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ORIGINAL PAPER

The Chemical Nature of Phosphorus in Subtropical Lake Sediments

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Abstract The phosphorus (P) composition of sediment profiles in three subtropical lakes of contrasting trophic state in Florida, USA, was determined by sequential fractionation and solution ^{31}P NMR spectroscopy. Sediment from Lake Annie, an oligo-mesotrophic sinkhole with moderately acidic sediment (pH 5.4; loss on ignition 58 %), contained higher total P concentrations than sediment from eutrophic Lake Okeechobee (pH 7.7, loss on ignition 36 %) and hyper-eutrophic Lake Apopka (pH 7.5, loss on ignition 69 %). The chemical nature of sediment P varied markedly among the three lakes, suggesting the predominance of different diagenetic processes. Lake Okeechobee sediment was dominated by inorganic P, indicating the dominance of abiotic reactions; Lake Annie sediment contained abundant organic P throughout the sediment profile, indicating the importance of organic P stabilization at acidic pH; Lake Apopka contained almost half of its sediment P in microbial biomass, indicating the importance of biotic processes in regulating P dynamics. Solution ^{31}P NMR spectroscopy of NaOH–EDTA extracts revealed that organic P occurred mainly as phosphomonoesters in all lakes. However, sediment from Lake Apopka also contained abundant phosphodiesters and was the only lake to contain detectable concentrations of polyphosphate, perhaps due to a combination of alternating redox conditions and high concentrations of inorganic phosphate and organic carbon. Organic P concentrations determined by sequential fractionation and solution ^{31}P NMR spectroscopy were similar for all lakes when microbial P was included in values for sequential fractionation. We conclude that the chemical nature of sediment P varies markedly depending on trophic state and can provide important information on the dominant processes controlling P cycling in subtropical lakes.

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1 Introduction

Phosphorus (P) is often the limiting nutrient for primary productivity in freshwater ecosystems. Sources of P to lakes can be external (allochthonous), originating from the drainage basin, or internal (autochthonous), originating from primary and secondary productivity within the lake. Sediment P is released to the water column (internal load) by a combination of chemical, physical, and biological processes, so sediments play a central role in P cycling by acting as a source or sink for P. This means that following a reduction or control of external nutrient loading to a water body, the sediment P pool can become important in regulating the trophic state of the water body and its recovery time (Petterson 1998).

Phosphorus accumulates in lake sediments in both inorganic and organic forms. Although organic P can constitute 30–80 % of the P in lake sediments (Williams and Mayer 1972; Boström et al. 1982), it has been relatively under-studied compared to inorganic phosphate (Mitchell and Baldwin 2005). In particular, organic P occurs in a variety of forms that behave differently in the aquatic environment (McKelvie 2005), so information on the relative abundance of P compounds in sediments is of importance for understanding P dynamics in lakes (Reitzel et al. 2007; Turner and Weckström 2009; Zhu et al. 2013). However, organic P is conventionally determined by sequential fractionation and detection by molybdate colorimetry (Turner et al. 2005; Condron and Newman 2011). Such procedures provide little compound-specific information and can overestimate organic P due to the presence of inorganic polyphosphates and, in some cases, to the apparent association of inorganic phosphate with humic compounds (Turner et al. 2006b).

Recent studies have determined the chemical nature of sediment organic P by alkaline extraction and solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy, which classifies organic P into a series of compounds and functional groups, including phosphomonoesters, phosphodiesters (e.g., DNA, lipids), and phosphonates (e.g., Carman et al. 2002; Ahlgren et al. 2005; Reitzel et al. 2007; Zhu et al. 2013). The technique also identifies inorganic phosphates, including orthophosphate, pyrophosphate, and polyphosphate (e.g., Hupfer et al. 2004). It has been applied widely in the study of P forms in lake sediments (e.g., Hupfer et al. 2004; Ahlgren et al. 2006; Zhang et al. 2009; Shinohara et al. 2012), although most studies have assessed temperate lakes, and limited information exists on the organic P composition of subtropical lake sediments.

Eutrophic and hyper-eutrophic lakes usually receive high external loads of nutrients, display high primary productivity and nutrient concentrations in the water column, and might therefore be expected to have high concentrations of organic matter and nutrients as compared with oligotrophic lakes (Flanery et al. 1982; Wisniewski and Planter 1985; Maassen et al. 2003). However, this is not always the case (Brenner and Binford 1988; Lopez and Morgui 1993; Gonsiorczyk et al. 1998), because sediment composition reflects the integrative effects of trophic state and diagenesis over long time periods. Moreover, the relative importance of the various P forms in sediments will depend on sediment composition, sedimentation rate, diagenesis, and physicochemical conditions (Lopez and Morgui 1993; Gonsiorczyk et al. 1998). Of significance is the fact that differences in the

abundance of P compounds with depth in lake sediments can provide information on mineralization rates and the contribution of the various compounds to the internal P load (Ahlgren et al. 2005; Reitzel et al. 2007).

Despite the importance of information on the P composition of freshwater lake sediments, the chemical nature of P in subtropical lakes remains poorly understood. To address this, we studied the chemical nature of P in sediment profiles of three subtropical lakes with different trophic states using two different techniques, solution ^{31}P NMR spectroscopy and sequential P fractionation, to assess factors controlling P dynamics in these lakes. Our aim was to determine whether the sediment P composition would reflect differences in trophic state and physio-chemical properties of lake sediments.

2 Methods

2.1 Study Sites

Three Florida lakes (USA) were selected for this study based on water quality and trophic status (Table 1; Fig. 1). Lake Annie is a small (0.37 km^2) oligo-mesotrophic lake located in south-central Florida (Highlands County) at the northern end of the Archbold Biological Station. The lake is characterized by pristine water quality with little surface water input (most is ground water) and low anthropogenic impact due to the absence of development

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

Parameter	Lake Annie	Lake Okeechobee	Lake Apopka
Site	Central	M9	West
Latitude (North)	27°12′27″	26°58′17.6″	28°38′01″
Longitude (West)	81°21′44″	80°45′38.4″	81°39′36″
Sampling date	June 2005	July 2005	May 2005
Sediment type	Mud/clay	Mud	Organic
Water column depth (m)	20	4.0	2.0
Secchi disk (m)	2.0	0.08	0.3
Temperature (°C)[a]	30.2	29.5	26.6
Electrical conductivity (μS cm^{-1})[a]	41.9	385	443
pH[a]	5.1	7.8	7.6
Dissolved oxygen (mg O_2 L^{-1})[a]	6.4	6.5	8.7
Dissolved oxygen (% saturation)	85.2	85.7	109.8
Dissolved organic C (mg C L^{-1})[b]	13.8	14.5	31.1
Total P (μg P L^{-1})[b]	33.2	255.9	69.7
Dissolved reactive P (μg P L^{-1})[b]	7.4	90.4	11.1
Total N (mg N L^{-1})[b]	1.81	3.44	11.15
Ammonium (mg N L^{-1})[b]	0.18	0.10	0.12

[a] Measured at 1 m depth in the water column

[b] Mean concentration in the water column. For Lake Annie, values are an average of samples taken at the following depths: 0.5, 1.0, 2.0, 5.0, 10, 20 m; for Lake Okeechobee and Lake Apopka, values are an average of samples taken at 0.5, 1.0, and 2.0 m

