

Degradation of organic phosphorus compounds in anoxic Baltic Sea sediments: A ^{31}P nuclear magnetic resonance study

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Abstract

The composition and abundance of phosphorus extracted by NaOH–ethylenediaminetetraacetic acid from anoxic Northwest Baltic Sea sediment was characterized and quantified using solution ^{31}P nuclear magnetic resonance. Extracts from sediment depths down to 55 cm, representing 85 yr of deposition, contained 18.5 g m^{-2} orthophosphate. Orthophosphate monoesters, teichoic acid P, microbial P lipids, DNA P, and pyrophosphate corresponded to 6.7, 0.3, 1.1, 3.0, and 0.03 g P m^{-2} , respectively. The degradability of these compound groups was estimated by their decline in concentration with sediment depth. Pyrophosphate had the shortest half-life (3 yr), followed by microbial P lipids with a half-life of 5 yr, DNA P (8 yr), and orthophosphate monoesters (16 yr). No decline in concentration with sediment depth was observed for orthophosphate or teichoic acid P.

Eutrophication is a major environmental problem in the Baltic Sea (e.g., Rönnerberg and Bonsdorff 2004), but there is a lack of consensus whether nitrogen (N) (Blomqvist et al. 2004) or phosphorus (P) (Hecky 1998) is limiting overall primary production in the Baltic proper. Contrary to N, P has no gaseous phase, meaning that the supply of P for primary production is dependent on external sources as well as internal recycling.

Until the mid-1980s, external P loading to the Baltic Sea had increased eightfold compared with a century ago (Larsson et al. 1985). Although total riverine input of P to the Baltic Sea showed a slight downward tendency during the 1990s (Ståhlhake et al. 1999), total external loading into the Baltic Sea has been fairly constant over the last decades (HELCOM 2005).

In spite of the stabilized external loading, water column P concentrations in the Baltic Sea show an increasing tendency, most likely due to internal P loading (Emeis et al.

2000). The amount and forms of organic sediment P that contribute to the internal loading process are basically not known, and such information would be of great importance for prediction of the future status of Baltic Sea water quality.

Most of the knowledge about P in sediment concerns inorganic P forms obtained from various sequential extraction procedures, but more specific information about the chemical forms of organic P is lacking, in spite of the quantitative importance of these compounds (Turner et al. 2005). ^{31}P nuclear magnetic resonance (^{31}P NMR) spectroscopy is a suitable method for assessing the general composition of P in a variety of matrixes since it, by using the resonance yielded by the P atom when it is placed in a strong magnetic field, can indicate what ligands are attached to the P atom. The method can indicate whether there is, for example, orthophosphate, pyrophosphate, or polyphosphate in the sample, or whether some of the P is situated in organic forms such as orthophosphate monoesters, diesters, or phosphonates. ^{31}P NMR can also be used to investigate possible changes in the P composition, thus making it possible to assess trends of P compounds in terms of degradation and mineralization (e.g., Ahlgren et al. 2005).

^{31}P NMR spectroscopy has been used by a number of researchers since the 1980s to identify organic P compounds in environmental samples, including sediments

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Acknowledgments

This investigation was supported by the Swedish Research Council for Environment, Agricultural Sciences, and Spatial Planning. Kasper Reitzel was supported by the Carlsberg Foundation by a postdoctoral grant and by the Danish Natural Science Research Council by grant 21020463.

(Sundareshwar et al. 2001; Hupfer et al. 2004; Ahlgren et al. 2005). To our knowledge, however, Carman et al. (2000) is the only study focusing on organic P in brackish sediments, using ^{31}P NMR spectroscopy to assess the phosphorus forms occurring in the surface sediment of the Baltic Sea.

In this study we used ^{31}P NMR spectroscopy to identify and quantify individual P compound groups in sediment depth profiles from the Landsort area in the Baltic proper. We used sediment accumulation rates to calculate degradation rates of the identified P compound groups, and we discuss the importance of these rates to the turnover of sediment P in the Baltic Sea and their possible environmental implications.

