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ORIGINAL PAPER

Degradation rates of organic phosphorus in lake sediment

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Abstract Phosphorus (P) binding groups were identified in phytoplankton, settling particles, and sediment profiles by ^{31}P NMR spectroscopy from the Swedish mesotrophic Lake Erken. The ^{31}P NMR analysis revealed that polyphosphates and pyrophosphates were abundant in the water column, but rapidly mineralized in the sediment. Orthophosphate monoesters and teichoic acids degraded more slowly than DNA-P, polyphosphates, and P lipids. Humic acids and organic acids from phytoplankton were precipitated from the NaOH extract by acidification and identified by ^{31}P NMR spectroscopy. The precipitated P was significantly more recalcitrant than the P compound groups remaining in solution, but does not constitute a major sink of P as it did not reach a stable concentration with depth, which indicates that it may eventually be degraded. Since P also precipitated from phytoplankton, the origin of humic-P can not be related solely to allochthonous P.

Keywords Organic P · ^{31}P NMR · Lake sediment · Degradation rates

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Introduction

Organic phosphorus (P) constitutes a major P input to the sediment in most lakes (e.g. Pettersson 2001) and originates from both allochthonous and autochthonous sources. Autochthonous organic P originates from in-lake primary production, whereas allochthonous organic P is supplied by the catchment. The bulk of allochthonous organic matter is dominated by recalcitrant compounds such as humic substances (Fenchel et al. 1998), which generally provide between 40% and 80% of the organic matter found in natural waters, thus they are major constituents of the lake ecosystem (Thurman 1985; Wetzel 2001).

Humic acids are degradation products that mainly result from vascular plant tissue. Autolysis of microorganisms is another source of humic-like substances (Fenchel et al. 1998; Wetzel 2001) such as phenols, quinones, phenolic carboxylic acids, and related compounds. Since humic acids

are recalcitrant, they tend to accumulate in soils and sediments. Hence, P associated with humic acids may accumulate as well, though the information about the forms and degradability's of these P compounds is limited.

Humic acids are extracted by NaOH in standard sediment extraction procedures together with other organic P species. Separation of humic acids from the NaOH extract serves as an important tool in determining the importance of these potentially recalcitrant P compounds in sediments (Paludan and Jensen 1995; Jensen et al. 2005; Reitzel et al. 2005).

In an attempt to precipitate P associated with humic acids, Paludan and Jensen (1995) included acidification (pH ~1) of the NaOH extract by 2 M H_2SO_4 in the extraction scheme for freshwater sediments originally proposed by Psenner and Pucsko (1988). Paludan and Jensen (1995) found that the precipitate contained up to 30% of the total phosphorus (TP) in sediments from a wetland area rich in organic matter and from a humic rich Danish lake. They hypothesized that the precipitate could constitute a large part of the TP in other lake sediments as well. Later, this hypothesis was supported by Reitzel et al. (2003) and Hansen et al. (2003) who found that the precipitate constituted 13% and 20% of the TP in the sediment of two non-stained Danish lakes.

Ahlgren et al. (2005) determined various P compound groups and their degradation rates in the NaOH extract of a dated sediment profile in the Swedish Lake Erken. This was accomplished using ^{31}P Nuclear Magnetic Resonance (^{31}P NMR) spectroscopy, a technique that distinguishes different P compound groups based on specific resonance frequencies. Hence, this method differentiates between P bound as orthophosphate, orthophosphate monoesters, orthophosphate diesters, pyrophosphate, polyphosphates, and phosphonates.

In this study, NaOH extracts from phytoplankton, settling particles and a dated sediment profile were analyzed using ^{31}P NMR spectroscopy to follow the diagenesis of the NaOH extractable P compound groups in a time span ranging from assimilation in phytoplankton to P compound groups subjected to 100 years of diagenesis in the sediment. In parallel, it is demonstrated that humic compounds can be studied seperately from more labile P compounds by using ^{31}P NMR spectroscopy on the precipitate of humic like organic P compounds in the NaOH extract.

Materials and methods

Study site

The mesotrophic Lake Erken (59°51′ N, 18°35′ E) is located in the south eastern part of Sweden, and has a surface area of 2400 ha and a maximum depth of 21 m, with an average depth of 9 m. The lake has been mesotrophic since measurements started in 1930 as the lake is situated on nutrient rich glacial and post glacial clay deposits. Summer average total P (TP) measured above the thermocline is 27 μg l^{-1} (range: 15–60 μg l^{-1}). Summer average pH is 8.2 (range: 8.0–8.5).

The study site was located at an accumulation bottom 18 m deep, situated in the southern part of the lake (59°50′25.0″ N 18°33′29.6″ E). Due to the small and constant number of inhabitants in the catchment area, 10% of which is low intensity farmland area, the lake is at equilibrium with the external load of P and organic matter, and thus serves as a suitable system for studying diagenetic transformations. The lake stratifies during the summer period, resulting in anoxia during this period. However, at the time of sampling the sediment surface was oxic.

Analyses of sediment, phytoplankton, and settling seston

Ten undisturbed sediment cores were collected in May 2004 with a sediment gravity core sampler. The sediment cores were brought to the laboratory, where they were sliced and pooled in 1 cm intervals for the upper 3 cm of the sediment. About 1-cm thick samples were also collected from the 5–6 cm and 9–10 cm depth intervals and for every 5 cm down to 40 cm depth.

In September 2004, four sediment traps were deployed to collect settling material at 8 m depth and 15 m depth, respectively, at a sampling site

positioned at 18 m depth. Thus, four traps (8 m) were situated in the thermocline, whereas four traps were situated 3 m above the sediment (15 m). As no inhibitors were added to the traps, changes in P groups integrate the effects of aging, chemical degradation, and microbial remineralization. The traps were emptied after 30 days and the contents immediately processed in the laboratory. Phytoplankton was collected, in amounts high enough for ^{31}P NMR analysis, with a 40 μm plankton net in the upper 1 m of the lake on the day that the sediment traps were deployed.

