiesterase activity; P forms measured by chemical fractionation (*FAP* fulvic acid bond-P; *HAP* humic acid bond P; labile inorganic P; labile organic P; inorganic P; MBP: microbial biomass phosphorus)

was found in the oligo-mesotrophic lake (Lake Annie). Phosphomonoesterase activity was lowest in eutrophic Lake Okeechobee, but displayed intermediate activity in hypereutrophic Lake Apopka. Lake Okeechobee had high concentrations of labile inorganic P (Torres et al., 2014), and lowest activities for both PMEase and PDEase. Lake Annie had high concentrations of labile organic P, FAP and HAP fractions, and phosphomonoester (Torres et al., 2014), and had high PMEase activity. Lake Apopka had high concentrations of MBP and phosphodiesters (lipids and DNA) (Torres et al., 2014), as well as PDEase activity. Statistical analyses support these results. There were higher correlation coefficients between PMEase and phosphomonoester ($r = 0.85$), labile organic P ($r = 0.88$), FAP ($r = 0.84$), and HAP ($r = 0.86$), while PDEase had high correlations with phosphodiesters (lipid-P $r = 0.87$, DNA-P $r = 0.93$) and MBP (r = 0.89). Linear regression analyses showed strong significant relations between PMEase and phosphomonoester, and between PDEase and phosphodiester concentrations (Figs. 2, 3). These results were corroborated by the two PCAs positioning these P forms and their respective related enzymes as clusters (Figs. 6, 7). Also, in relation to the PCAs, if enzyme production were only a reflection of microbial biomass and activity, both enzymes, CO_2 production and MBC would all cluster together, but there is clear separation of these variables. These results show that although microbial activity (CO_2), microbial biomass (MBC,) and enzyme activities are related, as expected, different P forms in sediments strongly influence enzyme production.

The lack of relationship between pore water DRP and enzyme activities in Lake Annie can be attributed to different mechanisms of enzyme production and sediment properties. Acid PMEase is usually regarded as a constitutive enzyme, and its production is not repressed by high orthophosphate availability (Siuda, 1984; Jasson et al., 1988). In acidified lakes, however, acid PMEase seem to have a similar role to alkaline PMEase in neutral systems (Jasson et al., 1981). We measured acid instead of alkaline PMEase in Lake Annie sediments to evaluate the maximum potential enzyme activity. Measurement of alkaline PMEase activity would probably be underestimated in Lake Annie, as it would be influenced by pH rather than P availability. In a study of acid PMEase in the water column of acid Lake Gårdsjön, Sweden, with high aluminum (Al) and iron (Fe) concentrations, Jasson (1981) showed that high acid PEMase activity was induced as a response of the plankton community to high Al concentration that blocks substrates by reacting with phosphate. Lake Annie sediments (central site) were characterized as having high Fe (3640 mg kg^{-1}) and Al (34640 mg kg^{-1}) concentrations (Thompson, 1981), suggesting that the P present in the sediments is sequestered in stable form.

In Lake Annie, high PMEase activity, unrelated to P availability, might be a result of several factors: (1)

high Al and Fe concentration in its sediment, (2) high P demand inside microorganism cells, (3) or presence of more stable phosphomonoester (i.e., inositol phosphate), the predominance of extracellular phosphatase stabilized on sediment surfaces (Burns, 1982). Some phosphomonoesters (e.g., inositol phosphate) are more resistant to degradation compared to phosphodiester (Makarov et al., 2002), probably due to higher charge density, which enables the phosphomonoester to form strong complexes with cations, protecting them from degradation (Celi et al., 1999). Inositol phosphate, which is considered to be stable in soils, was present in Lake Annie spectra (Torres et al., 2014). Moreover, both PDEase and PMEase are necessary for release of free phosphate from phosphodiesters (Turner & Haygarth, 2005).