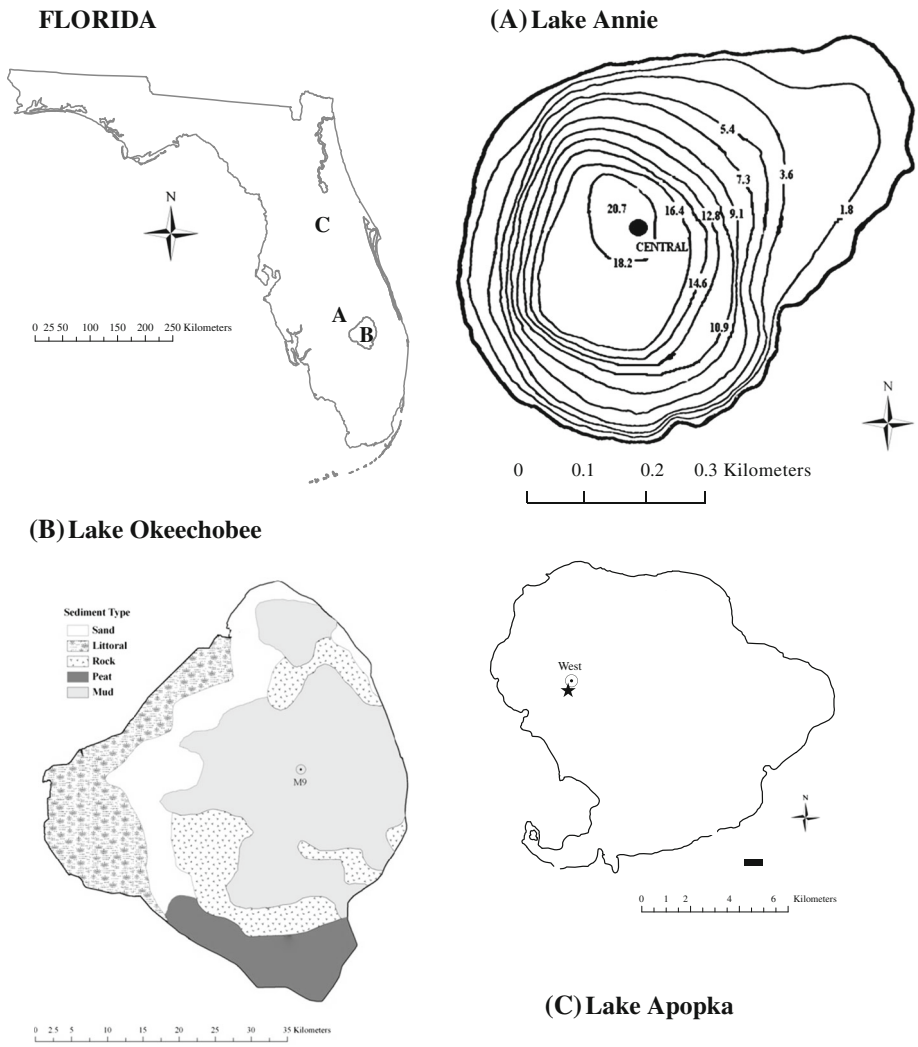

Fig. 1 Maps of the study sites, showing the location of the three lakes in Florida and the sampling sites within each lake

around the lake (Layne 1979). Lake Annie 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 periods of high rainfall (Battoe 1985). Lake sediments vary from organic to sandy in the littoral zone (Layne 1979).

Lake Okeechobee is a large (1,730 km^2), shallow, eutrophic lake located in south Florida, which has experienced anthropogenic eutrophication over the last 50 years (Engstrom et al. 2006). Benthic sediments are characterized as mud (44 % of the total lake surface area), sand and rock (28 %), littoral areas dominated by macrophytes (19 %), and peat or partially decomposed plant tissue (9 %) (Fisher et al. 2001).

Lake Apopka is a shallow lake (125 km^2) in central Florida. Once a clear-water macrophyte-dominated lake, it has changed since 1947 to a turbid, algal-dominated lake (Clugston 1963) following nutrient input from several sources, including agricultural drainage from adjacent vegetable farms (Schelske et al. 2000). Although these inputs were controlled and regulated to some degree, the eutrophication process has continued, and Lake Apopka is now considered hyper-eutrophic. Benthic sediments are characterized by unconsolidated material consisting mainly of algal deposits (Reddy and Graetz 1991).

2.2 Field Sampling

Sediment–water interface cores of variable lengths were collected using a piston corer (Fisher et al. 1992) or by SCUBA divers. Lake maps with sampling sites are shown in Fig. 1. One central site (80 cm core) was sampled in Lake Annie on June 25, 2005. Cores were collected at a site in the mud zone (M9 site, 70 cm core) of Lake Okeechobee, an area that has the highest concentration of P among the different sediment types of this lake (Fisher et al. 2001), on July 16, 2005. A western site (98 cm core) in Lake Apopka was sampled on May 28, 2005. 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 sealed in plastic bags and returned to the laboratory on ice. Nine cores were collected from each site. These were combined in sets of three to make composite replicate cores with sufficient material for all measurements. In other words, the nine cores yielded three replicate sets of composite sediments from each site.

2.3 Water Characteristics

Water parameters were measured to characterize the lakes in relation to their trophic status. For Lake Annie, water data are the average of measurements taken at 0.5, 1, 2, 5, 10 and 20 m, while for Lake Okeechobee and Lake Apopka, measurements were taken at 0.5, 1 and 2 m. Water transparency was determined using a Secchi disk, while water temperature (°C), electrical conductivity, pH, and dissolved oxygen concentration were measured with a YSI 556 Multi-Probe Sensor (YSI Environmental, Yellow Springs, OH). Water samples were collected from various depths at each site using a Van Dorn bottle (Table 1) and nutrient concentrations measured using standard procedures (EPA 1993). Total Kjeldahl nitrogen and total P were determined using EPA Methods 351.2 and 365.1, respectively. Each water sample was filtered through a 0.45-µm membrane and analyzed for dissolved reactive P (DRP) (EPA Method 365.1), ammonium (EPA Method 351.2), and dissolved organic carbon (automated Shimadzu TOC 5050 analyzer, EPA Method 415.1).

2.4 Sediment Properties

Sediment bulk density (g dry sediment cm^{-3}) was determined by drying a subsample of known volume at 70 °C for 72 h, and pH was determined on wet sediment in a 1:2 sediment/deionized water ratio, although this may not reflect accurately the in situ pH (Oman et al. 2007). Dried sediment was ground in a ball mill and sieved (<40 mesh). Organic matter content (loss on ignition) was determined by weight loss at 550 °C. Total P was measured by ignition at 550 °C followed by digestion in 6 mol L^{-1} HCl (Anderson 1976), with phosphate detection by automated molybdate colorimetry on a Bran+Luebbe

Technicon™ Autoanalyzer II (EPA Method 365.1). All measured sediment variables are reported on the basis of oven-dried sediment.

2.5 Sequential Phosphorus Fractionation

Due to high water content of sediments from Lake Annie and Lake Apopka, pore water was extracted by centrifuging at 10,000×*g* for 10 min prior to P fractionation. Pore water total P was measured after digestion in 5.5 mol L^{-1} H_2SO_4 and K_2SO_8 (EPA Method 365.1).

Phosphorus in the solid sediment was fractionated using a scheme described by Ivanoff et al. (1998). This involved sequential chemical extraction in a 1:50 dry sediment/solution ratio with (1) 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5), which represented labile inorganic and labile organic P, as well as microbial P (see below); (2) 1 mol L^{-1} HCl, which represented inorganic phosphate bound to aluminum, calcium, magnesium, and iron; (3) 0.5 mol L^{-1} NaOH, which represented organic P associated with fulvic and humic fractions (moderately available and highly resistant organic P, respectively). Phosphorus remaining in the sediment after sequential extraction was determined by ignition (as described above for total sediment P) and is termed 'residual P.'