The extraction scheme proposed by Psenner and Pucsko (1988) and modified by Paludan and Jensen (1995) for extraction of humic acids was followed, but the solid to solvent ratio was increased from 1:25 to 1:3 in order to obtain extract concentrations high enough for ^{31}P NMR analysis. The phytoplankton, settling particles, and sediment samples were pre-extracted in bicarbonate buffered dithionite solutions (BD) for 1 h to remove porewater P and P bound to reducible metals such as iron (Fe) and manganese (Mn). After the pre-extraction, the samples were separated from the BD solution by centrifugation at 4000 rpm for 10 min. The remaining solid sample was extracted in 0.1 M NaOH for 16 h at room temperature and the NaOH extract was collected following centrifugation. The NaOH extracts were divided into two subsamples: (1) a subsample for measurement of the TP concentration in the NaOH extract and ^{31}P NMR analysis, (2) a subsample for precipitation with 2 M H_2SO_4, and subsequently analysis of ^{31}P NMR, TP, TC, and TN.

The subsample for precipitation of humic acids were acidified to pH ~1 (Paludan and Jensen 1995) with 2 M H_2SO_4 and the precipitate was separated from the remaining solution by centrifugation, after 48 h of precipitation. The precipitate was washed in acidified water to avoid readsorption of P and subsequently centrifuged. The precipitate was collected and redissolved in 0.1 M NaOH in preparation for TP and ^{31}P NMR analyses.

Dry weight (DW) was measured by freeze drying until constant weight on all subsamples and on the precipitate from subsample 2. Loss on ignition (LOI) was measured on the same samples by combusting the freeze-dried samples for 6 h at 550°C.

Total carbon and total nitrogen were measured by gas chromatography with a NA 1500 Nitrogen/Carbon/Sulphur analyzer. Total P in phytoplankton, settling particles, and sediment was measured colorimetrically on combusted samples, according to Koroleff (1983). Total P in the extracts was measured by inductively coupled plasma (ICP) spectroscopy on a Spectroflame P from Spectro Analytical Instruments.

Sediment dating

Sediment accumulation rates were calculated after measuring the ^{137}Cesium (Cs) activity, on a sediment core sectioned into 1 cm intervals. ^{137}Cs activity was measured using an Intertechnique Model 4000 Gamma counting system equipped with a sodium iodine well detector. The layer with peak activity was assumed to represent the Chernobyl accident in 1986 and the amount of dry matter in above layers was divided by the number of years between 1986 and 2004 (18 years) to gain the average yearly deposition of matter.

^{31} P NMR analysis

Samples for the ^{31}P NMR analysis were preconcentrated (~10 times) by rotary evaporation (Hupfer et al. 2004). After concentration, the samples were frozen until analysis, a process which does not affect the composition of P compound groups (Hupfer et al. 2004). Prior to ^{31}P NMR analysis, the samples were centrifuged at 15,500 rpm for 10 min and 50 μl of BD solution was added to 1000 μl of the sample to reduce interference from potential remaining paramagnetic ions such as Fe(III) and Manganese (II) (Cade-Menun and Preston 1996). In order to obtain a stable lock signal, ~10% of D_2O was added to the sample. Peaks were assigned using standard solutions added to one of the sediment extracts ($Na_2HPO_4 \cdot 7H_2O$ for orthophosphate and $Na_2P_2O_7 \cdot 10H_2O$ for pyrophosphate) and comparisons with literature. The ^{31}P NMR spectra were measured at 121.5 MHz on a Varian MercuryPlus NMR spectrometer at ambient temperature. Spectra were recorded using a 63°

observe pulse, acquisition time 0.4 s, and relaxation delay 1.2 s, acquiring around 30,000 transients (12 h). Chemical shifts were indirectly referenced to external 85% H_3PO_4 (at $\delta = 0.0$) via the lock signal. To obtain peak areas, peaks in the raw spectrum with a signal to noise ratio exceeding 4, were fitted with Lorentzian line shapes using the deconvolution subroutine of the NMR software (Vnmr 6.1C). From these peak areas, the contribution of the individual P compound groups was calculated relative to the TP in the extracts. Spectra were plotted with a line broadening of 10 Hz.

Spectra were obtained from the precipitate and NaOH extract prior to precipitation, but it was not possible to obtain spectra from the NaOH supernatant (NaOH extract after acidification with H_2SO_4) due to line broadening. Line broadening of the supernatant was also found by Baldwin (1996), who clamed it to be a result of high ion strength in the supernatant. Therefore, the concentration of the supernatant was determined by subtracting the concentration of the precipitate from the concentration of the NaOH extract measured prior to precipitation. In this paper, the supernatant is used for the comparison of degradation rates with the precipitate while the NaOH extract without precipitation is used for describing diagenesis in the sediment profile and water column samples.

Statistical analyses

To determine differences in degradation rates of P compound groups in the supernatant and the precipitate, the relationship between P compound groups and age in the supernatant and precipitate from the sediment profile was evaluated by regression analysis. Analysis of four different regressions ($y = ax + b$, $y = a*e^{(-x*t)}$, $\ln y = ax + b$, $\ln y = a*e^{(-x*t)}$) was performed to optimize the best fit for both precipitate and supernatant simultaneously, as evaluated by the adjusted r^2-value. Subsequently the significance of the obtained regression was tested. The exponential decay regression ($y = a*e^{(-x*t)}$) was found to give the best fit. For the P compound groups where significant regressions for both precipitate and supernatant were found, a comparison of slopes was performed using Tukey's HSD test (Zar 1984). In all cases a significance level of $\alpha = 0.05$ was used.

Results

Sediment, phytoplankton, and settling seston

Table 1 presents the sediment characteristics of Lake Erken. In general, the TP in the sediment, LOI, TP in the supernatant, TP in the precipitate, TP extracted, and the proportion of TP extracted showed a decreasing trend with increasing sediment depth and water depth. The percentage of extracted P in the precipitate was lower (19%) in the phytoplankton sample, compared to the sample from the 15 m trap, which contained 81% of extracted P as precipitate. The same contribution in the sediment increased from 8% in the upper sediment layer to around 27% below 25 cm in depth. Based on the ratios between C, N, and P in the precipitate (Fig. 1), it was determined that P decreased faster than both C and N with increasing sediment depth.