Experiment

Study site and sampling—Samples were collected in February 2004 from an area in the Baltic proper (Fig. 1) close to the Landsort deep ($58^{\circ}42'36.68''\text{N}$, $17^{\circ}56'35.37''\text{E}$). This site is an accumulation bottom area outside the coastal zone and was chosen in order to obtain anoxic, laminated sediments. Salinity at the site varies between 7 and 8. Eight sediment cores were collected with a Gemini twin core sampler at a water depth of 91.5 m. The cores (inner diameter, 8 cm) were sliced into 1-cm segments (at every centimeter down to 10-cm depth, then at every fifth centimeter down to 55-cm depth) under a nitrogen atmosphere to ensure that no oxidation of the sediment would occur. Sediments from the same depth of all cores were pooled and homogenized in order to obtain a representative sample of sufficient size for extraction. Samples were immediately refrigerated at 4°C until analysis. Sediment dry weight (DW) was measured by freeze drying the sediment to constant weight, after which loss on ignition (LOI) was measured by combusting the dried sediment at 550°C for 6 h. Total P (TP) in the sediment was analyzed by autoclaving the combusted sediment in 1 mol L^{-1} HCl at 120°C for 1 h, followed by colorimetric measurement of orthophosphate.

Extraction—Sediment samples for the ^{31}P NMR analysis were extracted with a 1:1 mixture of 0.25 mol L^{-1} NaOH and 0.05 mol L^{-1} ethylenediaminetetraacetic acid (EDTA; e.g., Turner et al. 2003a) with a 1:3 (v:v) sediment to extractant ratio. After 16 h, the samples were centrifuged and the supernatants were concentrated to the same extent, relative to the dry weight, by rotary evaporation. The concentrated samples were frozen until analysis, a procedure proven not to affect the extracted P compounds (Hupfer et al. 2004). The total amount of P in the extracts was measured by inductively coupled plasma atomic emission spectroscopy, both before and after the concentration step, in order to quantify the amount of P in the extracts and the amount of P lost in rotary evaporation and the subsequent centrifugation. Losses were never more than 10% of the total extracted amount of P.

^{31}P NMR: analysis—Before ^{31}P NMR measurement, a small amount of dithionite solution (10% [v:v]) was added

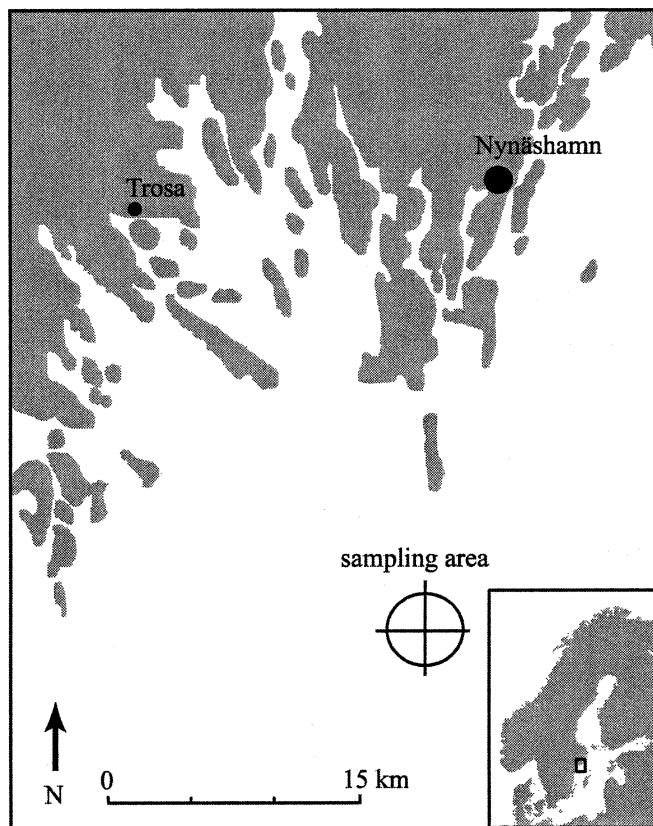


Fig. 1. The Landsort area, with the sampling station indicated.

to the extracts in order to reduce Fe(III) to Fe(II), since the paramagnetic Fe(III) otherwise could interfere with the NMR analysis. Assignment of peaks was done using standard solutions ($\text{Na}_2\text{HPO}_4 \times 7\text{H}_2\text{O}$ for orthophosphate and $\text{Na}_2\text{P}_2\text{O}_7 \times 10\text{H}_2\text{O}$ for pyrophosphate) that were added to one of the sediment extract solutions. Comparisons with previous studies were also conducted (Makarov et al. 2002a,b; Turner et al. 2003a). The ^{31}P NMR spectra were measured at 121.5 MHz on a Varian Mercury Plus NMR spectrometer at 20°C . An amount of D_2O sufficient to obtain a stable lock signal was added to each sample prior to measurement. Spectra were recorded using a 63° observe pulse, acquisition time 0.4 s, 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. Data were processed using a line broadening of 10 Hz.