Extracts from each fraction were centrifuged at 10,000×*g* for 10 min, and filtered through a 0.45 μm membrane. For the $NaHCO_3$ extracts, inorganic P was determined by molybdate colorimetry, and total P was determined by acid-persulfate digestion (Rowland and Haygarth 1997), with organic P calculated by difference. A second sample was treated by $ChCl_3$ fumigated to determine microbial P (Ivanoff et al. 1998). Briefly, a separate soil sample was treated with alcohol-free $CHCl_3$ (0.5 mL) to lyse microbial cells, placed in a vacuum desiccator, and incubated for 24 h. The sample was then extracted with 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) in a 1:50 dry sediment/solution ratio in an identical manner to the untreated $NaHCO_3$ extract. Extracts were analyzed for total P by acid-persulfate digestion, and microbial P was calculated as the difference between fumigated and non-fumigated samples without correction for unrecovered biomass P. The sequential fractionation procedure then continued with the fumigated sample.

The HCl extracts were analyzed for inorganic P only, and the NaOH extracts were analyzed for total P only. Humic acids were separated from the NaOH extracts by acidification to pH 0.2 with concentrated HCl, and the total P remaining in solution was assumed to be associated with fulvic acids. Humic acid P was then calculated as the difference between total P in the untreated NaOH extracts and the fulvic acid P. We did not determine molybdate-reactive P in the NaOH extracts because the majority of the inorganic P associated with metal oxides and carbonate is recovered in the preceding HCl extraction step (Ivanoff et al. 1998). We therefore assume that NaOH-extractable P in fulvic and humic acids is all organic in nature, although this likely leads to a slight overestimation of fulvic acid P. We also note that ^{31}P NMR analysis of NaOH extracts in the Ivanoff et al. (1998) procedure indicates that inorganic phosphate is present, although the majority of this does not react with molybdate, suggesting its occlusion within large organic molecules (Turner et al. 2006b).

2.6 Solution ^{31}P Nuclear Magnetic Resonance Spectroscopy

Samples from triplicate cores in each depth interval from each site were combined and extracted using a procedure described by Hupfer et al. (1995, 2004). Samples (approximately 30 g of wet sediment) were pre-extracted for 1 h with 100 mL of 0.067 mol L^{-1}

EDTA to reduce interference by calcium and iron in NMR spectroscopy. This pretreatment can remove small amounts of P, but we considered this to be negligible. After centrifuging (10,000×g, 30 min), the supernatant was discarded and the residue was extracted with 40 mL of a solution containing 0.2 mol L^{-1} NaOH and 0.067 mol L^{-1} EDTA (1:20 sediment/solution ratio). Samples were shaken for 2 h, centrifuged (10,000×g, 30 min), and an aliquot of each extract (2 mL) was used to determine total P by acid-persulfate digestion. The remaining sample was frozen immediately at −80 °C and lyophilized.

Samples were analyzed by solution ^{31}P NMR spectroscopy as described by Turner et al. (2006a). Each lyophilized extract (approx. 100 mg) was redissolved in 0.1 mL deuterium oxide (to provide an NMR signal lock) and 0.9 mL of a solution containing 1.0 mol L^{-1} NaOH and 0.1 mol L^{-1} EDTA (to raise the pH to >13 to ensure consistent chemical shifts and optimum spectral resolution), and transferred to a 5 mm NMR tube. Solution ^{31}P NMR spectra were determined using a 6-µs pulse (45°), a delay time of 2.0 s and acquisition time of 0.4 s, with a Bruker Avance DRX 500 MHz spectrometer operating at 202.456 MHz for ^{31}P. Chemical shifts of signals were expressed in parts per million (ppm) relative to an external standard of 85 % H_3PO_4. Signals were assigned to individual P compounds or functional groups based on literature reports (Turner et al. 2003).

2.7 Statistical Analysis

A Pearson correlation and a principal component analysis (PCA) were performed to determine relations among P forms. All statistical analyses were performed using Statistica 7.1 software (StatSoft 2006, Tulsa, OK).

3 Results

3.1 Water Column Properties

All three lakes had low water transparency. Water parameters were measured in several depths in each lake and the mean values are shown in Table 1. Electrical conductivity values were lowest for Lake Annie (42 µS cm^{-1}) and higher for Lake Okeechobee (385 µS cm^{-1}) and Lake Apopka (443 µS cm^{-1}). Lake Annie water column pH was acidic, while both Lake Okeechobee and Lake Apopka had near neutral pH water (Table 1). Daytime dissolved oxygen concentrations were similar for all lakes, with Lake Apopka (8.7 mg L^{-1}) presenting higher values. The dissolved oxygen levels in Lake Apopka were close to supersaturation due to the high phytoplankton biomass and low temperature. Values did not approximate supersaturation in Lake Okeechobee and Lake Annie due to the higher temperatures and low phytoplankton biomass in Lake Annie. Highest dissolved organic carbon values were found in Lake Apopka (31 mg C L^{-1}), while all other dissolved organic carbon values in the lakes were similar and around 14 mg C L^{-1}. Water total P and DRP were highest in Lake Okeechobee, while total nitrogen was highest in Lake Apopka.

3.2 Sediment Properties

Lake Annie sediments were acidic (pH 5.2–5.7), while Lake Okeechobee (pH 7.7–8.0) and Lake Apopka (pH 7.0–7.5) sediments were slightly alkaline (Table 2). Bulk density was

Table 2 Properties of the sediment profile in three subtropical lakes

Depth (cm)	Total P (mg P kg^{-1})	Pore water total P (mg P kg^{-1})	pH	Bulk density (g cm^{-3})	Loss on ignition (%)
Lake Annie (Central)					
0–5	1,439 ± 35	12 ± 3	5.4 ± 0.15	0.04 ± 0.006	58 ± 0.4
5–10	1,423 ± 28	7 ± 3	5.2 ± 0.05	0.04 ± 0.003	57 ± 1.2
10–15	1,459 ± 67	9 ± 3	5.3 ± 0.09	0.06 ± 0.003	55 ± 1.0
15–20	1,531 ± 80	15 ± 3	5.3 ± 0.11	0.07 ± 0.003	54 ± 1.6
20–30	1,484 ± 191	25 ± 8	5.4 ± 0.03	0.07 ± 0.003	52 ± 0.8
30–45	1,513 ± 258	24 ± 9	5.5 ± 0.09	0.08 ± 0.002	52 ± 0.1
45–60	1,133 ± 32	18 ± 4	5.5 ± 0.06	0.10 ± 0.006	50 ± 0.9
60–80	1,149 ± 51	8 ± 1	5.7 ± 0.06	0.11 ± 0.007	50 ± 1.0
Lake Okeechobee (M9)					
0–5	1,051 ± 39	nd	7.7 ± 0.16	0.11 ± 0.005	36 ± 1.7
5–10	924 ± 24	nd	7.7 ± 0.03	0.16 ± 0.003	37 ± 1.2
10–15	835 ± 16	nd	7.8 ± 0.04	0.20 ± 0.020	21 ± 1.3
15–20	732 ± 83	nd	7.8 ± 0.04	0.23 ± 0.020	26 ± 6.9
20–30	644 ± 57	nd	7.9 ± 0.03	0.26 ± 0.056	16 ± 3.8
30–45	575 ± 48	nd	7.9 ± 0.05	0.33 ± 0.040	25 ± 6.5
45–60	590 ± 30	nd	8.0 ± 0.01	0.30 ± 0.009	29 ± 4.7
60–80	497 ± 155	nd	8.0 ± 0.08	0.33 ± 0.043	35 ± 3.5
Lake Apopka (West)					
0–5	1,264 ± 53	10 ± 3	7.5 ± 0.07	0.01 ± 0.001	69 ± 0.7
5–10	1,303 ± 73	7 ± 1	7.3 ± 0.06	0.02 ± 0.001	67 ± 3.2
10–15	1,350 ± 37	5 ± 1	7.2 ± 0.02	0.02 ± 0.004	67 ± 1.1
15–20	1,275 ± 122	5 ± 1	7.2 ± 0.06	0.03 ± 0.006	65 ± 2.7
20–30	1,234 ± 153	6 ± 2	7.3 ± 0.04	0.03 ± 0.009	64 ± 1.0
30–45	990 ± 296	10 ± 2	7.2 ± 0.05	0.04 ± 0.014	66 ± 1.9
45–60	795 ± 156	12 ± 4	7.1 ± 0.10	0.06 ± 0.010	68 ± 1.3
60–80	615 ± 7	16 ± 4	7.0 ± 0.06	0.07 ± 0.005	69 ± 0.8
80–100[a]	694	7	7.0	0.07	71