Sediment dating

The activity of ^{137}Cs peaked in the 11–12 cm layer. The average deposition since 1986 was calculated to be 11.86 kg m^{-2} (656 g dry matter m^{-2} $year^{-1}$) which corresponds to a sedimentation rate of 10.4 mm $year^{-1}$. This average yearly deposition of matter corresponds well with the deposition rates found by Weyhenmeyer et al. (1996) who estimated an average deposition of 811 g dry matter m^{-2} $year^{-1}$ in accumulation areas of Lake Erken. Assuming a steady state, with a similar sedimentation of organic matter every year, the deepest sediment layer (39–40 cm) sampled here was determined to be 97-years old.

^{31} P NMR analysis

NaOH extract

The ^{31}P NMR analysis revealed eight different P compound groups in the NaOH extract Turner et al. (2003) from the settling particles and the

Table 1 Sediment characteristics for Lake Erken

Depth	Age (years)	LOI (%)	TP[a]	TP[a] NaOH extracted	TP[a] supernatant	TP NaOH extracted (%)	TP in precipitate[a]	TP precipitated (%)[b]	LOI Precipitate (%)[c]
Phytoplankton	n.d	43.0	144.6	89.3	72.4	62	16.9	18.9	n.d
8 m trap	n.d	20.4	64.9	20.6	13.4	32	7.2	35.0	n.d
15 m trap	n.d	21.6	62.6	11.3	2.2	18	9.1	80.5	n.d
0–1 cm	1	21.3	78.1	42.7	38.4	55	4.3	8.0	49.4
1–2 cm	2	21.3	64.1	26.4	21.3	41	5.1	14.8	50.4
2–3 cm	3	20.7	56.7	21.5	15.4	38	6.1	15.8	48.9
5–6 cm	8	19.7	42.5	17.1	12.8	40	4.3	17.5	48.5
9–10 cm	14	18.5	38.5	12.1	8.6	31	3.5	20.7	45.7
14–15 cm	24	17.2	31.5	9.0	5.5	29	3.5	21.1	41.2
19–20 cm	36	16.3	29.3	9.1	5.7	31	3.4	21.7	43.5
24–25 cm	48	15.0	29.3	6.8	3.9	23	2.9	27.9	41.8
29–30 cm	62	14.2	28.1	6.0	4.1	21	1.9	26.7	41.5
34–35 cm	78	14.1	29.4	4.5	2.2	15	2.3	27.3	41.5
39–40 cm	97	14.1	27.9	5.0	3.7	18	1.3	26.0	36.0

Sediment was sampled May 2004. Phytoplankton and settling particles were sampled September 2004. The designation n.d. denotes that no data was obtained

[a] μmol g^{-1}DW

[b] Percentage precipitated from the NaOH extract

[c] LOI of the precipitate

sediment, however only six P compound groups were found in the phytoplankton extract (Table 2; Fig. 2A). Orthophosphate, orthophosphate monoesters, a P compound group which could be teichoic acids (Makarov et al. 2002), P lipids, DNA–P, and pyrophosphate were all measured in the phytoplankton, settling particles, and the sediment.

Polyphosphate end-groups and polyphosphate middle-groups were below the detection limit in the phytoplankton. In the water column, the concentration of the P compound groups tended to be lowest in the 15 m sediment trap. The concentration of P compound groups in the sediment traps tended to be lower than in the phytoplankton (Table 2). The only exception was the amount of polyphosphate end-groups in the water samples, which were lower in the phytoplankton compared to the 15 m sediment trap.

In the sediment profile, orthophosphate was the dominant P form in the surface sediment, followed by orthophosphate monoesters, polyphosphates,

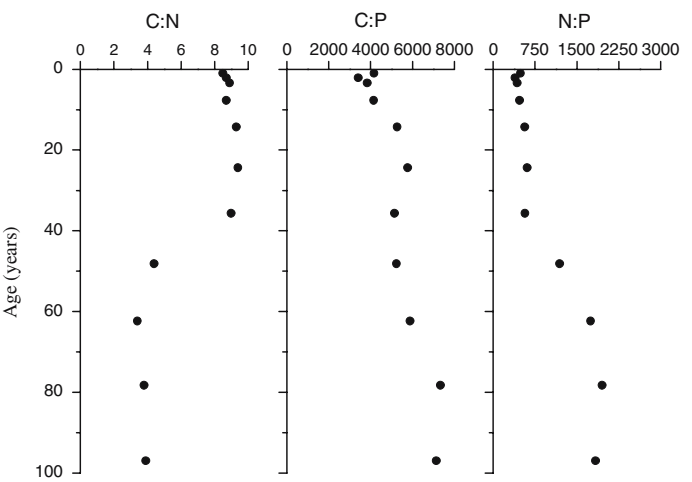

Fig. 1 Molar ratios between C, N, and P in the precipitate from Lake Erken

Table 2 Contributions (% of total extracted P) of P compound groups in the NaOH extract, and their amounts (μmol g^{-1} DW) in parentheses