The PMEase activity in Lake Apopka seems to be controlled by something other than microbial activity. In a study of alkaline phosphatase activity in Lake Apopka sediments (upper 30 cm, $n = 6$), Newman & Reddy (1993) reported that activity was negatively correlated with labile organic P and HAP, and not correlated with DRP. Here we found a negative relationship between pore water DRP and PMEase activity, and positive correlations with organic P forms (including HAP). We used the same method for enzyme assay used by Newman & Reddy (1993), but our sample size was larger. Several studies have shown that there is an inverse correlation between PMEase activity and DRP in sediments (Jasson et al., 1988; Barik et al., 2001; Wobus et al., 2003; Jin et al., 2006; Rejmankova & Sirova, 2007). However, Siuda & Chrost (2001) concluded from controlled experiments that PMEase is synthesized even during periods of high orthophosphate concentrations in lake water. They suggested that PMEase activity of bacteria is used for organic P hydrolysis and uptake of associated organic C moieties, concluding that bacterial PMEase contributes substantially to DOC decomposition in lake water. This seems to be the case of PMEase production in Lake Apopka, given the strong correlation here between enzyme activity and DOC concentration.

Conclusions

To the best of our knowledge, this is the first study that compared phosphatase activity and P forms measured by chemical P fractionation and ^{31}P NMR spectroscopy in lake sediments. This is also the first study to relate anaerobic respiration and phosphatase activities. We conclude that PMEase and PDEase activities are related to sediment microbial biomass and activity, as well as to the P composition and availability. Phosphatase activity decreased with greater depth in all lakes, reflecting lower microbial biomass and activity. Strong correlations between enzyme activities and anaerobic respiration indicated that bacterial enzymes dominate these sediments. Different P forms in sediments were also correlated with enzyme activity. The mechanisms controlling PMEase activity, however, seem to vary according to the difference in P contents and forms in lake sediment.

Acknowledgements The authors thank the following research staff of the UF Wetland Biogeochemistry Laboratory for their assistance in this work: Matt Fisher, Jason Smith, Andrea Albertin, and Kathleen McKee for field and sampling assistance: and Yu Wang and Jeremy Bright for laboratory assistance.

References

Anupama, V. N., P. N. Amrutha, G. S. Chitra & B. Krishnakumar, 2008. Phosphatase activity in anaerobic bioreactors for wastewater treatment. Water Research 42: 2796–2802.

Aaronson, S. & N. J. Patni, 1976. The role of surface and extracellular phosphatases in the phosphorus requirement of Ochromonas. Limnology and Oceanography 21: 838–845.

Alef, K., P. Nannipieri & T. Cepeda, 1995. Phosphatase Activity. In Alef, K. & P. Nannipieri (eds), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, New York: 335–344.

Barik, S. K., C. S. Purushothaman & A. N. Mohanty, 2001. Phosphatase activity with reference to bacteria and phosphorus in tropical freshwater aquaculture pond systems. Aquaculture Research 32: 819–832.

Battoe, L. E., 1985. Changes in vertical phytoplankton distribution in response to natural disturbance in a temperate and subtropical lake. Journal of Freshwater Ecology 3: 167–174.

Boon, P. I. & B. K. Sorrell, 1991. Biogeochemistry of billabong sediments. I. The effect of macrophytes. Freshwater Biology 26: 209–226.

Burns, R. G., 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biology and Biochemistry 14: 423–427.

Celi, L., S. Lamacchia, F. A. Marsan & E. Barberis, 1999. Interaction of inositol hexaphosphate on clays: adsorption and charging phenomena. Soil Science 164: 574–585.

