

Origin and diagenesis of polyphosphate in lake sediments: A ^{31}P -NMR study

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Abstract

Polyphosphate (poly-P) was detected with the use of ^{31}P nuclear magnetic resonance (NMR) spectroscopy in sediments from a large variety of lakes with different trophic state and morphometry. In the top 0.5 cm of sediment, poly-P was 1.5 to 11.4% of total P. Nonreactive phosphorus (NRP) in the NaOH fraction (often classified as organically bound phosphate) was up to 46% inorganic poly-P. In some surface sediments, the poly-P content equalled the iron-fixed phosphorus determined by chemical phosphorus fractionation. Sediments were probably supplied with poly-P by sedimentation because there were substantial amounts of poly-P in plankton and settling seston. As demonstrated with sediments of Lake Petersdorf, benthic organisms can also contribute to the formation of poly-P (up to 0.11 mg P [g dry weight] $^{-1}$) under favorable aerobic conditions. Poly-P is more rapidly transformed into single orthophosphate during diagenesis than other inorganic and organic P species. The transformation of organic P compounds and poly-P can contribute significantly to the release of P during diagenesis and should be considered along with the reductive dissolution of P sorbed to iron oxihydroxides.

Microbially induced changes in pH and redox potential affect the ability of lake sediments to retain inorganic phosphorus (Roden and Edmonds 1997; Gächter and Müller 2003). Although these changes are driven by organic matter decomposition, the direct role of sediment bacteria by the release of organically bound phosphorus often has been ignored. Many studies have shown that a substantial proportion of phosphorus in settling seston originates from biomass (e.g., Hupfer et al. 1995a; Pettersson 2001; Kleeberg 2002). The decreasing content of these organic P species in settled particles during early benthic diagenesis indicates that heterotrophic sediment bacteria mineralize sedimentary organic P to inorganic P (Wetzel 1999). However, some of the supplied P is assimilated during microbial growth. Studies with activated sludge and with mixed or pure cultures have shown that several types of microorganisms are able to take up P excessively and form intracellular polyphosphate (poly-P; Wentzel et al. 1991). In waste water treatment plants with biological P elimination, poly-P-accumulating organisms are dominant under oscillating redox conditions.

Acknowledgments

We thank Christiane Herzog for her help with the analytical work. René Gächter, Roland Psenner, Jörg Lewandowski, and two anonymous reviewers are acknowledged for critical reading and helpful comments on a former version of the manuscript. Sarah Poynton is acknowledged for the linguistic improvements of the text.

Given the high diversity of benthic microorganisms and considering that the surface of lake sediments often represents an aerobic/anaerobic transition layer with variable redox conditions, it has been speculated that poly-P-accumulating organisms might also be present in lake sediments (Uhlmann and Bauer 1988; Khoshmanesh et al. 2002). Redox-controlled poly-P metabolism has been proposed to contribute to benthic P fluxes in marine (Ingall and Jahnke 1997) and in lacustrine sediments (Gächter et al. 1988; Davelaar 1993).

^{31}P nuclear magnetic resonance (NMR), a well established method in the fields of environmental science and engineering, is used to identify biogenic phosphates such as phosphomonoesters, phosphodiester, and poly-P (e.g., Uhlmann et al. 1990; Feuillade et al. 1995; Turner et al. 2003). However, only two ^{31}P -NMR investigations have confirmed that microbial poly-P occurs in aquatic sediments (Hupfer et al. 1995b; Carman et al. 2000). In the present ^{31}P -NMR study, we (1) quantify the poly-P content of surface sediments of 22 European lakes, (2) induce poly-P formation under experimental conditions, and (3) describe the degradation of poly-P and loss of poly-P during early diagenesis.

Material and methods

Study sites—Our survey reports data from 22 European lakes with different trophic state, chemistry, hydrology, morphology, and catchment characteristics (see Table 1).

Table 1. Concentrations and portions of poly-P in surface sediments (0–0.5 cm) from the deepest point of 22 European lakes sorted according to trophic state. The oxygen conditions in the overlying water were estimated by monitoring data of water profiles (+, oxygen available; –, no oxygen available). The TP concentrations (TP_{lake}) are given for the sampling or the closest time interval.

Lake	Lake characteristics			Sampling date	Sampling depth (m)	Oxygen in overlying water	TP _{lake} (μg P L ⁻¹)
	Area (km ²)	Mixis	Special features				
Oligotrophic							
Alpnach (CH)	4.80	di	Prealpine lake	23 Jun 94	33	+	n.d.
Fuchskuhle (D)	0.015	di	Artificially divided acid bog lake	15 Apr 99	5.5	+	27
Lucerne (CH)	114	mo	Fjordlike lake system	27 Jan 94	112	+	8
Neunzehnhain R. (D)	0.29	di	Drinking water reservoir	4 Jul 93	35	+	11
Mesotrophic							
Froschhaus (D)	0.165	di	Agricultural catchment	16 Apr 99	9.5	+	19
Hopfen (D)	1.92	di	Agricultural catchment	21 May 98	10.5	–	49
Piburg (A)	0.13	me	Reoligotrophication	9 Nov 93	12	+	10
Piburg (A)	0.13	me		9 Nov 93	24	–	10
Saidenbach R (D)	1.46	di	Drinking water reservoir	20 Jan 95	45	+	14
Schliersee (D)	2.22	di	Artificial destratification	21 May 97	40	+	15
Zurich (CH)	68.2	mo	Densely populated catchment	30 Jun 94	133	–	34
Hufeisen (D)	0.70	me	Mining lake	14 Sep 94	24	–(H ₂ S)	28
Eutrophic							
Arendsee (D)	5.13	mo	Sediment capping	2 Jul 95	48	–	170
Baldegg (CH)	5.20	mo	Aeration/destratification	4 May 94	40	+	95
Baldegg (CH)	5.20	mo	Aeration/destratification	14 Oct 93	67	+	95
Hallwil (CH)	10.2	mo	Aeration/destratification	24 May 94	46	+	83
Kalksee (D)	0.84	di	Hardwater lake	1 Oct 98	11	–	132
Müggelsee (D)	7.30	po	Flushed by a river	18 Jan 99	7	+	142
Öschle (D)	0.37	di	Agricultural catchment	21 May 98	14.5	–	35
Petersdorf (D)	0.23	po	Slightly dystrophic	10 Dec 98	3.5	+	62
Plötzensee (D)	0.077	di	Urban catchment	4 Jun 97	6	–(H ₂ S)	178
Rotsee (CH)	0.47	di	Urban catchment	7 Apr 94	16	+	n.d.
Sempach (CH)	14.4	mo	Aeration/destratification	6 Oct 93	87	+	92
Tegel (D)	4.0	di	Hypolimnetic aeration	13 Mar 94	16	+	38

PP-EG, end groups of poly-P; PP-MG, middle groups of poly-P; LOI, loss on ignition; R, reservoir; A, Austria; CH, Switzerland; D, Germany; di, dimictic; mo, monomictic; me, meromictic; po, polymictic; d.l., below detection limit; n.d., not detected.