Compound groups	Orthophosphate	Orthophosphate monoesters	Orthophosphate diesters			Pyrophosphate	Polyphosphates	
Possible compounds			Teichoic acids	P lipids	DNA–P		Poly-P (end)	Poly-P (mid)
Phytoplankton	36 (32.0)	30 (27.1)	1 (0.5)	8 (7.2)	16 (14.2)	9 (8.2)	b.d.l.	b.d.l.
8 m trap	29 (6.1)	13 (2.7)	2 (0.4)	4 (0.9)	14 (3.0)	16 (3.4)	9 (1.9)	11 (2.3)
15 m trap	20 (2.3)	13 (1.5)	2 (0.2)	4 (0.5)	13 (1.4)	11 (1.2)	20 (2.2)	17 (1.9)
0–1 cm	54 (23.2)	13 (5.6)	1 (0.3)	3 (1.3)	8 (3.4)	6 (2.4)	9 (3.9)	6 (2.6)
1–2 cm	30 (8.0)	24 (6.5)	2 (0.6)	7 (1.9)	12 (3.2)	5 (1.4)	11 (2.8)	8 (2.1)
2–3 cm	28 (6.1)	29 (6.1)	3 (0.6)	6 (1.3)	17 (3.6)	4 (0.8)	7 (1.5)	7 (1.5)
5–6 cm	27 (4.6)	29 (5.0)	3 (0.4)	9 (1.5)	19 (3.2)	4 (0.7)	5 (0.9)	5 (0.8)
9–10 cm	23 (2.8)	36 (4.4)	5 (0.6)	9 (1.1)	18 (2.2)	5 (0.6)	3 (0.4)	b.d.l.
14–15 cm	23 (2.1)	41 (3.7)	5 (0.5)	7 (0.6)	18 (1.6)	5 (0.5)	b.d.l.	b.d.l.
19–20 cm	32 (3.0)	40 (3.6)	5 (0.4)	6 (0.6)	14 (1.3)	3 (0.3)	b.d.l.	b.d.l.
24–25 cm	25 (1.7)	40 (2.8)	5 (0.4)	13 (0.9)	15 (1.1)	b.d.l.	b.d.l.	b.d.l.
29–30 cm	37 (2.3)	41 (2.5)	5 (0.3)	6 (0.3)	12 (0.7)	b.d.l.	b.d.l.	b.d.l.
34–35 cm	30 (1.3)	41 (1.8)	5 (0.2)	10 (0.4)	14 (0.6)	b.d.l.	b.d.l.	b.d.l.
39–40 cm	50 (2.5)	35 (1.8)	5 (0.3)	b.d.l.	10 (0.5)	b.d.l.	b.d.l.	b.d.l.

Values below the detection limit are designated (b.d.l.)

and orthophosphate diesters in the form of DNA-P (Figs. 3, 4A, Tables 2). Orthophosphate constituted more than 50% of the P compound groups in the surface sediment, but decreased to a more constant fraction of 20% to 30% of the P compound groups, with the exception of a fraction of 50% orthophosphate in the oldest sediment layer (Fig. 4A). Below a sediment depth of 2 cm, the orthophosphate monoesters were the dominant P compound group, showing an increasing trend with depth to ~40% of the P extracted in the deeper sediment layers. The contribution of teichoic acids was lowest in the surface sediment and increased to 5% in the sediment layers below 10 cm (Fig. 4A). No obvious trend in P lipids was identified, but the concentration was below the detection limit in the deepest sediment layer. The contribution of DNA–P showed a slight decrease with increasing sediment depth, as well as the contributions of pyrophosphate and polyphosphates. The pyrophosphate and polyphosphates were not detectable in the sediment below 20 cm (pyrophosphate), 6 cm (polyphosphate middle-groups), and 10 cm (polyphosphate end-group).

Precipitate

In general, the major constituents of the precipitate in the water column samples were orthophosphate monoesters and DNA-P whereas less than 10% of the orthophosphate from the NaOH extract was precipitated (Tables 2, 3). The polyphosphate end-groups precipitated increased in concentration (Table 3) from the phytoplankton to the settling particles in the 15 m trap. Polyphosphate middle-groups and teichoic acids were not precipitated in detectable concentrations from the phytoplankton sample. However, both of these P compound groups were present in the samples extracted from settling particles, whereas only polyphosphate was precipitated in the extracts from the sediment (Table 3; Fig. 2B).

In general, all of the precipitated P compound groups decreased in concentration with increasing sediment depth (Table 3; Fig. 5), but orthophosphate monoesters and teichoic acids increased in their relative contribution to the precipitate (Fig. 4B). DNA–P, pyrophosphate and polyphosphate end-groups all decreased in their relative contributions while the relative contribution of orthophosphate remained fairly constant with depth (Fig. 4B). No P lipids and polyphosphate middle-groups were precipitated (Fig. 2B) in the extracts from the sediment profile.

By comparing the slopes from the exponential regressions of the supernatant and the precipitate in the sediment profile, orthophosphate monoesters, DNA-P and TP were found to have a