Values are the mean ± SD of three replicate samples, each a composite of three replicate cores

nd not determined

[a] No replicate cores

low in Lake Apopka (0.01–0.07 g cm^{-3}) and Lake Annie (0.04–0.11 g cm^{-3}) compared to Lake Okeechobee (0.11–0.33 g cm^{-3}), reflecting the higher mineral content of Lake Okeechobee sediment; values increased with depth in all three lakes. Loss on ignition (organic matter) was greatest in Lake Apopka (64–71 %), with lower values in Lake Annie (50–58 %) and Lake Okeechobee (16–36 %) (Table 2).

In surface sediment, Lake Annie had the highest total P concentration (1,439 mg P kg^{-1}), compared to Lake Apopka (1,264 mg P kg^{-1}) and Lake Okeechobee (1,051 mg P kg^{-1}), with concentrations declining with depth in all sediment profiles (Table 2). In Lake Okeechobee and Lake Apopka, the deepest sediment layer contained about half the total P concentration measured in surface sediments (Table 2).

3.3 Sequential Phosphorus Fractionation

Total P determined by sequential fractionation (i.e., the sum of all fractions including microbial P) was 80–93 % of the total sediment P determined by ignition for Lake Annie, 99–121 % for Lake Okeechobee, and 101–123 % for Lake Apopka (Table 3; Fig. 2). Recoveries >100 % probably reflect the accumulated error associated with the multiple extraction steps in the sequential fractionation procedure.

Table 3 Total extracted P determined as the sum of sequential fractions (mg P kg^{-1}) and the proportion (%) of each fraction in sediment profiles of three subtropical lakes

Depth (cm)	Total extracted P[a] (mg P kg^{-1})	Proportion of total extracted P (%)						
		$NaHCO_3$–P		Microbial P	HCl–P	Fulvic acid P	Humic acid P	Residual P
		Inorganic	Organic					
Lake Annie (Central)								
0–5	1,287 ± 42 (89)	5.6	3.9	6.1	28.7	28.8	26.4	0.5
5–10	1,219 ± 42 (86)	5.5	3.5	5.3	36.4	28.0	20.9	0.3
10–15	1,251 ± 99 (86)	4.4	3.2	3.5	43.8	30.3	14.8	nd
15–20	1,286 ± 40 (84)	4.0	2.7	2.9	46.6	29.4	14.3	0.2
20–30	1,377 ± 122 (93)	3.1	2.0	2.2	52.7	24.5	15.5	nd
30–45	1,211 ± 165 (80)	2.8	1.8	2.0	54.7	23.6	14.0	0.1
45–60	987 ± 10 (87)	2.2	2.0	2.3	55.1	19.8	18.7	nd
60–80	948 ± 17 (82)	1.6	1.7	1.9	56.9	21.7	16.2	nd
Lake Okeechobee (M9)								
0–5	1,155 ± 55 (110)	9.5	0.5	4.3	58.0	6.3	3.0	18.4
5–10	1,004 ± 21 (105)	8.7	0.8	3.4	58.0	6.4	3.5	19.3
10–15	1,009 ± 98 (121)	12.6	0.7	2.8	60.4	0.2	0.8	22.6
15–20	786 ± 25 (107)	7.0	0.5	2.2	73.4	0.6	0.5	16.0
20–30	722 ± 45 (112)	11.1	0.3	1.5	68.9	nd	nd	18.2
30–45	685 ± 41 (119)	8.0	0.5	1.3	74.4	nd	nd	15.8
45–60	584 ± 59 (99)	7.9	0.4	1.4	78.2	0.1	0.4	11.6
60–80	521 ± 40 (105)	6.8	0.3	0.5	81.1	0.8	0.9	9.6
Lake Apopka (West)								
0–5	1,518 ± 33 (120)	0.1	1.0	39.4	21.4	14.1	8.2	15.8
5–10	1,537 ± 157 (118)	0.1	1.2	38.7	22.6	13.0	7.6	16.8
10–15	1,655 ± 90 (123)	1.1	1.5	37.3	23.0	13.2	8.0	15.9
15–20	1,441 ± 146 (113)	1.4	1.6	36.0	25.4	10.2	6.5	18.9
20–30	1,358 ± 268 (110)	2.2	1.1	28.6	31.6	8.8	6.7	21.0
30–45	1,011 ± 366 (102)	4.0	1.1	23.3	36.3	8.8	6.3	20.3
45–60	810 ± 205 (102)	4.5	2.1	12.1	44.3	7.7	5.8	23.6
60–80	650 ± 47 (106)	6.5	1.1	4.8	53.8	5.7	4.4	23.7
80–100[b]	700 (101)	8.7	0.1	7.5	55.1	4.8	3.9	19.8

nd not detected

[a] Total P extracted in the sequential fractionation scheme. Values in parentheses are the proportion (%) of the total P determined by ignition and acid digestion

[b] No replicate cores

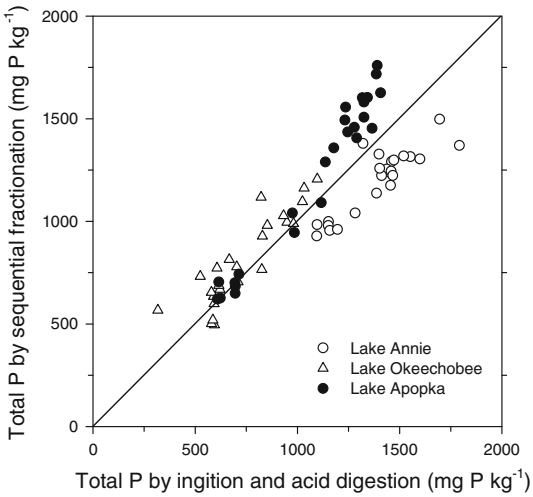

Fig. 2 Scatter plot showing the correlation between total P determined by ignition (*x* axis) and sequential fractionation (*y* axis). Points are Lake Annie (*open circles*), Lake Okeechobee (*open triangles*), and Lake Apopka (*closed circles*). The 1:1 *line* is shown