To obtain peak areas, peaks in the raw spectrum with a signal to noise ratio exceeding 4 were fit with Lorentzian line shapes using the deconvolution subroutine of the NMR software (Vnmr 6.1C). From these peak areas, the contribution of individual P compound groups was calculated relative to TP in the extracts.

Decay rates of identified P compounds—Sediment age was determined by counting lamina representing yearly deposition of matter (Morris et al. 1988). The sediment layer representing 1986 was determined by measuring the sediment activity of ^{137}Cs (Meili et al. 1998) and correlating

Table 1. Dry weight (DW), loss on ignition (LOI), total phosphorus (TP), total amount extracted with NaOH-EDTA, total amount extracted expressed as percentage of TP, sediment age, and dry matter load of a pooled sample of sediment depth profiles collected at the Landsort area in February 2004.

Sediment depth (cm)	DW (%)	LOI (%)	TP ($\mu\text{g g}^{-1}$ DW)	Total extracted amount ($\mu\text{g g}^{-1}$ DW)	Total extracted amount (% TP)	Sediment age (yr)	Dry substance ($\text{g m}^{-2} \text{yr}^{-1}$)
0–1	6.3	17	1,371	391	29	0.3	1,245
1–2	9.6	14	1,259	311	25	0.8	1,245
2–3	13.0	11	1,199	235	20	1.4	1,245
3–4	14.0	11	1,195	281	24	2	1,245
4–5	15.2	12	1,162	326	28	3	1,245
5–6	18.6	10	1,138	241	21	4	2,121
6–7	17.6	10	1,077	264	25	5	2,121
7–8	17.4	9	1,094	268	24	6	2,121
8–9	19.0	10	1,067	256	23	7	2,121
9–10	22.2	10	933	217	23	8	2,121
14–15	23.2	9	1,055	224	21	12	3,268
19–20	23.1	8	1,019	229	22	18	2,217
24–25	22.5	10	1,056	224	21	28	1,301
29–30	23.1	9	993	229	23	36	1,637
34–35	26.2	8	1,013	187	18	47	1,321
39–40	27.1	6	981	174	18	56	1,755
44–45	28.6	7	966	155	16	65	1,855
49–50	30.6	7	837	158	19	75	1,855
54–55	30.1	6	860	173	20	85	1,855

it to the Chernobyl accident. Half-life times were determined by plotting P concentrations of the respective P compound group versus sediment age and fitting exponential regression curves to each compound group's decline with age. The half-lives were then calculated using $\tau = \ln 2 / (k)$, where k is the rate constant (yr^{-1}) found in the equation of the regression curve.

Results

The total amount of extracted P declined from about $400 \mu\text{g g}^{-1}$ DW in the surface sediment to less than $200 \mu\text{g g}^{-1}$ DW in the deepest sediment layer (Table 1). LOI followed the same pattern, decreasing from 17% in the surface sediment to 6% in the deepest layer. Six different P compound groups were identified in the extracts (Fig. 2). These were orthophosphate, orthophosphate monoesters, and three different compound groups within the orthophosphate diester area. These were most likely deoxyribonucleic acid (DNA) P, microbial P lipids, and a signal likely caused by teichoic acid P but that could also be another form of phospholipid. In addition, pyrophosphate was detected. These groups are designated ortho P, monoester P, DNA P, P lipids, teichoic P, and pyro P, respectively.

Share of extracted P—Ortho P increased from 35% to 75% of the total extracted P with increased sediment depth (Table 2). Ortho P was, however, the only fraction to increase in proportion of extracted P, since all other P compound groups showed the opposite tendency. Monoester P decreased from 35% to 15%, whereas DNA P decreased from around 20% in the surficial sediment to less than 10% in the deepest sediment layer. The fraction of teichoic P and P lipids in the surface layer constituted about 1–2% and 8% of the TP extracted, respectively. P lipids

decreased slowly throughout the sediment profile, reaching the detection limit (i.e., a signal to noise ratio below 4) at 35-cm sediment depth (Table 2). Teichoic P was constant down to 30-cm sediment depth, below which it dropped under the detection limit. Pyro P constituted 5% of the extracted P in the surficial sediment but dropped below the detection limit at 4-cm depth.

Concentrations and amounts of extracted P—The concentration of ortho P was generally stable at $120\text{--}140 \mu\text{g g}^{-1}$ DW throughout the sediment profile, whereas the concentration of monoester P and DNA P decreased from 140 and $60 \mu\text{g g}^{-1}$ DW in the surficial sediment to 30 and $15 \mu\text{g g}^{-1}$ DW in the deepest layer, respectively. Teichoic P had a concentration of about $5 \mu\text{g g}^{-1}$ DW down to 30-cm sediment depth, below which the concentration was below the detection limit. P lipids ranged from $30 \mu\text{g g}^{-1}$ DW to $15 \mu\text{g g}^{-1}$ DW in the sediment profile down to 35 cm (Table 2), after which the concentration was below the detection limit. The concentration of pyro P ranged from $20 \mu\text{g g}^{-1}$ DW in the surficial sediment to below the detection limit below 4-cm sediment depth.