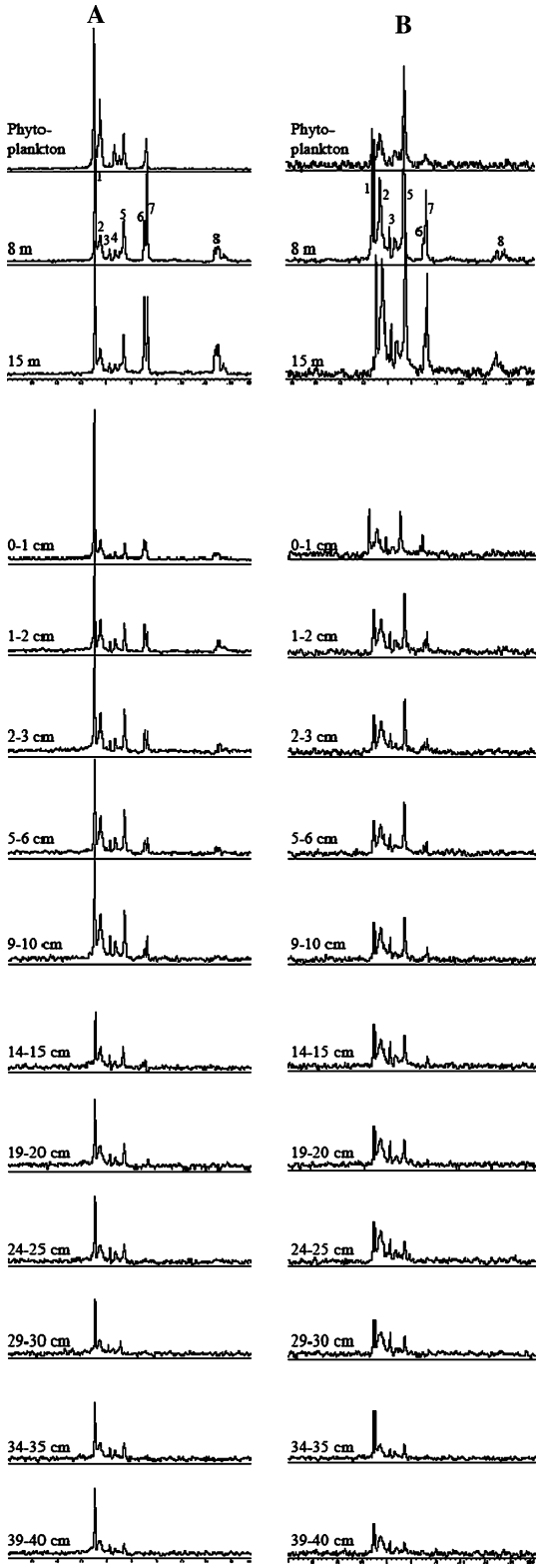

Fig. 2 ^{31}P NMR spectra of the NaOH extracts (**A**) and the precipitate (**B**). Numbers denotes position of chemical shifts: 1) orthophosphate, 2) orthophosphate monoesters, 3) teichoic acids, 4) P lipids, 5) DNA-P, 6) polyphosphate end-groups, 7) pyrophosphate, 8) polyphosphate middle-groups

significantly higher decay rate in the supernatant relative to the precipitate (Table 4).

Discussion

Total P concentrations along with the various P compound groups in phytoplankton, settling particles and the sediment showed a decreasing trend with increasing age, indicating mineralization of P (Tables 1, 2). However, the relative contribution of the different P compound groups showed distinct patterns, revealing different degradation rates (Tables 2, 4; Fig. 4A, B). For the orthophosphate monoesters, DNA-P, and TP there were significantly lower degradation rates in the precipitate relative to the supernatant, demonstrating that the precipitate in general isolated the more recalcitrant constituents of the organic P compound groups (Table 4). However, our results did not prove that the precipitate constitutes a permanent sink for P, as the concentration of the precipitate did not reach an obvious stable level with sediment age. Still, it is evident that the precipitate makes up a significantly more recalcitrant part of the sediment P compared to the supernatant.

Water column samples

Differences in P compound groups were identified between the phytoplankton and the settling particles (Table 2). Indications of a rapid initial degradation of P compound groups in the water column was present, although our results can not definitively clarify this, as the origin of the trap material may not nessecarily be identical to the phytoplankton sampled, due to e.g., resuspension and selective regeneration of P in settling particles. Lower percentages of orthophosphate, orthophosphate monoester, P lipids, DNA-P, and

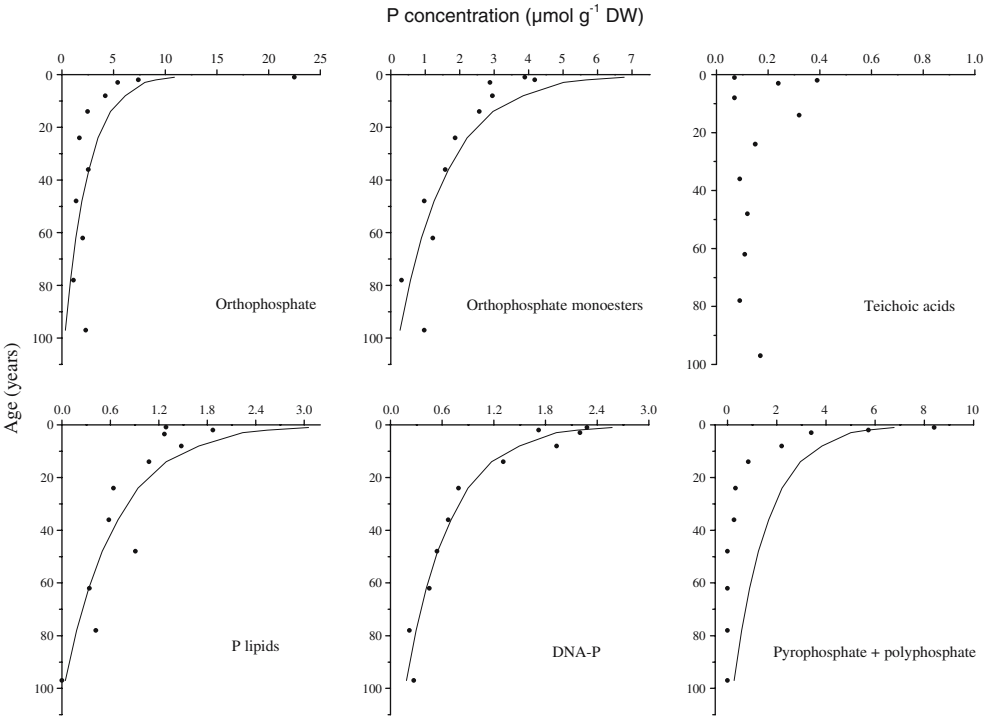

Fig. 3 Sediment phosphorous profiles and regressions (solid lines) of the P compound groups in the supernatant

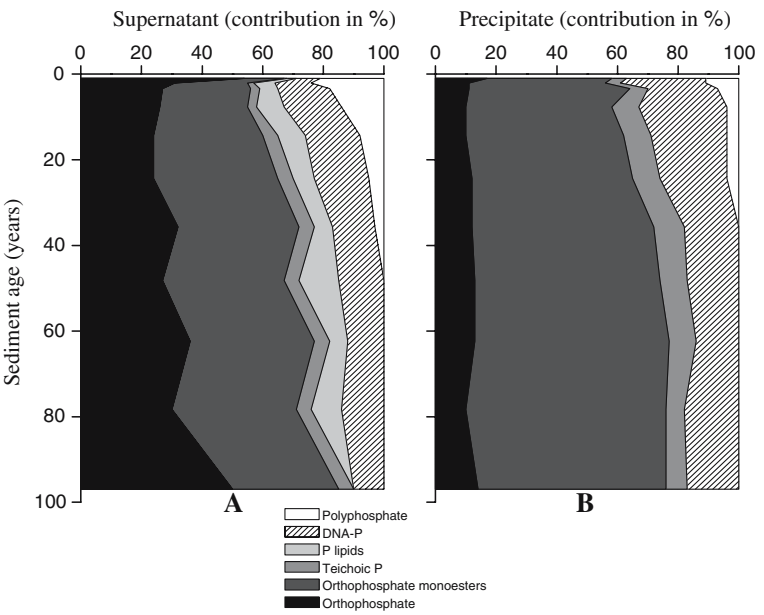

Fig. 4 (**A**) Shows the contribution of the P compound groups in the supernatant, whereas (**B**) shows the contribution of the P compound groups in the precipitate. Polyphosphates and pyrophosphates are combined as polyphosphates