Chrost, R. J. & W. Siuda, 2002. Ecology of microbial enzymes in lake ecosystems. In Burns, R. G. & R. P. Dick (eds),

Enzymes in the Environment: Activity, Ecology and Applications. Marcel Dekker Inc, New York: 35–72.
Clugston, J. P., 1963. Lake Apopka, Florida, A changing lake and its vegetation. Quarterly Journal of the Florida Academy of Sciences 26: 168–174.
Davelaar, D., 1993. Ecological significance of bacterial polyphosphate metabolism in sediments. Hydrobiologia 253: 179–192.
Davis, S. H. & R. Goulder, 1993. Deterioration in bacteriological quality of water through fish farms. The Journal of Applied Bacteriology 74: 336–339.
Elser, J. J. & B. L. Kimmel, 1986. Alteration of phytoplankton phosphorus status during enrichment experiments: implications for interpreting nutrient enrichment bioassay results. Hydrobiologia 133: 217–222.
Engstrom, D. R., S. P. Schottler, P. R. Leavitt & K. E. Havens, 2006. A reevaluation of the cultural eutrophication of Lake Okeechobee using multiproxy sediment records. Ecological Applications 16: 1194–1206.
EPA, 1993. Methods for the Determination of Inorganic Substances in Environmental Samples. Environmental Monitoring Systems Lab, Cincinnati.
Fisher, M. M., M. Brenner & K. R. Reddy, 1992. A simple inexpensive, piston corer for collecting undisturbed sediment/water interface profiles. Journal of Paleolimnology 7: 157–161.
Fisher, M. M., K. R. Reddy & R. T. James, 2001. Long-term changes in the sediment chemistry of a large shallow subtropical lake. Journal of Lake and Reservoir Management 17: 217–232.
Gächter, R. & J. S. Meyer, 1993. The role of microorganisms in mobilization and fixation of phosphorus in sediments. Hydrobiologia 253: 103–121.
Gächter, R., J. S. Meyer & A. Mares, 1988. Contribution of bacteria to release and fixation of phosphorus in lake sediments. Limnology and Oceanography 33: 1542–1558.
Hedley, M. J. & J. W. B. Stewart, 1982. Method to measure microbial phosphate in soil. Soil Biology and Biochemistry 14: 377–385.
Hino, S., 1989. Characterization of orthophosphate release from dissolved organic phosphorus by gel filtration and several hydrolytic enzymes. Hydrobiologia 174: 49–55.
Jasson, M., 1981. Induction of high phosphatase activity by aluminum in acid lakes. Archiv für Hydrobiologia 93: 32–44.
Jasson, M., H. Olsson & O. Broberg, 1981. Characterization of acid phosphatases in the acidified Lake Gårdsjön. Sweden. Archiv für Hydrobiologie. 92: 377–395.
Jasson, M., H. Olsson & K. Pettersson, 1988. Phosphatases; origin, characteristics and function in lakes. Hydrobiologia 170: 157–175.
Jin, C. J., S. R. Wang, H. C. Zhao, Q. Y. Bu, J. Z. Chu, Z. Cui, X. N. Zhou & F. C. Wu, 2006. Effect of lake sediments of different trophic states on alkaline phosphatase activity. Journal of Lake and Reservoir Management 11: 169–176.
Kobori, H. & N. Taga, 1979. Phosphatase activity and its role in mineralization of organic phosphorus in coastal sea water. Journal of Experimental Marine Biology and Ecology 36: 23–39.
Kuenzler, E. J., 1965. Glucose-6-phosphate utilization by marine algae. Journal Phycology 1: 156–164.
Layne, J. N., 1979. Natural Features of the Lake Annie tract, Highlands County. Florida, Archbold Biological Station.
Makarov, M. I., L. Haumaierb & W. Zechb, 2002. Nature of soil organic phosphorus: an assessment of peak assignments in the diester region of ^{31}P NMR spectra. Soil Biology and Biochemistry 34: 1467–1477.
Massik, Z. & M. J. Cotello, 1995. Bioavailability of phosphorus in fish farm effluents to freshwater phytoplankton. Aquaculture Research 26: 607–616.