Interpolating the results of Table 2 for every centimeter of sediment depth, the total amount of each identified P compound group in the investigated sediment profile can be derived. The sediment down to 55-cm depth in the investigated area thus contains 18.5 g ortho P, 6.7 g monoester P, 0.3 g teichoic P, 1.1 g P lipids, 3.0 g DNA P, and 0.03 g pyro P per square meter.

Decline rates—Sediment dating revealed the age of the deepest investigated sediment (54–55 cm) to be around 85 yr (Table 1), and laminae, indicating anaerobic conditions, were observed down to 47-cm sediment depth. Below this layer, representing 1934, aerobic conditions were

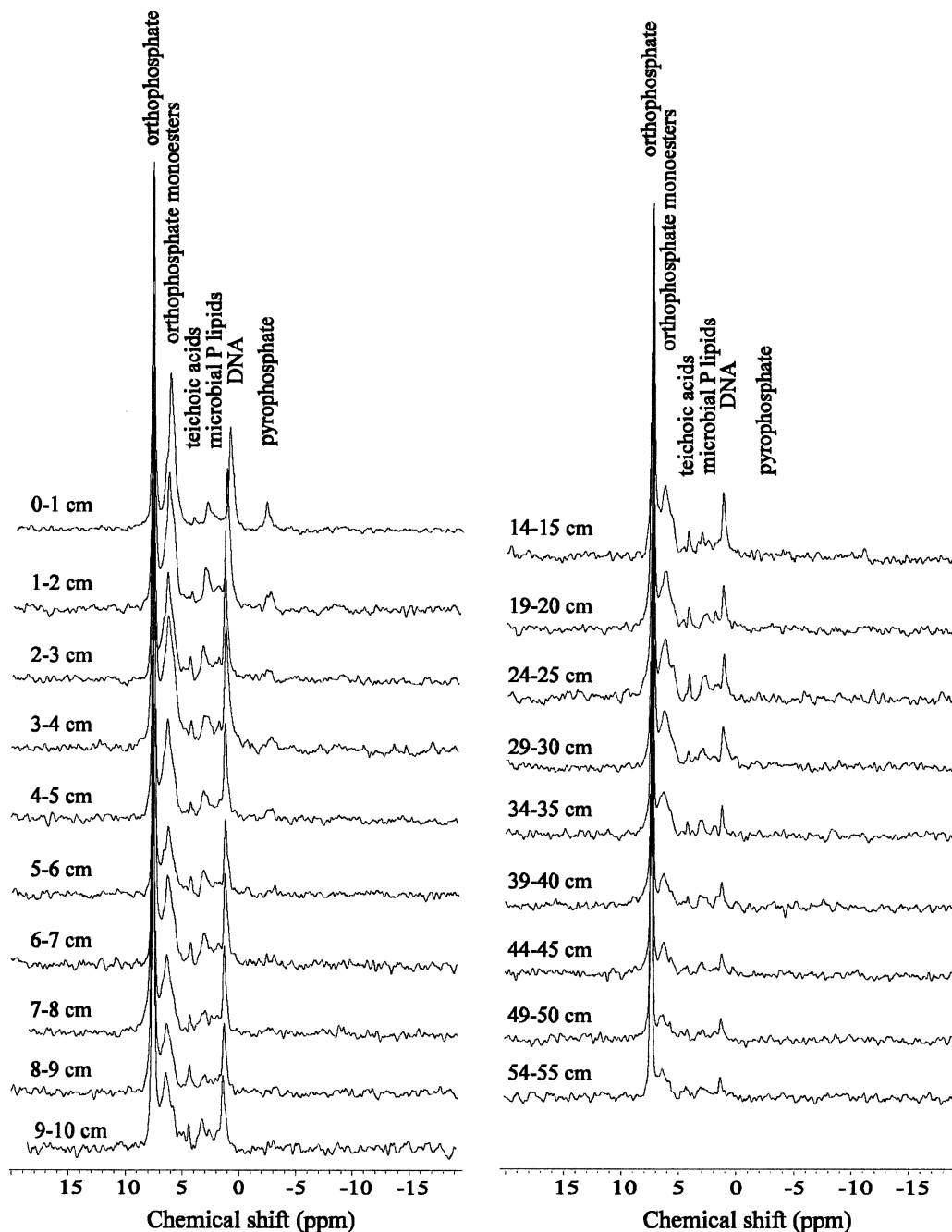


Fig. 2. ^{31}P NMR spectra of a depth profile of pooled sediment samples ($n = 8$) from the Landsort area, collected in February 2004.