correspondingly higher percentages of pyrophosphates and polyphosphates, were observed in the settling particles relative to the phytoplankton. Since no polyphosphates and a minimal amount of pyrophosphate were observed in the phytoplankton sample, the increasing fractions of these compounds from the 8 m trap to the 15 m trap indicate polyphosphates and pyrophosphate originating from the bacterial community colonizing and mineralizing the settling particles.

Table 3 Contribution (% of total precipitated P) of P compound groups in the precipitate and their amounts (μmol g^{-1} DW) in parentheses

Compound groups	Orthophosphate	Orthophosphate monoesters	Orthophosphate diesters			Pyrophosphate	Polyphosphates	
Possible compounds			Teichoic acids	P lipids	DNA–P		Poly-P (end)	Poly-P(mid)
Phytoplankton	10 (1.6)	27 (4.6)	b.d.l.	11 (1.8)	45 (7.7)	7 (1.1)	b.d.l	b.d.l
8 m trap	14 (1.0)	31 (2.3)	3 (0.2)	8 (0.6)	25 (1.8)	9 (0.7)	3 (0.2)	6 (0.4)
15 m trap	11 (1.0)	32 (1.5)	4 (0.2)	10 (0.5)	25 (1.4)	9 (0.8)	4 (0.4)	7 (0.6)
0–1 cm	17 (0.6)	41 (1.4)	5 (0.2)	b.d.l	26 (0.9)	6 (0.2)	5 (0.2)	b.d.l
1–2 cm	11 (0.4)	45 (1.7)	5 (0.2)	b.d.l	29 (1.1)	7 (0.3)	4 (0.2)	b.d.l
2–3 cm	11 (0.4)	53 (1.8)	6 (0.2)	b.d.l	23 (0.8)	3 (0.1)	4 (0.1)	b.d.l
5–6 cm	10 (0.3)	48 (1.4)	9 (0.3)	b.d.l	29 (0.9)	4 (0.1)	b.d.l	b.d.l
9–10 cm	9 (0.2)	52 (1.3)	9 (0.2)	b.d.l	25 (0.6)	4 (0.1)	b.d.l	b.d.l
14–15 cm	12 (0.2)	52 (1.0)	9 (0.2)	b.d.l	22 (0.4)	4 (0.1)	b.d.l	b.d.l
19–20 cm	12 (0.2)	60 (1.2)	10 (0.2)	b.d.l	18 (0.4)	b.d.l	b.d.l	b.d.l
24–25 cm	13 (0.2)	61 (1.2)	9 (0.2)	b.d.l	17 (0.3)	b.d.l	b.d.l	b.d.l
29–30 cm	13 (0.2)	64 (1.0)	9 (0.1)	b.d.l	14 (0.2)	b.d.l	b.d.l	b.d.l
34–35 cm	10 (0.1)	66 (0.8)	6 (0.1)	b.d.l	18 (0.2)	b.d.l	b.d.l	b.d.l
39–40 cm	15 (0.2)	62 (0.8)	7 (0.1)	b.d.l	17 (0.2)	b.d.l	b.d.l	b.d.l

Values below the detection limit are designated b.d.l

However, it should be noted that the 15 m trap, to some extent, may reflect resuspended surface sediment (Weyhenmeyer 1996; Pettersson 2001). However, low amounts of polyphosphates in the surface sediment in this period (unpublished data) may indicate that the sediment was not the source of polyphosphate.

Among the samples from the water column, the greatest difference in P composition was observed between the phytoplankton sample and the settling particles in the 8 m trap. If the origin of settling particles is similar to the sampled phytoplankton, this indicate that early diagenesis in the water column is a rapid process through

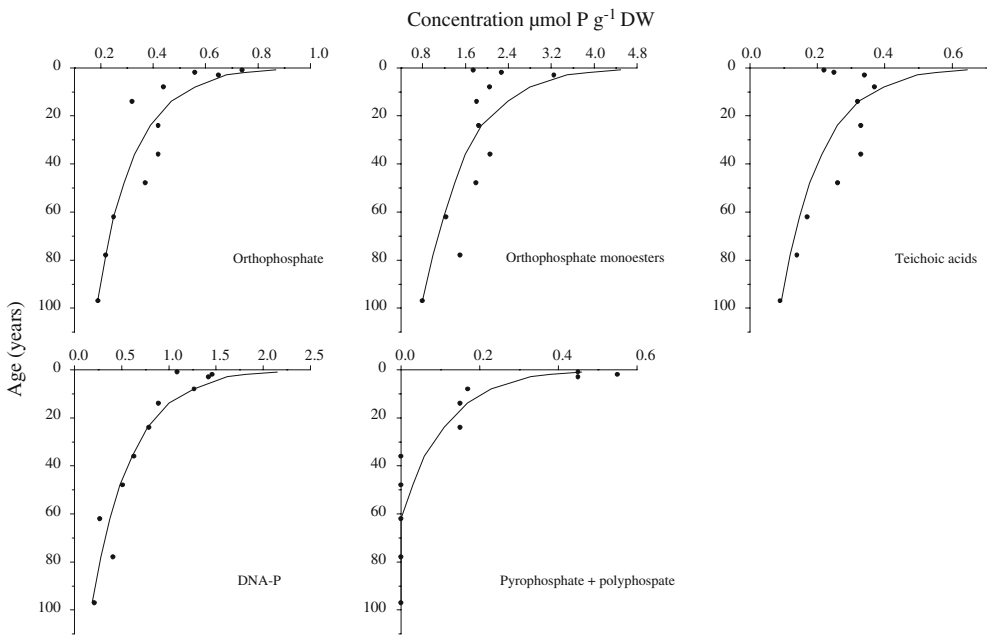

Fig. 5 Sediment phosphorous profiles and regressions (solid lines) of the P compound groups in the precipitate