Sampling—Sediment cores (diameter 5.5 cm) were collected with a modified Kajak sampler (UWITEC®) from the profundal zone of each lake during 1993 and 1999. Immediately after sampling, the uppermost 0.5-cm-thick sediment layer was separated from the remainder of the core. In order to evaluate diagenetic changes, one or more deeper layers below the sediment surface to a depth of 4–5 cm were additionally sampled in eight lakes. To obtain a representative composite sample, the samples of four to five replicate cores per site were combined. Seasonal variations in surface sediments were investigated in Lake Arendsee ($n = 5$) and Lake Baldegg ($n = 10$).

Settling seston was collected in sediment traps (diameter 9 cm, height 70 cm) exposed at 40 m (Lake Baldegg, September 1993) and 80 m depth (Lake Sempach, February 1994).

Spot checks of seston samples (0–5 m) were taken from five lakes—Arendsee (3 July 1995), Baldegg (9 July 1993), Fuchskuhle (28 October 1998), Froschhaus (16 April 1999), and Müggelsee (30 May 1997)—with a planktonic net (10 μm diameter, HYDROBIOS).

All samples were stored at 4°C during transport from lake

to laboratory. Samples were processed immediately after return to the laboratory.

Phosphorus uptake experiments—Surface sediment of Lake Petersdorf was diluted with oxygen-free (N₂ bubbled) distilled water to a particle concentration of 2% dry matter in a total volume of 600 ml. This suspension was then divided into six glass flasks and duplicates were closed after bubbling with N₂ for 0.5 h and shaken and permanently aerated. Two flasks were shaken and permanently aerated after an initial addition of 100 mg C₂H₃NaO₂ (sodium acetate) as the C source, stimulating the heterotrophic activity. After 24 h, 1.0 mg P (g dry weight [dw])⁻¹ was added as KH₂PO₄ (potassium hydrogen phosphate) to all flasks. All flasks were incubated at room temperature (22°C) for 10 d, and oxygen concentration and pH were monitored with WTW probes. After incubation, P binding forms in each flask were analyzed by the sequential extraction scheme proposed by Psenner et al. (1984). For NMR analysis the replicates were pooled.

Preparation of NMR samples—In this study, a wet mass (equivalent to a mass of at least 0.5 g dry matter) was treated

Table 1. Extended.

Sediment				NMR				
LOI (%)	TP (mg [g dw] ⁻¹)	Fe (mg [g dw] ⁻¹)	NaOH/EDTA-NRP (mg [g dw] ⁻¹)	o-PO ₄ ³⁻ (mg [g dw] ⁻¹)	PP-EG (mg [g dw] ⁻¹)	PP-MG (mg [g dw] ⁻¹)	EG+MG (% of TP)	EG+MG (% of NaOH-NRP)
9.1	0.71	35.7	0.083	0.195	d.l.	d.l.	—	—
83.0	1.32	4.8	0.460	0.129	0.035	0.090	9.4	27.1
11.5	2.13	23.9	0.230	0.346	0.01	0.102	5.3	48.7
32.7	1.43	27.6	0.170	0.040	0.028	d.l.	2.0	16.5
32.9	1.24	17.0	0.237	0.085	0.026	0.049	6.1	31.9
13.0	1.39	12.0	0.153	0.123	d.l.	d.l.	—	—
41.2	1.92	19.1	0.130	0.213	0.014	0.045	3.1	45.4
40.1	1.38	14.5	0.380	0.114	n.d.	0.038	2.8	10.0
17.8	4.00	25.3	0.355	0.688	0.083	0.022	2.6	29.6
7.4	1.45	17.0	0.180	0.058	0.018	0.014	2.2	18.0
14.7	0.96	14.2	0.302	0.091	0.034	0.075	11.4	36.1
15.6	1.06	30.6	0.126	0.091	d.l.	d.l.	—	—
29.1	2.60	4.0	0.751	0.142	0.076	0.097	6.7	23.0
9.6	1.69	12.5	0.264	0.153	0.029	0.071	5.9	37.9
11.5	1.21	8.8	0.220	0.179	0.019	0.046	5.4	29.5
15.3	1.41	8.2	0.341	0.131	0.053	0.095	10.5	43.4
18.4	3.53	17.5	0.269	0.305	0.074	0.000	2.1	27.4
25.9	5.64	55.7	0.315	0.280	0.049	0.055	1.8	33.1
25.6	1.44	7.0	0.086	0.108	d.l.	d.l.	—	—
32.9	1.81	17.8	0.473	0.163	0.026	0.058	4.7	17.8
41.5	2.15	14.4	0.276	0.142	0.024	0.024	2.2	17.4
16.9	1.28	13.7	0.343	0.135	0.018	0.088	8.3	30.9
14.8	2.01	12.5	0.240	0.147	0.011	0.041	2.6	21.7
22.8	5.24	32.0	0.398	0.408	d.l.	0.077	1.5	19.3

with 0.2 mol L⁻¹ NaOH/67 mmol L⁻¹ edetic acid (EDTA, Titriplex III, Merck 1.08418) after pre-extraction with 67 mmol L⁻¹ EDTA (as described by Hupfer et al. 1995b). Most previous ³¹P-NMR studies also have used alkaline extraction procedures for the analysis of organic P and poly-P because P in biological materials dissolves in alkaline extracts (e.g., Uhlmann et al. 1990). The yield and diversity of P forms are increased by addition of the chelator EDTA to NaOH (Cade-Menun and Preston 1996; Robinson et al. 1998), and the middle groups of poly P can only be detected by NaOH/EDTA extraction (Cade-Menun and Preston 1996). It is assumed that the chelating ability of EDTA increases the efficiency of NaOH by breaking P-containing organometal complexes. The pre-extraction with EDTA reduces interference in the NMR spectra by minimizing iron and other paramagnetic metals. Furthermore, metal ions catalyzing fragmentation of poly-P were removed during pre-extraction or inactivated during alkaline extraction by complexing with EDTA (Hupfer et al. 1995b).