apparently prevailing because the lack of lamina indicates bioturbation. By converting sediment depth to sediment age and applying a first-order decay model, the half-life of each extracted P compound was determined. Monoester P, P lipids, DNA P, and pyro P had an exponential decline in concentration with increased sediment depth (Table 3). Pyro P showed the fastest decline, with a half-life of 3 yr, followed by P lipids with a half-life of 5 yr. DNA P and monoester P had half-life times of 8 and 16 yr, respectively (Table 3). For ortho P and teichoic P, the depth distribution could not be explained by the first-order decay model (Table 1). For monoester P, P lipids, and DNA P the

fit of the model was very good ($r^2 \geq 0.80$). For pyro P, 55% of the variation was explained by the first-order model, suggesting other factors also are important for the depth distribution.

Discussion

Of the identified compound groups, monoester P, P lipids, DNA P, and pyro P declined in concentration with age. If the TP concentration and the individual P compound groups of newly settled matter have remained roughly constant throughout the study period (even though

Table 2. Relative composition, and concentration ($\mu\text{g g}^{-1}$ DW) in parentheses, of identified P compound groups in pooled samples of sediment from the Landsort area, collected in February 2004. ND = not detected.

Sediment depth (cm)	Sediment age (yr)	Ortho P % ($\mu\text{g g}^{-1}$ DW)	Monoester P % ($\mu\text{g g}^{-1}$ DW)	Teichoic P % ($\mu\text{g g}^{-1}$ DW)	P lipids % ($\mu\text{g g}^{-1}$ DW)	DNA P % ($\mu\text{g g}^{-1}$ DW)	Pyro P % ($\mu\text{g g}^{-1}$ DW)
0–1	0.3	35.8 (140)	35.3 (138)	0.9 (4)	7.7 (30)	16.0 (62)	4.4 (17)
1–2	0.8	33.3 (104)	33.8 (105)	1.2 (4)	8.1 (25)	20.8 (65)	2.8 (9)
2–3	1.4	36.0 (85)	33.1 (78)	1.7 (4)	9.7 (23)	16.9 (40)	2.6 (6)
3–4	2	38.5 (108)	36.6 (103)	1.0 (3)	6.5 (18)	16.2 (46)	1.2 (3)
4–5	3	39.3 (128)	33.5 (109)	1.5 (5)	9.1 (30)	16.6 (54)	ND
5–6	4	46.8 (113)	28.2 (68)	1.9 (5)	9.0 (22)	14.1 (34)	ND
6–7	5	46.0 (121)	29.3 (77)	1.7 (5)	7.8 (21)	15.2 (40)	ND
7–8	6	55.7 (149)	25.4 (68)	1.1 (3)	5.7 (15)	12.1 (32)	ND
8–9	7	54.5 (140)	22.7 (58)	3.1 (8)	8.1 (21)	11.6 (30)	ND
9–10	8	46.8 (102)	28.7 (62)	2.2 (5)	8.3 (18)	14.0 (30)	ND
14–15	12	48.7 (109)	28.6 (64)	2.7 (6)	6.9 (15)	13.1 (29)	ND
19–20	18	57.0 (131)	23.4 (54)	2.8 (6)	4.7 (11)	12.1 (28)	ND
24–25	28	57.4 (129)	25.0 (56)	2.2 (5)	6.6 (15)	8.8 (20)	ND
29–30	36	54.8 (125)	24.9 (57)	1.9 (4)	6.6 (15)	11.8 (27)	ND
34–35	47	64.3 (120)	24.4 (46)	ND	5.6 (10)	5.8 (11)	ND
39–40	56	73.8 (128)	17.6 (31)	ND	ND	8.6 (15)	ND
44–45	65	73.7 (115)	18.1 (28)	ND	ND	8.2 (13)	ND
49–50	75	75.6 (119)	15.7 (25)	ND	ND	8.7 (14)	ND
54–55	85	73.6 (127)	17.8 (31)	ND	ND	8.6 (15)	ND

the loading rate of matter was variable), a decline of any of these groups with sediment depth represents degradation that either will result in a release of P to the water column or formation of inert P compounds not extracted by NaOH-EDTA.