Table 4 Regression equations, half-life times ($T_{1/2}$ (years)) and comparison of regressions between slopes in supernatant and precipitate

P compound group	Supernatant			Precipitate			Supernatant versus precipitate
	$T_{1/2}$	R^2	Regression	$T_{1/2}$	R^2	Regression	
Orthophosphate	1	0.85	*	50	0.59	*	NS
Orthophosphate monoesters	29	0.89	*	87	0.75	*	*
Teichoic acids			NS	88	0.64	*	NS
DNA–P	22	0.93	*	30	0.89	*	*
Poly P	2	0.93	*	6	0.80	*	NS
TP	6	0.75	*	54	0.81	*	*

* Indicates significance ($P < 0.05$). NS denotes that the regression was not significant

which more than 50% of the TP is released before the settling material reaches the 8 m trap. Moreover, if the data from the water column are representative, they indicate that the settling material is processed, so that the fraction of precipitate in the NaOH extract increases with water depth. Thus, 80% of the P compound groups in the NaOH extract were precipitated in the 15 m trap (Table 1).

Sediment profile

The sediment consisted of a variety of different P compound groups including organic as well as inorganic P. In addition to the P compound groups detected by Ahlgren et al. (2005) with ^{31}P NMR (orthophosphate, orthophosphate monoesters, orthophosphate diesters, and pyrophosphate), teichoic acids, P lipids, and polyphosphates were discovered, as well, most likely due to improved instrumentation (i.e. new ^{31}P NMR instrument, which is more sensitive) and the use of a BD pre-extraction prior to the NaOH extraction. In the alkaline NaOH extract, orthophosphate reflects P exchangeable with OH^-, P hydrolyzed from labile organic P species, and vacuolar orthophosphate (Paytan et al. 2001).

The discovery of polyphosphates in the sediment from Lake Erken could be a result of the BD pre-extraction step which removes paramagnetic Fe^{3+} and possibly Ca^{2+} (Jensen et al. 1998) prior to the extraction in NaOH. This possibility is based on e.g., Hupfer's et al. (2004) suggesting that the degradation of polyphosphates in the NaOH extract is believed to be caused by the action of free ions (e.g., Ca^{2+}), which may catalyze the breakdown of polyphosphates to pyrophosphates (Van Wazer 1958). Thus, pre-extraction with BD may result in an enhanced preservation of the polyphosphates in the NaOH extract. Despite the use of the BD pre-extraction, some of the pyrophosphate measured may still originate from degradation of polyphosphates in sediments with high Ca^{2+} concentrations.

As the concentration of the P compound groups declined with increasing depth, the increased contribution of the orthophosphate monoesters and the teichoic acids indicate that these P compound groups are degraded more slowly relative to the DNA–P, pyrophosphate, and polyphosphate or that orthophosphate monoesters and teichoic acids are degradation products of other P compound groups, such as for instance orthophosphate diesters.

The highly inconsistent pattern of the P lipids makes it difficult to predict their role in the sediment. Makarov et al. (2002) found P lipids to be labile P compound groups in soil extracts, which may be the case in lake sediments as well. Furthermore, since P lipids are essentially hydrophobic compounds, the extraction of P lipids with the aqueous NaOH is probably low, and as a consequence P lipids may be underestimated in the NaOH extract.

The polyphosphates and pyrophosphates rapidly declined with increasing sediment depth, supporting the findings by Ahlgren et al. (2005) who estimated half-life times of ~10 years for pyrophosphate in Lake Erken sediments. These findings agree with Gächter et al. (1988) who stated that polyphosphates can be synthesized by algae, bacteria, and fungi in response to oxic

conditions and favourable nutrient concentrations, conditions which prevail in the surface sediment.

Polyphosphates and pyrophosphates comprised more than 45% of the non-reactive P (nrP) in the surface sediment based on the assumption that orthophosphate is the only molybdenum reactive P compound group in the NaOH extracts. Obviously, the polyphosphates and pyrophosphates play an important role in the sediment P release, as can be deduced from the rapid decline in the uppermost sediment layers. This finding is in agreement with the results of Hupfer et al. (2004) and Ahlgren et al. (2005). As a result, these inorganic P compound groups must be considered as highly labile constituents of the NaOH nrP which is consistent with the findings of Törnblom and Rydin (1998) and Pettersson (2001). The findings of highly labile polyphosphates are in sharp contrast to the results presented by Kenney et al. (2001), who claimed polyphosphates to be suitable indicators of eutrophication, as these P compounds should be chemically inert. Based on our findings, their conclusion can not be supported.

Precipitate

Humic substances are complex mixtures of organic compounds, thus no clear definition of origin or degradability can be provided (Perdue 1998). The mechanism of precipitation is not fully understood, but a probable explanation is that the NaOH solution extracts charged organic compounds which remain in suspension due to intermolecular repulsions. When acid is added to the NaOH extract, it will neutralize the negatively charged organic compounds and Van der Waals forces will attract the compounds, which then form colloids and precipitate due to size (Wetzel 2001).

Figures 3 and 5 show that none of the P compound groups in either the supernatant or the precipitate can be considered strictly recalcitrant, since the concentrations decrease with increasing sediment depth. However, there is a tendency for the teichoic acids in the supernatant to remain fairly stable with age. Whether this P compound group are in fact recalcitrant to degradation, or produced by bacteria, Grant (1979) can not be concluded from this study. When considering the relative proportion of the P compound groups with increasing sediment depth, some general trends can be identified (Fig. 4A, B). The low amount of orthophosphates found in the precipitate supports the theory of precipitation due to size. Furthermore, the relatively constant percentage of orthophosphate with depth in the precipitate could indicate orthophosphate trapped in the hydrophobic core of humic colloids or it could reflect orthophosphate in intracellular compartments, as suggested by Paytan et al. (2003). A third possibility is that the orthophosphate found in the precipitate is sorbed to metals which are complexed in the humic material. Finally, the orthophosphate could be an artifact reflecting P adsorbed to the precipitate during the precipitation process. However, Jensen et al. (2005) spiked sediment samples with ^{32}P and concluded that the P associated with the precipitate was not an artifact, since the precipitate did not contain significant amount of ^{32}P.