After 10-fold concentration in a rotary vacuum evaporator (30°C), the extracts were stored at -20°C until NMR analysis. Repeated NMR analysis of the same samples over several years have shown that freezing and subsequent storage

of extracts does not alter the phosphorus composition (Hupfer unpubl. data).

³¹P-NMR analysis—Most NMR measurements were carried out on a Bruker DRX 600 spectrometer operating at 242 MHz for ³¹P (acquisition time 0.65 s, pulse width 14 μs, relaxation time 2 s, sweep width 25,000 Hz). The addition of 5% of pure D₂O added to the extracts provided the lock signal. Scans (4,000–12,000) were accumulated to achieve an acceptable signal to noise ratio. The minority of ³¹P-NMR spectra for samples from Swiss lakes were measured with a Bruker AC 200 spectrometer operating at 81 MHz (see Hupfer et al. 1995b). The chemical shift of spiked KH₂PO₄ (potassium hydrogen phosphate) to a subsample was calibrated to 0 ppm. Shifts of other P compounds were identified relative to that signal and were largely independent of pH (Carmann et al. 2000).

Peaks were assigned by data reported in the literature (e.g. Bedrock et al. 1994; Cade-Menun and Preston 1996). Chemical shifts of linear polyphosphate were validated with Graham salt (Na-poly-P; Merck 1.06529): the middle groups of poly-P provide a characteristic, well-defined signal at a position of approximately -26 ppm. Only the phosphoryl

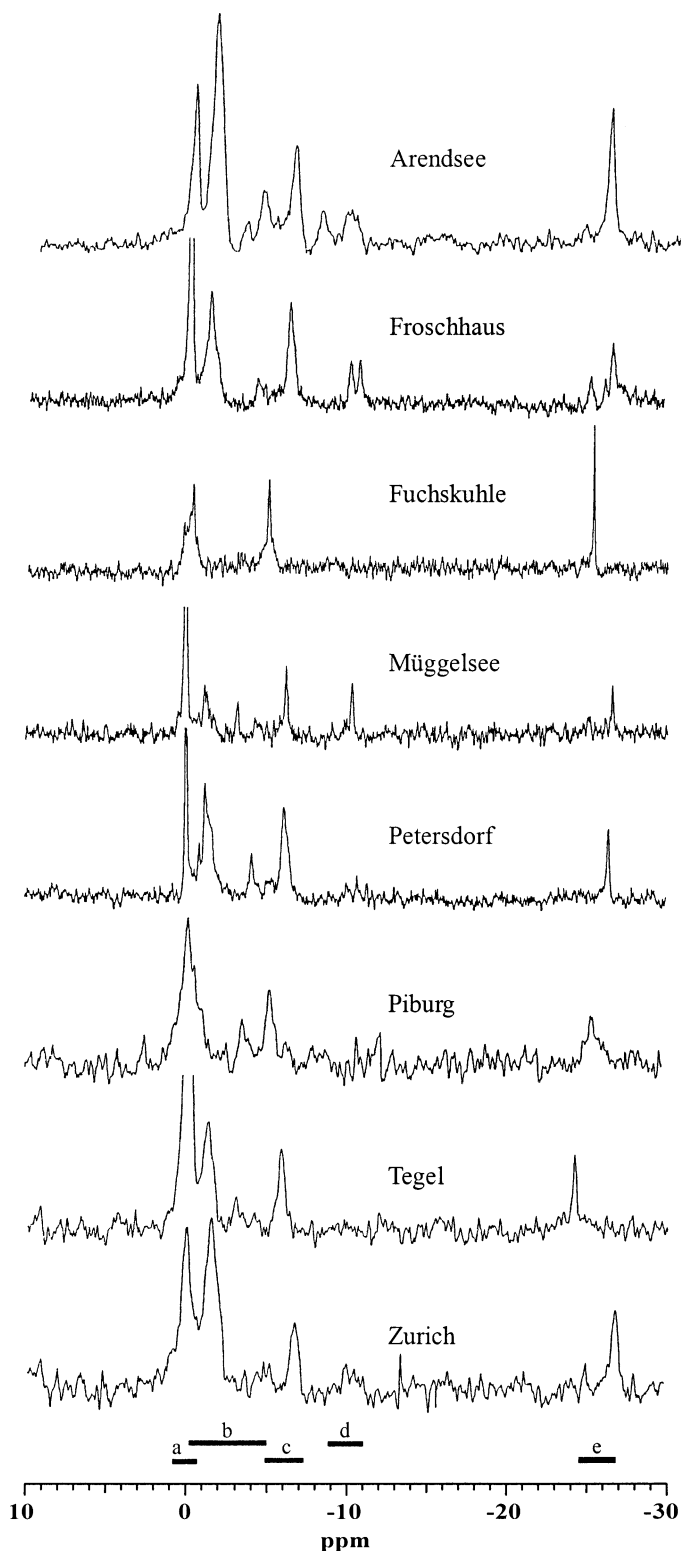


Fig. 1. Poly-P in NaOH/EDTA extracts from surface sediments (0–0.5 cm) of eight lakes detected by ^{31}P -NMR spectroscopy. The chemical shifts for the P compounds relative to inorganic P were based on published data (see text). (a) Orthophosphate, (b) phosphomonoester, (c) phosphodiester, (d) pyrophosphate, poly-P/end groups, (e) poly-P/middle groups. All examples show distinct signals of poly-P middle groups.

group at the β position of adenosine triphosphate (ATP) has a resonance signal in this region at approximately -23 ppm. If this signal is mainly due to ATP, similar signal intensities will be expected from two other phosphoryl groups in α and γ position, resulting in shifts of about -13 and -8 ppm, respectively (Cade-Menun and Preston 1996). Furthermore, ATP is not of quantitative importance compared to other organic P compounds in living cells. Resonances of other important organic P compounds, such as phosphomonoester (e.g., nucleotides, sugar phosphate, phosphatidic acid, inositol hexaphosphate, choline phosphate) and phosphodiester (nucleic acid, phospholipids), do not interfere with the resonance signals of poly-P (Fig. 1). Therefore, a single outstanding signal at -26 ppm strongly indicates the presence of inorganic poly-P.