Decreases in both TP and individual P compound groups with age (or sediment depth) had a different trend from that indicated by external P input over the last century (Fig. 3). If external P loading is the major influence on sediment P composition, an increase in TP in the sediment profile down to the layers from the 1970s would be expected, followed by a slow steady decrease toward the sediment surface. Instead, the P concentration peak is found in the surface sediment, followed by an exponential decrease with increasing sediment depth. This indicates that processes other than external P input determine the P composition and concentration in the sediment. One prominent mechanism most likely regulating this is internal loading, including the degradation of organic P compounds and subsequent P release to the water. Assuming that the measured decline in concentration of these P forms with

increasing sediment depth (Table 2) represents degradation and subsequent release from the sediment, this decrease corresponds to a release rate of $0.28 \text{ g m}^{-2} \text{ yr}^{-1}$ for monoester P, $0.22 \text{ g m}^{-2} \text{ yr}^{-1}$ for DNA P, $0.14 \text{ g m}^{-2} \text{ yr}^{-1}$ for the P lipids, and $0.007 \text{ g m}^{-2} \text{ yr}^{-1}$ for pyro P, altogether resulting in a release rate of approximately $0.6 \text{ g P m}^{-2} \text{ yr}^{-1}$. This corresponds well with previous investigations, since Matthiesen et al. (1998) found a release of $0.3\text{--}0.9 \text{ g P m}^{-2} \text{ yr}^{-1}$ from Gotland Deep sediment.

However, it cannot be excluded that the phosphorus was transformed into inert compounds that escaped our extraction procedure. Hence, these figures represent the maximum release of P from the sediment due to the decay of these P forms. The formation of inert compounds is supported by the fact that a smaller percentage of TP is extracted from the deepest sediment layers compared with the surface sediments, indicating a larger fraction of nonextractable P compounds at depth. This might, on the other hand, be interpreted as a lower amount of labile P compounds at greater depth, since they already have been degraded and released to the water. Since sediment TP increases with decreasing sediment depth (Table 1) in a pattern not consistent with the development of the external loading over the time span represented by the sediment profile (Fig. 3), it appears plausible that P has been released from the sediment within this period.

Ahlgren et al. (2005) determined half-life times for degradation of organic P compounds in lake sediments and found degradation rates almost twice as slow as those in the Baltic proper sediments presented here. Part of this discrepancy might be due to enhanced degradation of inositol, a monoester P, under anoxic conditions (*see following*). The high degradation rate might also indicate chemical degradation, since anoxia might induce chemical processes leading to alteration, and possibly complete breakdown, of organic phosphorus compounds (Baldwin

Table 3. Half-life times of P compound groups identified in a pooled sediment sample from the Landsort area, including r^2 value and p value. Note the low r^2 value of ortho P and teichoic P, indicating different behavior of these compounds, and thus the lack of calculated half-life time and potential release.

Compound group	r^2	Half-life time (yr)	p
Monoester P	0.87	16	<0.001
Microbial P lipids	0.80	5	<0.001
Teichoic P	—	—	—
DNA	0.89	8	<0.001
Pyro P	0.55	3	<0.001
Ortho P	—	—	—