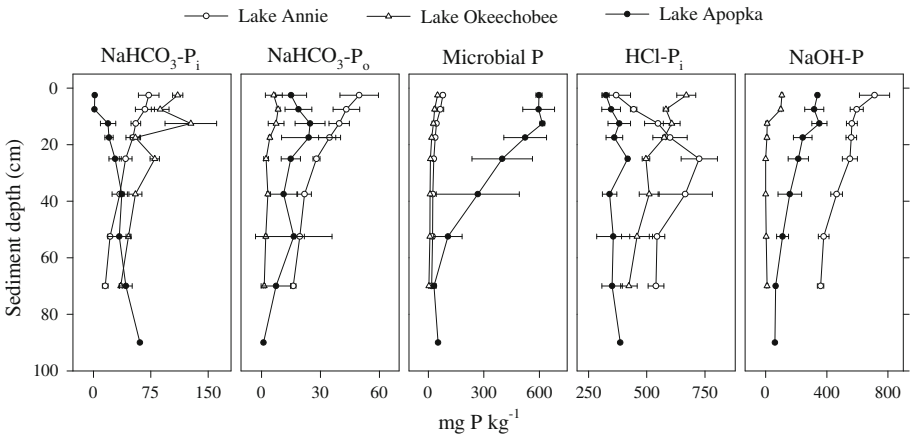

Fig. 3 Concentrations (mg P kg^{-1} dry weight) of P fractions determined by sequential fractionation in sediment from three subtropical lakes in Florida

Labile inorganic phosphate ($NaHCO_3$-extractable inorganic P) concentrations in surface sediments were highest in Lake Okeechobee (110 ± 7 mg P kg^{-1}) and Lake Annie (72 ± 13 mg P kg^{-1}) and declined with depth, whereas concentrations in Lake Apopka (1.6 ± 0.4 mg P kg^{-1}) were low in surface sediments and increased with depth (Fig. 3). As a proportion of the total P determined by sequential fractionation, labile inorganic phosphate constituted as little as 0.1 % in surface sediment Lake Apopka, but between 7.0 and 12.6 % in Lake Okeechobee; values declined with depth in Lake Annie and increased in Lake Apopka (Table 3).

Labile organic P ($NaHCO_3$-extractable organic P) concentrations in surface sediments were higher in Lake Annie (50 ± 10 mg P kg^{-1}) than Lake Apopka (15 ± 8 mg P kg^{-1}) and Lake Okeechobee (6 ± 4 mg P kg^{-1}) and generally declined with depth in all lakes (Fig. 3). Labile organic P was a greater proportion of the total P determined by sequential fractionation in Lake Annie (1.7–3.9 %) compared to the other two lakes (Table 3).

Table 4 Phosphorus composition of the sediment depth profile determined by ^{31}P NMR spectroscopy

Depth (cm)	Total extracted P[a] (mg P kg^{-1})	Proportion of total extracted P (%)					
		Phosphate	Phosphomonoester	Phospholipid	DNA	Pyrophosphate	Polyphosphate
Lake Annie (Central)							
0–5	1,559 (108)	51	35	tr	10	nd	nd
5–10	1,525 (107)	54	36	tr	9	tr	nd
10–15	1,468 (101)	58	33	tr	9	nd	nd
15–20	1,498 (98)	58	34	tr	6	nd	nd
20–30	1,584 (107)	71	23	tr	6	tr	nd
30–45	1,296 (86)	60	34	nd	8	nd	nd
45–60	1,041 (92)	61	31	nd	10	nd	nd
60–80	854 (74)	61	29	nd	8	nd	nd
Lake Okeechobee (M9)							
0–5	896 (85)	68	24	nd	9	tr	nd
5–10	547 (57)	67	27	nd	7	tr	nd
10–15	405 (48)	100	nd	nd	nd	nd	nd
20–30	174 (24)	100	nd	nd	nd	nd	nd
45–60	228 (40)	100	nd	nd	nd	nd	nd
Lake Apopka (West)							
0–5	1,139 (90)	28	28	3	31	10	nd
5–10	824 (63)	32	22	4	29	2	11
10–15	670 (50)	35	20	3	31	3	9
15–20	858 (67)	33	22	4	30	3	8
20–30	349 (28)	47	25	tr	28	nd	nd
30–45	224 (23)	63	14	tr	23	nd	nd
45–60	150 (19)	64	12	tr	25	nd	nd
60–80	135 (22)	80	nd	nd	21	nd	nd
80–100	102 (15)	85	nd	nd	15	nd	nd

nd not detected, *tr* trace (i.e., not quantifiable)

[a] Total P extracted in NaOH–EDTA. Values in parentheses are the proportion (%) of the total P determined by ignition and acid digestion

Microbial P concentrations were much greater in Lake Apopka (598 ± 17 mg P kg^{-1} in surface sediment) compared to Lake Annie (78 ± 9 mg P kg^{-1}) and Lake Okeechobee (50 ± 2 mg P kg^{-1}) (Fig. 3). Concentrations decreased with depth in all sediment profiles, and in Lake Apopka sediment, the values were similar to those in the other two lakes by 60–80 cm (Fig. 3). Microbial P constituted up to 39 % of the total P determined by sequential fractionation in surface sediment of Lake Apopka, declining to 5 % at 60–80 cm (Table 3). Microbial P was 6 and 4 % of the total P determined by sequential fractionation in surface sediment of Lake Annie and Lake Okeechobee, respectively, and values declined with depth (Table 3).

In surface sediments, HCl-extractable inorganic P concentrations were greater in Lake Okeechobee (670 ± 41 mg P kg^{-1}) compared to the other two lakes, although concentrations were high in both lakes (367 ± 62 mg P kg^{-1} in Lake Annie and 325 ± 14 mg P kg^{-1} in Lake Apopka) (Fig. 3). Concentrations declined with depth in Lake Okeechobee sediment were relatively constant with depth in Lake Apopka, but increased markedly to 726 ± 78 mg P kg^{-1} at 30 cm in Lake Annie, before subsequently declining (Fig. 3). HCl-extractable inorganic phosphate accounted for 21–57 % of the total

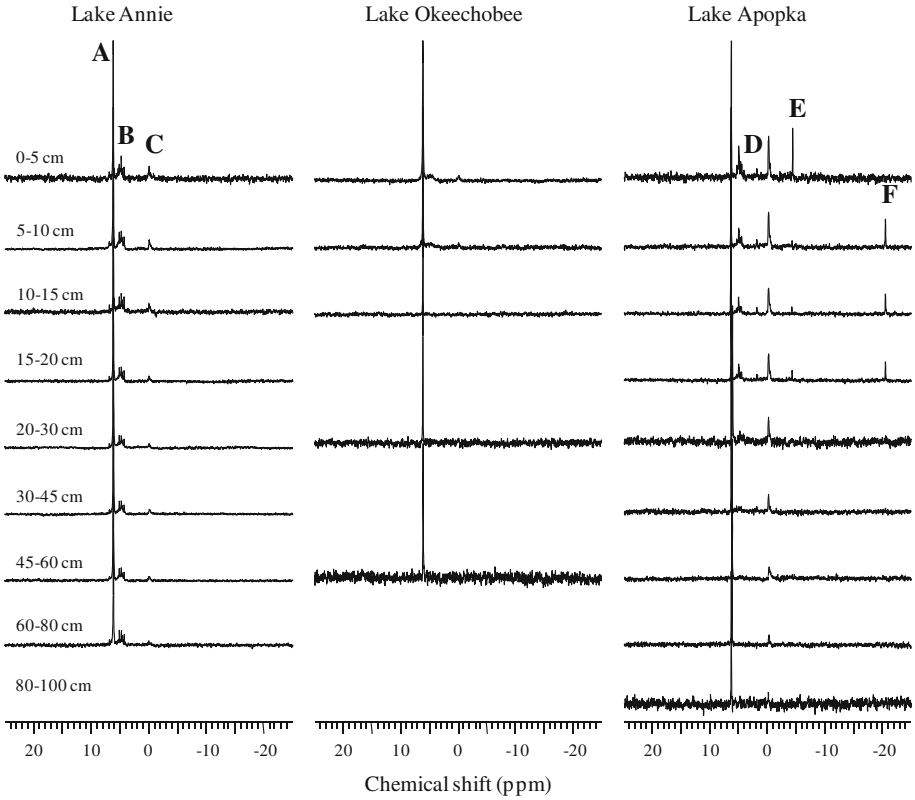

Fig. 4 Solution ^{31}P NMR spectra of NaOH–EDTA extracts of three subtropical lakes in Florida. Signals are assigned as follows: *A* inorganic orthophosphate, *B* phosphomonoesters, *C* DNA, *D* phospholipids, *E* pyrophosphate, *F* polyphosphate

P determined by sequential fractionation in Lake Annie and Lake Apopka, but 58–81 % in Lake Okeechobee, with values increasing with depth in all three lakes (Table 3).