NMR spectra were evaluated with the MestRe-C 2.3 for Windows (University of Santiago de Compostella, Spain). The area of single peaks was used to determine the relative proportion of the assigned species relative to the total P (TP) concentration in the extract (Robinson et al. 1998; Carman et al. 2000). Internal poly-P and KH_2PO_4 standards added to alkaline extracts confirmed that the strong relationship between peak intensity and concentration was independent of the chemical shift (Hupfer et al. 1995b; Robinson et al. 1998). A relatively small portion of compounds near the detection limit is a precondition for an exact quantification. Broadening or overlapping of signals increases the uncertainty of quantification.

Chemical analysis—Sequential extraction of phosphorus was carried out as suggested by Psenner et al. (1984) and modified by Hupfer et al. (1995a). The soluble reactive phosphorus (SRP) and nonreactive phosphorus (NRP) were determined according Hupfer et al. (1995a). The TP of solid sediment was determined as SRP after digestion of 5–10 mg dry sediment in a solution of 2 ml 5 mol L^{-1} H_2SO_4 , 2 ml 30% H_2O_2 , and 20 ml distilled water at 150°C for 16 h. Total iron was analyzed with a flame atomic absorption spectrometer (Perkin Elmer) after aqua regia digestion. Loss on ignition was calculated as loss of weight after combustion of dried sediment (450°C , 3 h).

Results

Poly-P in surface sediments—The ^{31}P -NMR spectra of surficial lake sediments show signals of orthophosphate, phosphomonoester, phosphodiester, pyrophosphate, and end and middle groups of poly-P (Fig. 1). On the basis of middle and end-group signals, poly-P was present in most surficial sediments of investigated lakes, irrespective of whether the overlying water was oxic or anoxic (Table 1). Only in four out of 24 samples was no poly-P found. The maximum contribution of poly-P to the NaOH/EDTA-NRP in sediment samples were seen in Lakes Hallwil (43.4%), Lucerne (48.7%), and Piburg (45.4%). Detectable poly-P contributed 1.5–11.4% to the total P content in all lakes. However, the poly-P content was not correlated either with organic matter or with total phosphorus.

In the uppermost sediment layer (0–0.5 cm) sampled in Lake Baldegg at a water depth of 40 m, the poly-P content

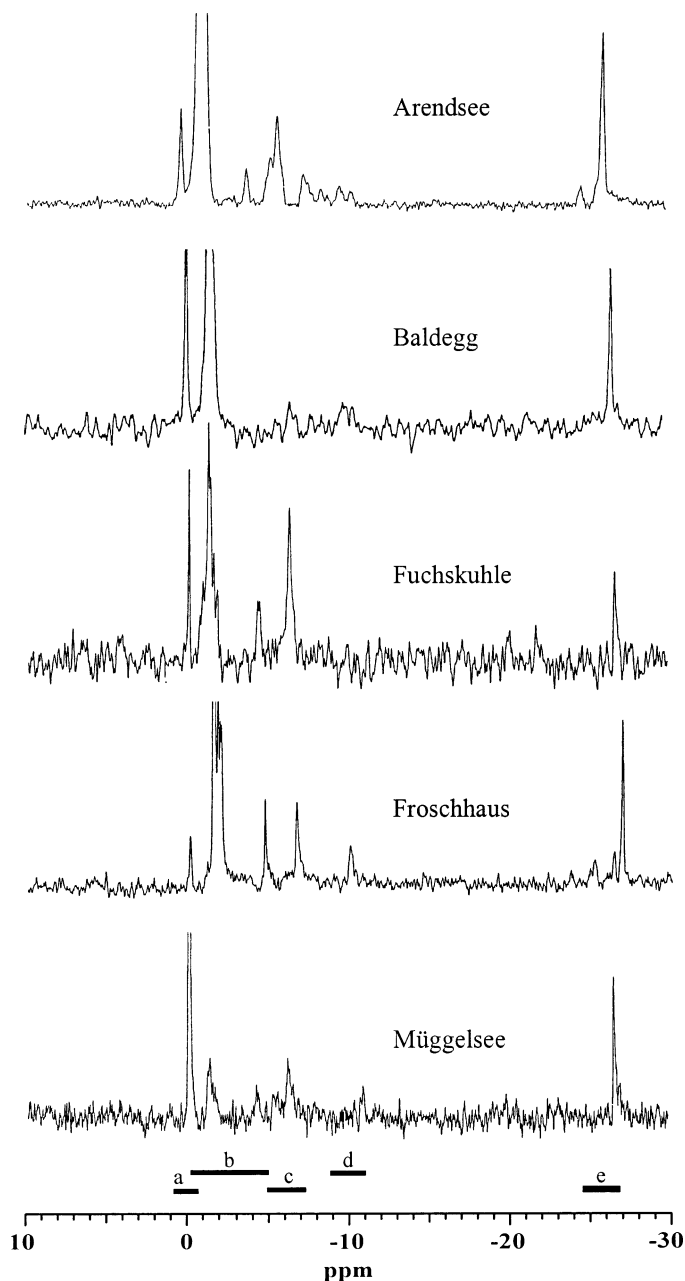


Fig. 2. ^{31}P -NMR spectra of seston samples (0–5 m) from Lake Arendsee (3 July 1995), Lake Baldegg (9 July 1993), Lake Fuchskuhle (29 October 1998), Lake Froschhaus (16 April 1999), and Lake Müggelsee (30 May 1997) taken by a 10- μm plankton net. For ranges of signals (a–e) for P compounds, see Fig. 1.

ranged between 0.04 and 0.18 mg P (g dw) $^{-1}$, with minimum values observed in winter (February 1994) at turnover, when productivity and sedimentation were low, and the maximum value observed in summer (July 1993). Also in Lake Arendsee, poly-P content was substantially lower during turnover (January 1995, 0.093 mg P [g dw] $^{-1}$). In this lake, the highest values—0.173 and 0.143 mg P (g dw) $^{-1}$ —were observed in July and August 1995 when the sediment was overlaid with anoxic water.