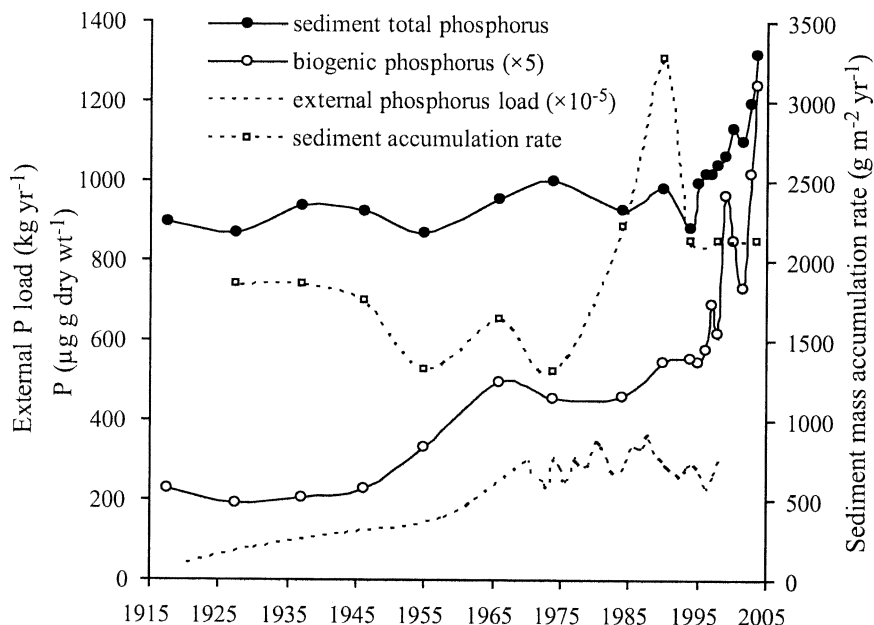


Fig. 3. Total phosphorus, biogenic phosphorus (the sum of the P compound groups showing exponential decrease with increasing sediment depth: orthophosphate monoesters, microbial P lipids, DNA P, and pyrophosphate), and sediment mass accumulation rate at sediment depths corresponding to different years. Dashed line indicates estimated external P load in the Baltic proper over the last century (data from Larsson et al. 1985 and Conley et al. 2002).

et al. 2001). Once phosphate has been mobilized from, e.g., organic compounds in the anoxic sediment, sorption to iron, a major inorganic P retention process, is absent. Formation of iron sulfide complexes inhibits iron from sorbing phosphate in marine sediments, resulting in a more pronounced P release from marine sediments compared with freshwater ones (Blomqvist et al. 2004).

Monoester P—The most abundant of the identified organic P compound groups was monoester P, a group including mononucleotides, sugar phosphates, inositol P of varying phosphorylation degrees, phospholipids, and possibly degradation products of ribonucleic acid (RNA) (Turner et al. 2003a,b). RNA, an orthophosphate diester, has been shown to degrade completely to an orthophosphate monoester within 24 h in an alkaline environment (Makarov et al. 2002b; Turner et al. 2003a) leading to a potential overestimation of the monoesters and subsequently to an underestimation of orthophosphate diesters in the extractants used in these studies. Inositol phosphate compounds, consisting of a six-carbon atom ring with varying numbers of phosphate groups attached, are considered to be the most abundant organic component in sediments, both in terms of spatial distribution (Degroot and Golterman 1993) and relative abundance (Turner et al. 2003b). This group of compounds, even though chemically inert, has been found to degrade to orthophosphate in marine sediments under anaerobic conditions (Suzumura and Kamatani 1995).

DNA P—DNA P had a half-life of less than a decade and can thus be considered to be more labile than

monoester P. According to Makarov et al. (2002b), orthophosphate monoesters are more resistant to degradation in soil samples than orthophosphate diesters. This is probably due to a higher charge density of the orthophosphate monoesters, compared with orthophosphate diesters, which enables the orthophosphate monoesters to form strong complexes with cations (Celi et al. 1999), protecting them from degradation. The same could be the case for monoester P in sediments and would be an explanation for the difference in decomposition rates, or half-lives, between the monoester P and the DNA P. Recent investigations have also shown that extracellular DNA can substantially contribute to P cycling in deep-sea sediments, accounting for an estimated 17% of total organic P regeneration (Dell'Anno and Danovaro 2005).

P lipids—As one of the minor constituents of sediment, P lipids have a half-life of only 5 yr, making them one of the most labile P compound groups found in the sediment. The small amount of P lipids compared with the total P extracted might be an indicator of low bacteria levels and thus low microbial degradation of organic matter (Makarov et al. 2002a).

Pyro P—The P compound group degrading most rapidly with increased sediment depth was pyro P, which was depleted at 5-cm sediment depth. This short half-life of only a few years indicates this compound is highly susceptible to biological and/or chemical breakdown. The pyro P may originate from esters being hydrolyzed during the alkali extraction (Anderson and Russell 1969), or it may be a remnant of polyphosphates present in bacteria in the

surface sediment. Hupfer et al. (1995) showed that a 2-h extraction in 0.1 mol L⁻¹ NaOH could hydrolyze polyphosphate into smaller fragments. It is possible the 16-h extraction used in this investigation might thus reduce polyphosphate to pyro P and perhaps even partially to ortho P. However, all pyro P found in sediment is not likely to be remnants of larger compounds being hydrolyzed during extraction. Carman et al. (2000) found significant amounts of pyro P in Baltic Sea sediments and concluded that polyphosphate was absent because of the low redox potential of anoxic sediment. This indicates that decomposition of polyphosphate during extraction is not the source for the pyro P found in this study. Additionally, Sundareshwar et al. (2001) showed the importance of pyro P in the biogeochemical phosphorus cycle in brackish estuarine sediments, where it serves as a reservoir of orthophosphate, and related its presence to human impact.