NaOH-extractable P was greater in Lake Annie (359–713 mg P kg^{-1}) than in Lake Apopka (61–339 mg P kg^{-1}), while significant concentrations were detected in Lake Okeechobee sediments only in the surface 10 cm (99–106 mg P kg^{-1}) (Fig. 3). Concentrations of NaOH-extractable P declined with depth in all three lakes (Fig. 3). NaOH-extractable P accounted for 37–55 % of the total P determined by sequential fractionation in Lake Annie, 9–22 % in Lake Apopka, but <10 % in Lake Okeechobee, with values decreasing with depth in all samples (not shown). The proportion of P associated with fulvic acids tended to be greater than proportion than the proportion associated with humic acids in all three lakes (Table 3).

Residual P accounted for a considerable proportion of the total P determined by sequential fractionation in Lake Okeechobee (10–23 %) and Lake Apopka (16–24 %), but <1 % in Lake Annie (Table 3).

3.4 Solution ^{31}P Nuclear Magnetic Resonance Spectroscopy

Phosphorus extracted by the NaOH–EDTA procedure was 74–108 % of the total sediment P determined by ignition for Lake Annie, 24–85 % for Lake Okeechobee, and 15–90 % for

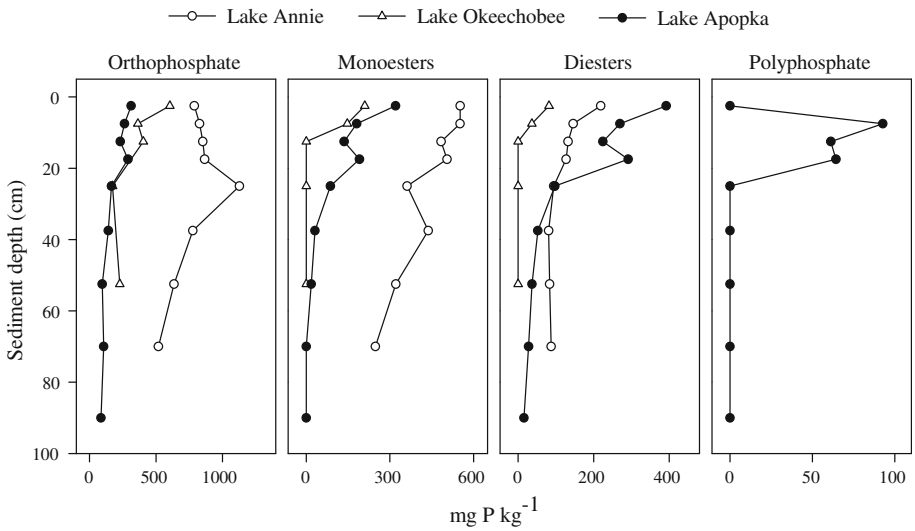

Fig. 5 Concentrations (mg P kg^{-1} dry weight) of P compounds determined by NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy in sediment from three subtropical lakes in Florida. Polyphosphate includes both pyrophosphate (chain length $n = 2$) and long-chain polyphosphate and was detected only in the Lake Apopka sediments

Lake Apopka (Table 4). Recoveries were generally greatest in the surface sediment layers and decreased with depth. Low recoveries, particularly for Lake Okeechobee and in deeper sediment layers, likely reflect the relative absence of organic P in the sediments, because the NaOH–EDTA extraction is designed to recover organic and condensed P forms and not stable inorganic phosphate (Bowman and Moir 1993; Turner et al. 2005). We interpret recoveries slightly >100 % to the error associated with the two procedures.

A series of P compounds were detected in the NaOH–EDTA sediment extracts, including orthophosphate, phosphomonoesters, phosphodiesters (mainly DNA), pyrophosphate, and polyphosphate (Fig. 4). Orthophosphate was the major P compound in sediments of Lake Okeechobee (67–100 % of extracted P) and Lake Annie (51–71 % of extracted P) (Fig. 5; Table 4). Concentrations were greatest in Lake Okeechobee sediments, and orthophosphate was the only compound detected in the deeper sediment layers of this lake (Table 4). The remaining compounds in the surface layers of the Okeechobee sediment profile were phosphomonoesters (24–27 %) and DNA (7–9 %), with a trace of pyrophosphate (Fig. 4; Table 4).

In Lake Annie, approximately one-third of the extracted P was phosphomonoesters and 6–10 % was DNA throughout the sediment profile (Table 4). Lake Annie sediment contained the highest concentrations of phosphomonoesters of the three lakes (Fig. 5), but pyrophosphate was detected in trace amounts only in two samples (Table 4).

Lake Apopka contained the richest range of P compounds (Fig. 4). Orthophosphate concentrations were the lowest of the three lakes (Fig. 5), although the proportion of the extracted P in this form increased markedly with depth from 28 % in the surface layer to 85 % at 80–100 cm. Phosphomonoesters (20–28 % of extracted P) and DNA (28–31 %) were present in approximately equal proportions in the upper 30 cm of Lake Apopka sediments (Table 4), but while phosphomonoesters declined to undetectable levels at >60 cm, DNA represented 15–21 % of the extracted P (Table 4). Phospholipids and

Table 5 Total organic P determined by sequential fractionation (sum of $NaHCO_3$–-organic P, fulvic acid P, and humic acid P) and by NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy (sum of phosphomonoesters and phosphodiesters)

Depth (cm)	Sequential fractionation		^{31}P NMR spectroscopy	
	mg P kg^{-1}	% of total P	mg P kg^{-1}	% of total P
Lake Annie (Central)				
0–5	762	53	771	54
5–10	639	45	697	49
10–15	603	41	616	42
15–20	595	39	632	41
20–30	579	39	456	31
30–45	486	32	519	34
45–60	399	35	405	36
60–80	375	33	336	29
Lake Okeechobee (M9)				
0–5	113	11	292	28
5–10	107	11	184	19
10–15	17	2	<1	<1
15–20	12	2	nd	nd
20–30	2	<1	<1	<1
30–45	3	<1	nd	nd
45–60	5	<1	<1	<1
60–80	10	2	nd	nd
Lake Apopka (West)				
0–5	354	28	713	56
5–10	336	26	451	35
10–15	376	28	360	27
15–20	267	21	482	38
20–30	229	19	184	15
30–45	169	17	83	8
45–60	127	16	55	7
60–80	73	12	28	5
80–100	62	9	16	2

nd not determined

pyrophosphate were detected at low concentrations to 20 cm, while Lake Apopka was the only sediment profile to contain detectable concentrations of polyphosphate (Figs. 4, 5; Table 4). Although not detected in the uppermost sediment layer, polyphosphate occurred in concentrations up to 93 mg P kg^{-1} at 5–10 cm, equivalent to 11 % of the extracted P, declining to undetectable levels at >20 cm (Table 4).