The proportions of orthophosphate monoesters and DNA–P were high in the precipitate (Fig. 4B), indicating that these P compound groups are part of the molecules constituting the humic matrix, supporting findings by Bedrock et al. (1994). The relative accumulation of orthophosphate monoesters indicate that the diagenesis of this P compound group is slower than the diagenesis of the DNA-P, thereby supporting literature findings from soil science stating that orthophosphate monoesters can be considered more recalcitrant than orthophosphate diesters (Makarov et al. 2002).

Phytic acid, an orthophosphate monoester, is suggested to be a major constituent of organic P in many sediment and soil types (e.g. Cosgrove 1967; Thurman 1985; Degroot and Golterman 1993; Turner et al. 2002) and possibly a major constituent of the humic matrix. Suzumura and Kamatani (1993) suggested that phytic acids were associated with humic acids and consequently protected from degradation. Thus, phytic acids may be a major constituent of the orthophosphate monoesters in the NaOH extract.

Teichoic acids are found in gram positive bacteria (Makarov et al. 2002) where they can

constitute up to 85% of the total P in the cell wall. The teichoic acids constituted a minor part of the P in the NaOH extract, but the relatively high percentage precipitated from the NaOH extract indicates that this P compound group could be part of the humic P (Tables 2, 4; Fig. 4B).

The increased contribution of the teichoic acids with increasing depth in the precipitate indicates that the diagenesis of this P compound group is relatively slow.

The presence of pyrophosphates and polyphosphates in the precipitate was unexpected since these compounds are regarded as small highly labile P compound groups (Hupfer et al. 2004; Ahlgren et al. 2005). The presence of polyphosphate end-groups, but no polyphosphate middle-groups, in the precipitate reveals the absence of long chained polyphosphates in the precipitate. According to Wetzel (2001), an important property of humic colloids is their ability to associate with organic and inorganic materials via adsorption or peptization. Therefore, it seems likely that the low molecular weight compounds precipitated reflect P compound groups incorporated in or associated with larger compounds.

Comparison of the regression slopes of the supernatant and the precipitate revealed significant differences between orthophosphate monoesters, DNA–P, and TP (Table 4). A significant difference in the decay rates of the P compound groups is evident and indicates more recalcitrant P compound groups in the precipitate than in the supernatant. The results show that the use of exponential regressions are highly suitable in explaining the decline in concentration of the organic P compound groups in the supernatant, indicating that a first order exponential decay, most likely caused by the microbial community, is responsible for the diagenesis.

The most apparent difference in degradability occured in the orthophosphate monoesters and TP, where the half life time ($T_{1/2}$ (years)) is around 3–9 times higher in the precipitate than the supernatant (Table 4), documenting that the precipitation of humic acids leads to a separation of more recalcitrant P compound groups.

The $T_{1/2}$ rates found in this study were in general comparble to the $T_{1/2}$ rates found in Lake Erken by Ahlgren et al. (2005), with the relative degradation rates of the P compound groups being similar. Thus, orthophosphate monoesters had higher $T_{1/2}$ rates compared to DNA–P, whereas pyrophosphate/polyphosphate had the lowest degradation rates.

The C:P ratio and the N:P ratio in the precipitate both increased throughout the sediment profile indicating that P is lost from the precipitate more quickly than C and N (Fig. 1). A mechanism for this could be selective mineralization. As mentioned by Perdue (1998) and Wetzel (2001), plant-derived humic matter generally contains low amounts of N and consequently displays high C:N ratios (~50:1). Thus, the precipitate can not be true allochthonous hardly degradable humic matter, since relatively low C:N ratios (<9) in the precipitate from the surface sediment were found. Instead, the low C:N ratios indicate that the NaOH extractable organic P compound groups included in the precipitate originate from autochthonous matter.

However, the NaOH may not extract all of the organic P. Due to diagenetic transformations, a part of the organic P becomes hard to extract with the NaOH solution. Thus, the decline in the P compound groups with increasing age may reflect release, but also diagenetic transformations resulting in more recalcitrant P compound groups not extracted. As it is difficult to make a reliable quantification of the total amount of organic P in sediments, these two processes can not be adequately distinguished. However, sequential extraction of the sediment in Lake Erken, showed that the amount of, what is believed to be recalcitrant organic P (Residual P), did not increase significantly with depth (Rydin 2000; Ahlgren et al. 2005), whereas the HCl extractable P increased with increasing age. This indicates that the lower extraction efficiency of the NaOH solution with increasing age may be due to a lower extraction of inorganic P rather than a lower extraction of organic P, which validates the degradation rates determined in this study.

It is also possible that some P compound groups are replenished by the breakdown of other P compound groups. Thus, orthophosphate diesters may be expected to be mineralized to orthophosphate monoesters. Even though, this will enhance the impression of orthophosphate

monoesters being more recalcitrant than the orthophosphate diesters, this should not present an important artifact as an accumulation of orthophosphate monoesters, relative to orthophosphate diesters, will only occur if the diagenesis is slower than the diagenesis of the orthophosphate diesters.

The results presented in this paper suggest that the precipitate from the sediment of Lake Erken is dominated by autochthonous material originating from phytoplankton, as revealed by the low C:N ratios. The study further demonstrates that within several of the P compound groups extracted, there are pools of different recalcitrance. Finally, this study shows that it is possible to separate a more recalcitrant part of the organic P compound groups by using a modified extraction method for the separation of humic acids. The finding of a rather specific P compound group, such as DNA–P in the precipitate, suggests that these compounds are strongly bound to the humic fraction even after harsh treatment such as NaOH extraction.

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