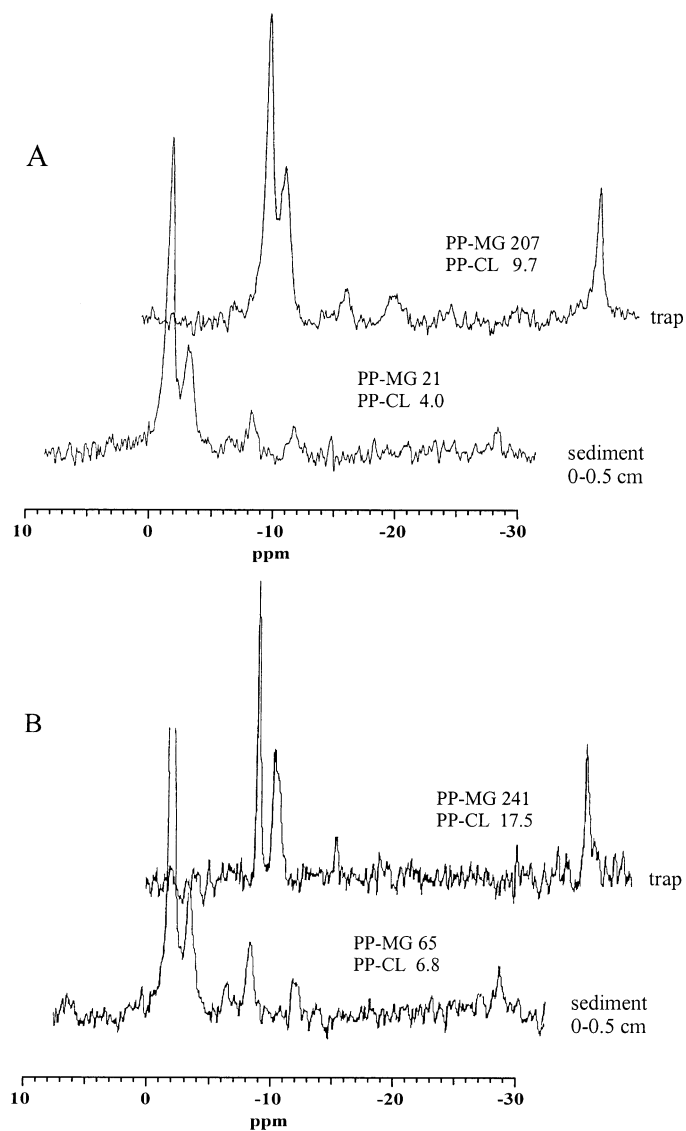


Fig. 3. ^{31}P -NMR spectra of settling seston and surface sediments of two Swiss lakes. (A) Material from 80-m trap (22 February 1994) and sediment (87 m, 0–0.5 cm, 2 March 1994) from Lake Sempach; (B) material from 40-m trap (20 September 1993) and sediment (67 m, 0–0.5 cm, 15 October 1993) from Lake Baldegg. PP-MG, concentration of middle groups ($\mu\text{g P [g dw]}^{-1}$); PP-CL, chain lengths. For chemical shifts for P compounds, see Fig. 1.

Evidence of poly-P in seston samples—In Lakes Fuchskuhle, Froschhaus, Arendsee, Baldegg, and Müggelsee, 10- μm -net samples were analyzed by ^{31}P -NMR spectroscopy (Fig. 2). In addition to poly-P, several unspecified organic P compounds, such as phosphomonoester and phosphodiester, were detected. Investigated seston samples were rich in total phosphorus (3.52–6.17 mg P [g dw] $^{-1}$) and organic matter (63.1–86.4% dw). Poly-P contents ranged between 0.160 and 0.650 mg P (g dw) $^{-1}$.

High poly-P contents of the planktonic seston, however, does not necessarily mean that this material is the source of poly-P in lake sediments, because a part of the P species in planktonic seston underlies intensive transformation in the

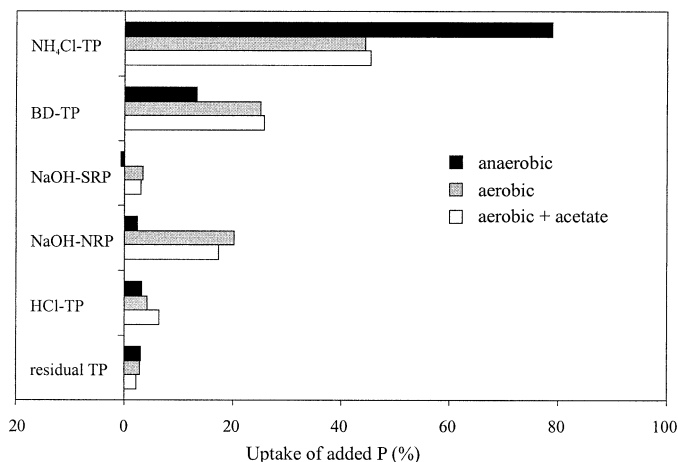


Fig. 4. Percent distribution of added KH_2PO_4 ($1.0 \text{ mg P [g dw]}^{-1}$) within the P fractions (sequential extraction scheme according Psenner et al. [1984]) in the sediment (0–0.5 cm) from Lake Petersdorf (mean values of duplicates). The sediment were incubated under anaerobic and aerobic conditions (with and without acetate) for 10 d.

waterbody. Therefore, the ^{31}P -NMR spectra of material collected in sediment traps exposed in Lakes Baldegg and Sem-pach are compared with those of the surface sediment in Fig. 3. Qualitative similarities in the spectra of seston, trap material, and sediments indicate that the seston strongly affects the P speciation of the surface sediment. The concentration of poly-P, however, decreased quickly after sedimentation.