3.5 Relationships Among Phosphorus Forms

Concentrations of organic P determined by sequential fractionation and solution ^{31}P NMR spectroscopy were similar in sediments of Lake Annie and Lake Okeechobee, but differed

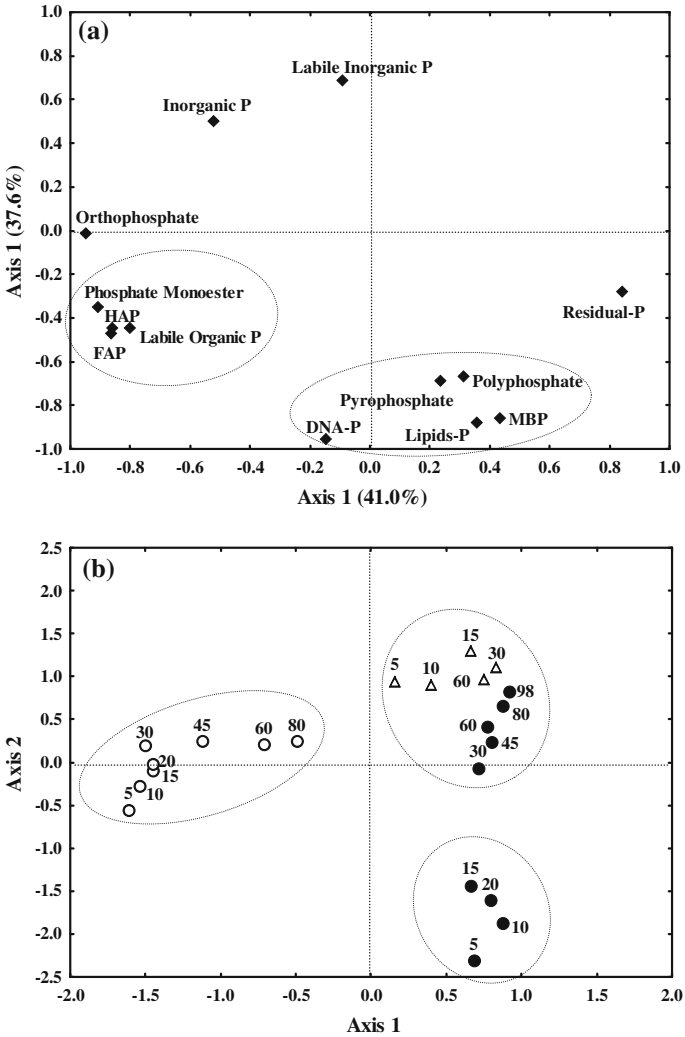

Fig. 6 Results of a principal component analysis, showing **a** loadings of the different P compounds measured by solution ^{31}P NMR spectroscopy and sequential fractionation ($n = 25$), and **b** the plot of the scores of the sites and sediment depth (*numbers* are cm) from Lake Annie (*open circles*), Lake Okeechobee (*open triangles*), and Lake Apopka (*closed circles*). *MBP* microbial P, *FAP* fulvic acid P (moderately labile organic P), *HAP* humic acid P (highly resistant organic P)

markedly for Lake Apopka (Table 5). In particular, values by NMR spectroscopy were higher than those by sequential fractionation for surface layers, but lower for deeper layers. Comparisons of P forms determined by the two different methods showed that orthophosphate determined by solution ^{31}P NMR spectroscopy was correlated positively with HCl-extractable inorganic P ($r = 0.68$, $p < 0.001$), fulvic acid P ($r = 0.82$, $p < 0.001$), and humic acid P ($r = 0.80$, $p < 0.001$) determined by sequential fractionation. Phosphomonoesters were correlated positively with fulvic acid P ($r = 0.94$, $p < 0.001$) and humic acid P ($r = 0.90$, $p < 0.001$), as expected from the dominance of

phosphomonoesters in these operationally defined extracts (Turner et al. 2006b). Microbial P was correlated positively with DNA-P ($r = 0.75$, $p < 0.001$).

A principal component analysis (PCA) was conducted to assess relationships between P forms in sediments from the three different lakes (Fig. 6). The PCA had 41.0 % of the data variability explained by Axis 1. Axis 2 explained 37.6 % of the data variability, and the selected variables were phospholipids, microbial P, and DNA (Fig. 6a). Orthophosphate, phosphomonoesters, labile organic P, fulvic acid P, humic acid P, and residual P were the variables selected by Axis 1. The results of the PCA showed that Axis 1 represented phosphomonoesters and Axis 2 phosphodiesters. The position of the sites and sediment depth in relation to the variables loadings in the PCA showed that the three lakes are separated into different groups (Fig. 6b). Lake Apopka surface sediments corresponded with the position of phosphodiester compounds (parameters selected by Axis 2) and Lake Annie corresponded with the position of phosphomonoester compounds (parameters selected by Axis 1). Lake Okeechobee was placed closer to the position of inorganic P forms (i.e., HCl-extractable and labile inorganic phosphate) and with deeper sediments of Lake Apopka (Fig. 6b).

4 Discussion

There were marked differences in the forms and concentrations of sediment P among three lakes of contrasting trophic status. Lake Okeechobee sediments were dominated by inorganic orthophosphate and contained low organic P concentrations. Phosphorus dynamics in Lake Okeechobee mud sediments are relatively well-studied, and it is known that iron phosphate precipitation controls the behavior of phosphate under oxidizing conditions, while calcium phosphate precipitation governs phosphate solubility under reducing conditions (Moore and Reddy 1994). Phosphorus solubility in this lake is also influenced by pH, with DRP concentrations in the water column being low at neutral pH (6.5 and 7.5), but higher under slightly acidic (pH 5.5) or basic (pH 8.5) conditions (Moore and Reddy 1994). Furthermore, the DRP concentration can increase exponentially following a decrease in redox potential (Olila and Reddy 1997). It therefore seems likely that P dynamics in Lake Okeechobee mud sediments are controlled by abiotic processes linked to changes in pH and redox potential (Moore and Reddy 1994; Olila and Reddy 1997).

In contrast to Lake Okeechobee, P dynamics in the Lake Annie appear to be regulated to a greater extent by biotic processes. Lake Annie sediments contained large concentrations of organic P to considerable depth, mainly in the form of phosphomonoesters. Lake Annie water originates from ground water (90 %) and direct rainfall (10 %), with little anthropogenic impact and negligible inputs from surface runoff (Swain and Gaiser 2005), so the relatively high total P concentration in the sediment is a natural phenomenon and reflects the low sediment bulk density. Lake Annie sediment is permanently anoxic and has the lowest pH of the three lakes studied ($\leq$5.7 in sediments and 5.1 in the water column), as well as high concentrations of metal oxides and clays (Thompson 1981). These factors may favor the stabilization of organic P, in particular phosphomonoesters such as the inositol phosphates (Celi and Barbaris 2005). Indeed, a marked difference in the organic P composition of the three lakes was that sediment from Lake Annie appeared to contain inositol phosphates (Fig. 5), as indicated by signals in the phosphomonoester region from *myo*- and *scyllo*-inositol hexakisphosphate, as well as signals upfield of orthophosphate that were recently assigned to *neo*- and D-*chiro*-inositol hexakisphosphate (Turner et al. 2012). Inositol phosphates are abundant in mineral soils and have been detected in a number of

Scandinavian lakes (Jørgensen et al. 2011). Their absence in subtropical wetland sediments in the Florida Everglades (Turner and Newman 2005; Turner et al. 2006a) does not appear to be due to anaerobic conditions (e.g., Suzumura and Kamatani 1995), because *myo*-inositol hexakisphosphate persisted for many years at depth in the anaerobic sediment of a Helsinki embayment (Turner and Weckström 2009). The results presented here therefore indicate that pH and mineral content are possible additional factors promoting the persistence of organic phosphorus in lake sediments.