Megonigal, J. P., M. E. Hines & P. T. Visscher, 2004. Anaerobic Metabolism linkages to trace gases and aerobic processes. In Schlesinger, W. H. (ed.), Biogeochemistry. Elsevier-Pergamon, Oxford: 317–424.
Newman, S. & K. R. Reddy, 1993. Alkaline phosphatase activity in the sediment-water column of a hypereutrophic lake. Journal of Environmental Quality 22: 832–838.
Pant, H. K. & P. R. Warman, 2000. Enzymatic hydrolysis of soil organic phosphorus by immobilized phosphatases. Biology and Fertility of Soils 30: 306–311.
Reddy, K. R. & D. A.Graetz, 1991. Internal nutrient budget for lake Apopka. Special Publ. SJ91-SP6. St Johns River Water Mgt District. Palatka, FL, USA.
Rejmankova, E. & D. Sirova, 2007. Wetland macrophyte decomposition under different nutrient conditions: relationships between decomposition rate, enzyme activities and microbial biomass. Soil Biology and Biochemistry 39: 526–538.
Schelske, C. L., M. F. Coveney, F. J. Aldridge, W. Kenney & J. E. Cable, 2000. Wind or nutrients: historical development of hypereutrophy in Lake Apopka, Florida, USA. Archiv für Hydrobiologie Special Issues in Advanced Limnology 55: 543–563.
Sinke, A. J., A. A. Cornelese & T. E. Cappenberg, 1991. Phosphatase activity in sediments of the Loosdrecht lakes. Verhandlundgen der Internationale Vereinigung Limnologie 24: 719–721.
Siuda, W., 1984. Phosphatases and their role in organic phosphorus transformation in natural waters. A review. Polskie Archiwum Hydrobiologii 31: 207–233.
Siuda, W. & R. J. Chrost, 2001. Utilization of selected dissolved organic phosphorus compounds by bacteria in lake water under non-limiting orthophosphate conditions. Polish Journal of Environmental Studies 10: 475–483.
Tabatai, M. A., 1994. Soil Enzymes. In Weaver, R. W. (ed.), Methods of soil analysis: Microbiological & biochemical properties, Part 2. Soil Science Society of America, Madison: 903–931.
Thompson, D. M., 1981. Distribution of heavy metals in selected Florida lake sediments. Master dissertation, University of Florida, Gainesville, FL, USA.
Torres, I. C., B. L. Turner & K. R. Reddy, 2014. The chemical nature of phosphorus in subtropical lake sediments. Aquatic Geochemistry. doi:10.1007/s10498-014-9228-9.
Turner, B. L. & P. M. Haygarth, 2005. Phosphatase activity in temperate pasture soils: potential regulation of labile organic phosphorus turnover by phosphodiesterase activity. Science of the Total Environment 34: 27–36.
Vance, E. D., P. C. Brookes & D. S. Jenkinson, 1987. An extraction method for measuring microbial biomass C. Soil Biology and Biochemistry 19: 703–707.

Wetzel, R. G., 1991. Extracellular enzymatic interactions; storage, redistribution, and interspecific communication. In Chrost, R. J. (ed.), Microbial Enzymes in Aquatic Environments. Springer, New York: 6–28.

Wetzel, R. G., 1999. Organic phosphorus mineralization in soils and sediments. In Reddy, K. R., G. A. O'Connor & C. L. Schelske (eds), Phosphorus Biogeochemistry of Subtropical Ecosystems. Lewis, Boca Raton: 225–241.

Wobus, A., C. Bleul, S. Maassen, C. Scheerer, M. Schuppler, E. Jacobs & I. Röske, 2003. Microbial diversity and functional characterization of sediments from reservoirs of different trophic state. FEMS Microbial Ecology 46: 331–347.

Wright, A. L. & K. R. Reddy, 2001. Heterotrophic microbial activity in northern Everglades wetland soils. Soil Science Society American Journal 65: 1856–1864.

Yiyong, Z., L. Jianqi & Z. Min, 2001. Vertical variations in kinetics of alkaline phosphatase and P species in sediments of a shallow Chinese eutrophic lake (Lake Donghu). Hydrobiologia 450: 91–98.