Benthic poly-P formation—The increase of NaOH-NRP in the laboratory experiment with sediments from Lake Petersdorf demonstrated that benthic microorganisms are able to incorporate a portion of added phosphate into their biomass. The distribution of added P in the different chemical P fractions is shown in Fig. 4. The $\text{NH}_4\text{Cl-TP}$ mainly represents the remaining P in the pore water. The aerobic P uptake in the other fractions was about twice as high as the anaerobic uptake. Under aerobic conditions, 45% of total P uptake in solid structures were fixed as bicarbonate-dithionite (BD)-TP and 36% as NaOH-NRP. Table 2 indicates that under these conditions, the poly-P content was increased by about $0.075 \text{ mg P (g dw)}^{-1}$ (without acetate) and $0.110 \text{ mg P (g dw)}^{-1}$ (with acetate). The relative contribution of poly-P to all biogenic P forms (NaOH/EDTA-NRP) increased from 20% to 31% (without acetate) and to 36% (with acetate). Additionally, the increase of chain lengths also indicated a

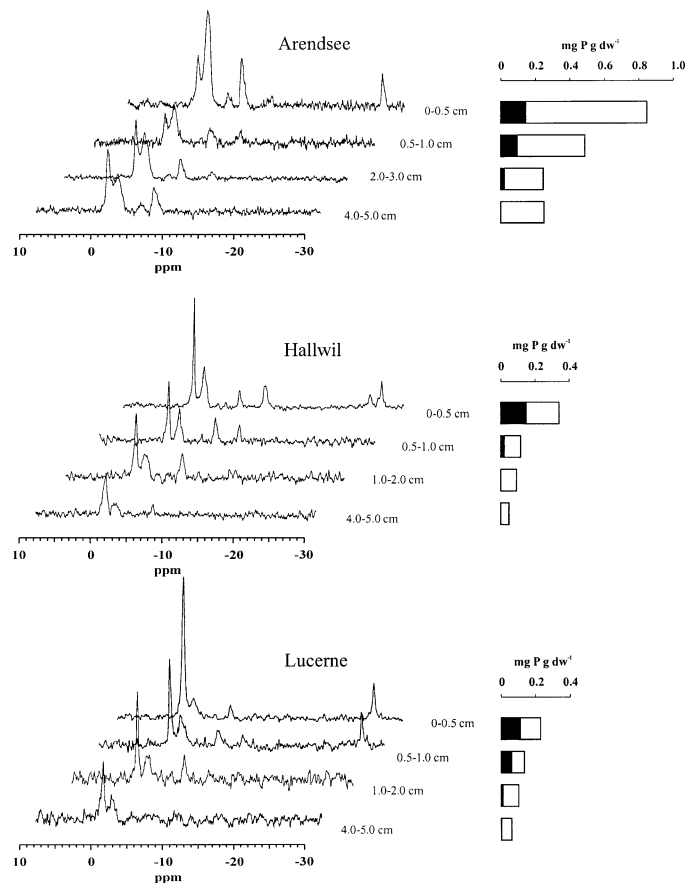


Fig. 5. Changes of poly-P and other P compounds during diagenesis. (Left) ^{31}P -NMR spectra of NaOH/EDTA extracts of different sediment horizons in Lake Lucerne (27 January 1994, 112 m), Lake Hallwil (24 May 1994, 46 m), and Lake Arendsee (15 August 1998, 48 m). For chemical shifts for P compounds, see Fig. 1. (Right) Contribution of inorganic poly-P (black) on the depth-dependent changes of nonreactive P in the NaOH/EDTA extracts. Poly-P content was determined as the sum of middle and end groups from the spectra.

synthesis of poly-P (Table 2). Under anaerobic conditions, both poly-P content and chain lengths were slightly decreased.

Changes of poly-P during early diagenesis—Comparison of ^{31}P -NMR spectra of samples collected at different sediment horizons yields information on the diagenetic transformation of various P species. In the sediments of Lakes Ar-

Table 2. Influence of redox conditions on the yield of NaOH/EDTA-NRP, poly-P (PP-EG, endgroups; PP-MG, middle groups), and the poly-P chain length (PP-CL) during P uptake experiments with sediment samples from Lake Petersdorf (0–0.5 cm, 19 Apr 99).

Sample	NaOH/EDTA-NRP (mg P [g dw]^{-1})	PP-EG (mg P [g dw]^{-1})	PP-MG (mg P [g dw]^{-1})	PP-CL	Portion of PP on NaOH/EDTA-NRP (%)
Reference	0.220	0.013	0.030	6.5	19.7
Anaerobic	0.171	0.012	0.015	4.4	15.9
Aerobic	0.384	0.023	0.096	10.5	30.9
Aerobic + acetate	0.424	0.036	0.117	8.4	36.2

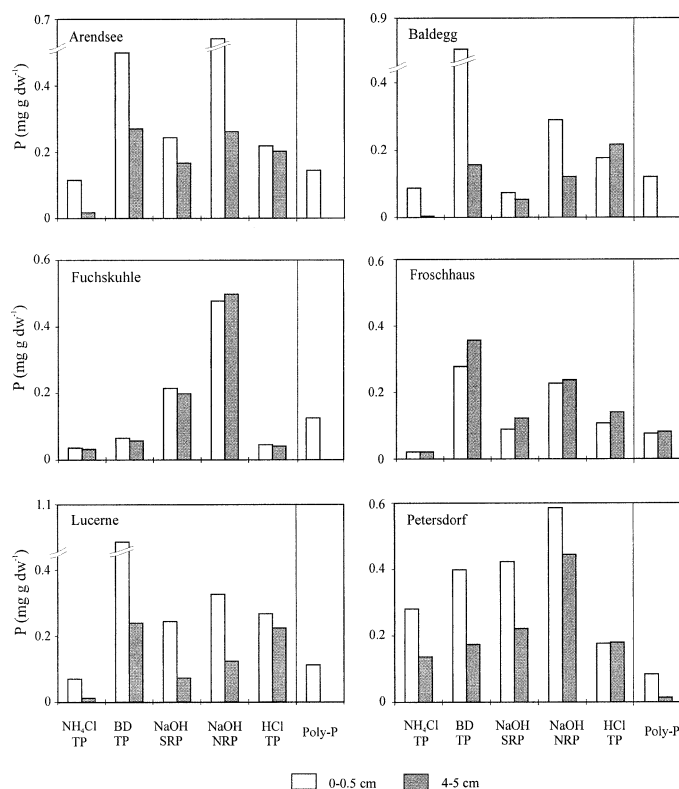


Fig. 6. Fractional P composition (sequential extraction scheme of Psenner et al. [1984]) in the uppermost sediment layer (0–0.5 cm) compared to a deeper sediment layer (4–5 cm) in Lakes Baldegg (22 February 1994), Lucerne (27 January 1994), Arendsee (7 November 1995), Fuchskuhle (15 April 1999), Petersdorf (10 December 1998), and Froschhaus (16 April 1999). The contribution of poly-P to diagenetic changes was determined by ^{31}P -NMR investigations. In the cases of Lakes Baldegg and Arendsee, the sampling dates for NMR investigations (2 February 1994 and 15 August 1995, respectively) differ slightly from the dates for analysis of the P fractions.