In contrast to Lake Annie and Lake Okeechobee, in which sediment P was dominated by either organic or inorganic P, respectively, the sediment P composition in Lake Apopka appeared to be regulated in large part by the presence of a large microbial biomass concentration. This presumably reflects the high primary production and subsequent deposition of labile organic matter from algal biomass in sediment, fueling microbial activity. Gale and Reddy (1994) reported gross primary productivity in Lake Apopka of 1,400 g C m^{-2} $year^{-1}$ of which approximately 1,034 g C m^{-2} $year^{-1}$ was deposited in sediments. The Lake Apopka phytoplankton community is dominated by cyanobacteria (*Synechococcus* sp., *Synechocystis* sp., and *Microcystis incerta*), with little variation throughout the year (Carrick et al. 1993). Brunberg (1995) simulated a *Microcystis* sedimentation event in a eutrophic lake deposit and found that organic P was the dominant fraction in the cells (74 % of total P). After 15 days of incubation, most of the total P was transformed into labile (inorganic and organic) P and NaOH-extractable organic P.

In Lake Apopka's calcareous sediments, abiotic phosphate uptake and solubility are controlled by pH (Olila et al. 1995; Olila and Reddy 1997). Phosphorus release is associated with dissolution of calcium phosphate, and a six-fold increase in pore water DRP concentration occurred with a 0.5 decrease in pH (Olila et al. 1995; Olila and Reddy 1997). However, biotic P control is also important: Olila and Reddy (1997) reported a large increase in labile phosphate under highly reducing conditions and suggested it was caused by lysed microbial cells or degradation of stored polyphosphate (see also Gächter et al. 1988).

Almost 40 % of the total P in Lake Apopka surface sediments is contained within microbial biomass, and the presence of polyphosphate and pyrophosphate indicates the activity of microorganisms involved in biological P cycling (Hupfer et al. 2004; Ahlgren et al. 2005; Reitzel et al. 2006a). This is also reflected in the relatively high concentrations of phosphodiesters, particularly DNA, in Lake Apopka sediments. Although the microbial contribution to the total organic P was lower in the other two lakes studied, it has important implications for the use of solution ^{31}P NMR spectroscopy to calculate organic P degradation rates in sediment profiles (e.g., Ahlgren et al. 2006; Reitzel et al. 2007). These are likely to overestimate the true degradation rates, particularly for compounds such as DNA, phospholipids, and polyphosphates, which are abundant in microbial cells.

Lake Apopka was the only lake in which polyphosphate was detected. This compound has been detected in surface sediment of a large number of lakes (Hupfer et al. 1995, 2004; Carman et al. 2002; Reitzel et al. 2006a, b, 2007), although the mechanisms of its formation remain poorly understood (Hupfer et al. 2007). Although the absence of polyphosphate in the uppermost 5 cm of sediment may be a methodological artifact, a similar phenomenon has been reported elsewhere (Carman et al. 2002; Reitzel et al. 2006a, 2007). Bacteria can store polyphosphate if sufficient organic carbon and phosphate are available under alternating aerobic/anaerobic conditions (Gächter and Meyer 1993; Mino et al. 1998; Seviour et al. 2003), which probably accounts for differences in the occurrence of polyphosphate in the lakes studied here: Lake Apopka sediments contain large amounts of labile carbon and experience alternating aerobic–anaerobic conditions, whereas Lake

Annie sediments contain high organic carbon but are always anaerobic and Lake Okeechobee sediments undergo oscillating aerobic–anaerobic conditions but contain little labile organic carbon (Torres 2007). Similar results were reported for a series of Scandinavian lakes (Carman et al. 2002): high polyphosphate concentrations were detected in Lake Gömmaren, with well-oxidized sediments and high organic matter content; smaller concentrations of polyphosphate were detected in Lake Långsjön, with oscillating aerobic–anaerobic conditions; and no polyphosphate was detected in Lake Flaten, with constant anoxic conditions.

Based on a procedure involving heat extraction and colorimetric phosphate detection, Kenney et al. (2001) suggested that 25–90 % of the total P in Lake Apopka sediment may be sequestered in intact phytoplankton cells as polyphosphate, which they detected to 50 cm depth in the sediment. However, the heat-extractable P procedure used in that study is not specific to polyphosphate, so it is likely that various sources of P were included in the measurements. Furthermore, some studies reported significant correlations between bacterial biomass and NaOH-extractable organic P and suggested that the latter could be used as a proxy measure of bacterial polyphosphate (Uhlman and Bauer 1988; Waara et al. 1993; Goedkoop and Petterson 2000). Based on our results, this would markedly overestimate polyphosphate concentrations.

Several studies in Lake Apopka and Lake Okeechobee have indicated that the increase in total P concentration in upper sediment layers is the result of anthropogenic P inputs (Schelske et al. 2000; Kenney et al. 2002; Waters et al. 2005; Schottler and Engstrom 2006). However, a decrease in total P with sediment depth has been observed in many lakes unaffected by pollutant P (e.g., Søndergaard et al. 1996; Gonsiorczyk et al. 1998; Ahlgren et al. 2005) and may simply represent the recycling of sediment P following sediment deposition.

Although solution ^{31}P NMR spectroscopy yields information on the functional groups of organic P present in sediments, it does not provide information on the physical nature of the compounds within the sediment matrix. Principal component analysis revealed a link between the phosphomonoester pool and P associated with humic and fulvic acids (i.e., stable organic P) plus labile organic P. This is consistent with phosphomonoesters occurring in both labile (e.g., sugar phosphates, mononucleotides) and stable (e.g., inositol phosphates) forms in sediments (e.g., Turner and Weckström 2009; Jørgensen et al. 2011). In contrast, DNA and inorganic polyphosphates were correlated strongly with the microbial P pool. This suggests that these compounds are associated with microbial activity in the sediments or extracted directly from live microbial cells rather than occurring as extracellular compounds (Cheesman et al., 2012). The latter explanation might explain in part the rapid decline in phosphodiesters and polyphosphate with depth in the sediment profile in this and other studies (e.g., Ahlgren et al. 2005; Reitzel et al. 2007).

In general, organic P determined by sequential fractionation and solution ^{31}P NMR spectroscopy were similar for Lake Annie, but less so for Lake Apopka. This discrepancy arises because the values for total organic P determined by sequential fractionation do not include microbial P (which is extracted in a separate step of the procedure). In contrast, microbial P is included in the NaOH–EDTA extracts and measured by solution ^{31}P NMR spectroscopy in large part as phosphomonoesters and phosphodiesters, the dominant forms of P in microorganisms (Oberson and Joner 2005). Polyphosphate is also contained in some sediment microbes and is quantified separately by solution ^{31}P NMR spectroscopy, but is included in the organic P fraction by sequential fractionation (Turner et al. 2006b). If microbial P is added to the total organic P pool measured by chemical fractionation for Lake Apopka sediments, then total organic P values agree more closely with those from

solution ^{31}P NMR spectroscopy. A combination of procedures appears to provide a more detailed assessment of sediment P than either procedure alone (Reitzel et al. 2006b).

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