endsee, Lucerne and Hallwil organic P compounds and inorganic poly-P decreased with increasing sampling depth (Fig. 5). We could not detect poly-P middle groups at sediment depths below 0.5 cm except in Lake Lucerne. Poly-P decreased faster than the sum of the other nonreactive P compounds. At the sediment surface of Lakes Hallwil and Lucerne, poly-P amounted to >40% of the NRP, but 1 cm below the surface, it decreased to <10%. In Lake Arendsee, less poly-P was found at the sediment surface (17% of NaOH/EDTA-NRP), but traces of end groups were observed down to a sediment depth of 3 cm.

The composition of various P fractions found in the uppermost layer are compared with that at 4–5 cm depth (Fig. 6). In Lakes Lucerne and Baldegg, the reductant soluble Fe-bound P (BD-TP) was the dominating P fraction at the sediment surface. This fraction contributed most to the total P loss between the sediment surface and the 4–5-cm layer. In Lakes Arendsee and Petersdorf, several fractions contributed evenly to total P loss. In both lakes, vertical poly-P losses were more or less equal to the losses of BD-TP and organic P (NaOH-NRP minus poly-P). The dystrophic Lake Fuch-

skuhle was the only lake, in which poly-P exceeded the BD-TP in surficial sediment. In Lake Froschhaus, all P fractions reached about equal concentrations at both sediment depths, probably because of intensive bioturbation.

Table 3 summarizes the vertical changes of poly-P compared with the losses of TP and NaOH/EDTA-NRP between the surface sediment and a deeper sediment layer. In Lake Arendsee, surface sediment was collected shortly before the profundal sediment was covered with littoral calcareous material (Seekreide) to restore the lake in 1995. At that time, the sediment surface poly-P content was $0.145 \text{ mg P (g dw)}^{-1}$. Four years later, the layer below the calcite cover was sampled again, and its poly-P content had decreased to only $0.017 \text{ mg P (g dw)}^{-1}$, confirming poly-P breakdown during early diagenesis. The gradients of P forms between 0–0.5 and 4–5 cm in the sediment of Lake Fuchskuhle cannot be interpreted as a result only of diagenetic processes because the poly-P decrease is higher than the NaOH/EDTA-NRP attenuation. The bioturbation in Lake Froschhaus disturbs the formation of gradients and an evaluation of diagenetic changes (see Fig. 4). In all other lakes, loss of 8.2–28.6% of the TP during the time when 4 cm of sediment accumulated could be attributed to diagenetic losses of poly-P (Table 3).

Discussion

Occurrence of poly-P in surface sediments—Our study shows that inorganic poly-P can be found in different types of lake sediments and can contribute substantially to the operationally defined organic P. To date, the evidence was based on direct observations of P-rich structure in sediment bacteria by electron microscopy and X-ray spectroscopy (Uhlmann and Bauer 1988), rare ^{31}P -NMR investigations (Bedrock et al. 1994; Hupfer et al. 1995a; Carman et al. 2000), interpretations of experimental phenomena (Gächter et al. 1988; Waara et al. 1993; Brunberg 1995; Khoshmanesh et al. 2002), and theoretical considerations (Davelaar 1993; Gächter and Meyer 1993). The common finding of poly-P in our present study shows that the occurrence of this P species in surface lake sediments is not a single or episodic phenomenon.

In contrast to the opinion of Golterman (2001), we now show that ^{31}P -NMR spectroscopy is a powerful tool for benthic poly-P identification. Because poly-P cannot be generated during the extraction procedure and because enzymatic hydrolysis of free poly-P is fast under natural conditions, detected poly-P can occur only in living or dead microorganisms. The poly-P contents presented here are underestimations because the $0.2 \text{ mol L}^{-1} \text{ NaOH}/67 \text{ mmol L}^{-1} \text{ EDTA}$ is a less efficient extractant than the usually applied $1 \text{ mol L}^{-1} \text{ NaOH}$ for P extraction. In this study, the mean extraction efficiency of $0.2 \text{ mol L}^{-1} \text{ NaOH}/67 \text{ mmol L}^{-1} \text{ EDTA}$ was equivalent to 79.5% of $1 \text{ mol L}^{-1} \text{ NaOH}$ ($n = 16$) in the sequential extraction according to Psenner et al. (1984). However, a short extraction time and mild alkalinity were chosen to prevent fragmentation of poly-P during extraction (Hupfer et al. 1995b). The rapid fragmentation of poly-P middle groups could explain why they were rarely detected in other NMR studies (see Cade-Menun and Preston 1996).

Table 3. Relative contribution of poly-P dissolution to the diagenetic losses of total phosphorus and NaOH/EDTA-NRP from surface sediments to older sediment layers (4–5 cm). The dating by calcite capping in Lake Arendsee permitted an analysis of changes by a direct comparison of the same layer 32 months later. d.l., below detection limit; EG, end groups; MG, middle groups.

Lake	Sampling date	Sediment depth (cm)	TP (mg [g dw] ⁻¹)	NaOH/EDTA-NRP (mg [g dw] ⁻¹)	Poly-P EG+MG (mg [g dw] ⁻¹)	Portion of poly-P dissolution on decrease of	
						NaOH/EDTA-NRP (%)	TP (%)
Arendsee	15 Aug 95	0–0.5	2.70	0.847	0.145		
		4–5	1.12	0.251	d.l.	24.3	8.9
Baldegg	19 Apr 99	below SK*	1.22	0.169	0.017	18.9	8.6
		6 Sep 93	0–0.5	1.12	0.240	0.120	
Fuchskuhle	15 Apr 99	0–0.5	1.32	0.460	0.125	60.2	28.6
		4–5	1.11	0.359	d.l.	123.0†	60.3
Froschhaus	16 Apr 99	0–0.5	1.24	0.237	0.076		
		4–5	1.25	0.295	0.082	No gradient	
Hallwil	24 May 94	0–0.5	1.41	0.341	0.148		
		4–5	0.51	0.048	d.l.	50.5	16.4
Lucerne	27 Jan 94	0–0.5	2.13	0.230	0.112		
		4–5	0.77	0.060	d.l.	65.9	8.2
Petersdorf	10 Dec 98	0–0.5	1.81	0.473	0.084		
		4–5	1.43	0.288	d.l.	38.0	18.5
Rotsee	7 Apr 94	0–0.5	1.28	0.343	0.106		
		4–5	0.86	0.130	d.l.	49.8	25.2

* Material corresponds to the surface layer before sediment capping with calcareous mud (SK) in autumn 1995.

† Loss of NaOH/EDTA-NRP by poly-P is partly compensated by an increase in P monoester (*see text*).

Origin of poly-P—The ubiquitous occurrence of poly-P is not surprising, considering the high abundance and diversity of heterotrophic and chemoautotrophic benthic microorganisms. It is assumed that the alternating redox conditions prevailing at the sediment–water interface are especially advantageous to facultative anaerobic poly-P–storing bacteria (Davelaar 1993). The potential of nonphototrophic microorganisms to form poly-P in the sediment was demonstrated by the increase of nonreactive P and poly-P during aerobic incubation experiments with sediment from Lake Petersdorf (Fig. 4; Table 2). Contrary to this, an increase of organic P or poly-P was not observed under anaerobic conditions. Waara et al. (1993) stimulated bacterial growth in sediments of Lake Vallentunasjön and also found an increase of P extracted as NaOH-NRP. According to Khoshmanesh et al. (2002), two prerequisites must be fulfilled for biotic poly-P accumulation: aerobic conditions and acetate availability. In our aerobic P uptake experiments, the NaOH-NRP and poly-P content were not substantially enhanced by addition of acetate (Table 2). Hence, in these organic, P-rich sediments of Lake Petersdorf, microbial poly-P formation was not limited by carbon. Evidently, benthic bacteria synthesize poly-P if conditions are favorable. Abiotic poly-P formation under our experimental conditions with a relatively low temperature is excluded.

Poly-P might also be imported to the sediment by settling particles. Our investigation of seston and trap material (Fig. 3) supports the hypothesis of a partly pelagic origin of the sedimentary poly-P pool. It is well known that seston contains aggregated structure of living algae, residues from zooplankton and algae, that could be colonized by bacteria, fungi, and micrograzers (Grossart et al. 1997). All these

organisms are potential carriers for poly-P. The life cycle of some algae includes benthic and pelagic stages (Brunberg 1995; Pettersson 2001). For example, it is well documented that *Microcystis* colonies are able to overwinter and survive for extended periods in sediments (Preston et al. 1980). In Lake Vallentunsjön, the benthic biomass of *Microcystis* constituted >90% of total benthic living biomass (Brunberg 1995). *Microcystis* cells can store a large amount of phosphorus in poly-P granules (Jacobson and Halman 1982). Poly-P–containing *Microcystis* preferentially occurred in sedimenting colonies (Oliver 1994). Brunberg (1995) has shown that the coupling of P release with the fate of *Microcystis* colonies in Lake Vallentunasjön corresponded to changes of sedimentary “organic-bound phosphorus” in the chemical fractionation, which could be better defined as non-reactive.

Diagenesis of poly-P—Because organic phosphorus compounds are supplied to the sediment surface and mineralized by heterotrophic decomposition, they decrease with increasing sediment depth (e.g., Hupfer et al. 1995a; Penn et al. 1995; Søndergaard et al. 1996). Bacteria are able to synthesize and store poly-P in an aerobic environment, which is normally restricted to the uppermost few millimeters of the sediment. Compared to organic biogenic P compounds, poly-P is decomposed even more quickly with increasing sediment depth (Fig. 4). The rapid mobilization of poly-P can be explained by (1) cellular regulation or lysis of facultative anaerobic poly-P–storing bacteria (*see Davelaar 1993*) and (2) coupling of microbial mineralization of freshly settled detritus of pelagic origin with release of stored poly-P.

If the poly-P gradients are steep in the upper sediment

layers and the spatial sampling resolution is not fine enough, poly-P might be missed, which could be one reason why poly-P was not detected in some sediment investigations (e.g., Carman et al. 2000).

The contribution of poly-P to total P release is difficult to estimate by our data because the 0.5-cm layer represents different time periods after deposition and is partly influenced by the seasonal variability of sedimentation. NaOH-NRP is mobilized faster than other fractions (Hupfer et al. 1995a), so that the NaOH-NRP and poly-P concentrations in the 0–0.5-cm layer do not represent initial conditions. Therefore it is assumed that the contribution of easily mobilizable poly-P to the P loss after sedimentation is higher, as quantified by comparison of the two investigated sediment layers (0–0.5 cm and 4–5 cm, Table 3). The time periods for diagenesis represented by comparison of these two layers are different in the lakes, which restricts more quantitative consideration by sediment core analysis.

The P released by microbial degradation of organic P and poly-P can be chemically sorbed to sediment particles depending on their binding capacity. Our results, however, show that none of the solid chemical P fractions distinctly increased during diagenesis (Fig. 6) to compensate the loss by dissolution of poly-P. The importance of microbial poly-P can be assessed not only by its concentration and its changes, but also by its fate during microbial transformation or in the benthic food chain.

Our ^{31}P -NMR study has shown that poly-P can be found in many types of lake sediments where it can contribute substantially to the operationally defined nonreactive P. In some surface sediments, the poly-P content amounted to about 10% of total phosphorus and was similar to the amount of iron-bound phosphorus. The detected poly-P must be a part of microorganisms that can enrich phosphorus as a nutrient or energy reserve. Benthic microorganisms have the potential to synthesize poly-P under favorable conditions; however, the quantitative importance of the metabolism of benthic organisms for the regulation of P fluxes between sediment and water cannot yet be assessed because planktonic and settling seston also contain substantial amounts of poly-P. Compared with other organic and inorganic P forms, poly-P is rapidly transformed during early diagenesis. From our ^{31}P -NMR investigations, it was evident that the contribution of poly-P to the release of P during diagenesis should not be disregarded compared with the reductive dissolution of P sorbed to iron oxihydroxides.

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Received: 7 May 2003

Accepted: 29 July 2003

Amended: 25 August 2003