Ortho P—Most of the ortho P extracted originates from stable inorganic complexes dissolved at high pH. On average, only 10 µg P g⁻¹ DW was extracted as dissolved phosphate under anoxic conditions from the layers between 0- and 30-cm sediment depth (data not shown) during P fractionation run in parallel to extractions for NMR analyses presented here. This corresponds to less than 10% of the ortho P detected in the NaOH-EDTA extracts (Table 3).

Teichoic P—The teichoic P differed from the other organic P compound groups in the extract by lacking the exponential decrease pattern with age, showing instead a constant concentration throughout the sediment profile, which indicates a recalcitrant character in the sediments, until it reached the detection limit at 35-cm depth (approximately 1955). The presence of teichoic P at detectable concentrations in sediment younger than 1955 might be explained by an increase in external input due to the modernization of agricultural methods during this period. Bacteria will use teichoic acids to build cell walls when the P supply is large enough, but they also can use P-free teichuronic acid in cell walls in P-limited systems (Lang et al. 1982).

We show that degradation of organic P compounds in the sediment is substantial and may be an important source of P to the water column of the Baltic proper. Although the detected P groups most likely are mixtures of numerous compounds with varying lability, our results suggest major differences in reactivity among broad classes of P compounds. Accordingly, these different compound groups may contribute differently to internal P loading and eutrophication. Besides losses via the outflow, burial of P in the sediment is the major loss process from the P cycle. We suggest that transformations of organic P to forms that are either buried permanently in the sediment or released to the water column are a crucial step in the P cycle of the Baltic Sea. Based on the fact that sediment TP decreased by nearly 40% during the time span investigated here, we suggest that internal loading from the P compounds presented in this study is extensive.

References

- AHLGREN, J., L. TRANVIK, A. GOGOLL, M. WALDEBÄCK, K. MARKIDES, AND E. RYDIN. 2005. Depth attenuation of biogenic phosphorus compounds in lake sediment measured by ³¹P NMR. *Environ. Sci. Technol.* **39**: 867–872.
- ANDERSON, G., AND J. D. RUSSELL. 1969. Identification of inorganic pyrophosphate in alkali extracts of soil. *J. Soil Food Agric.* **20**: 78–81.
- BALDWIN, D. S., J. K. BEATTIE, L. M. COLEMAN, AND D. R. JONES. 2001. Hydrolysis of an organophosphate ester by manganese dioxide. *Environ. Sci. Technol.* **35**: 713–716.
- BLOMQUIST, S., A. GUNNARS, AND R. ELMGREN. 2004. Why the limiting nutrient differs between temperate coastal seas and freshwater lakes: A matter of salt. *Limnol. Oceanogr.* **49**: 2236–2241.
- CARMAN, R., G. EDLUND, AND C. DAMBERG. 2000. Distribution of organic and inorganic phosphorus compounds in marine and lacustrine sediments: A ³¹P NMR study. *Chem. Geol.* **163**: 101–114.
- CELLI, L., S. LAMACCHIA, F. A. MARSAN, AND E. BARBERIS. 1999. Interaction of inositol hexaphosphate on clays: Adsorption and charging phenomena. *Soil Sci.* **164**: 574–585.
- CONLEY, D. J., C. HUMBORG, L. RAHM, O. SAVCHUK, AND F. WULFF. 2002. Hypoxia in the Baltic Sea and basin-scale changes in phosphorus biogeochemistry. *Environ. Sci. Technol.* **36**: 5315–5320.
- DEGROOT, C. J., AND H. L. GOLTERMAN. 1993. On the presence of organic phosphate in some Camargue sediments—evidence for the importance of phytate. *Hydrobiologia* **252**: 117–126.
- DELL'ANNO, A., AND R. DANOVARO. 2005. Extracellular DNA plays a key role in deep-sea ecosystem functioning. *Science* **309**: 2179.
- EMEIS, K.-C., U. STRUCK, T. LEIPE, F. POLLEHNE, H. KUNZENDORF, AND C. CHRISTIANSEN. 2000. Changes in the C, N, P burial rates in some Baltic Sea sediments over the last 150 yr—relevance to P regeneration rates and the phosphorus cycle. *Mar. Geol.* **167**: 43–59.
- HECKY, R. E. 1998. Low N:P ratios and the nitrogen fix: Why watershed nitrogen removal will not improve the Baltic, p. 85–115. *In* A.-G. Dahlberg [ed.], *Effects of nitrogen in the aquatic environment*, Report 1. The Royal Swedish Academy of Sciences.
- [HELCOM] Helsinki Commission 2005. Nutrient pollution to the Baltic Sea in 2000. *Balt. Sea Environ. Proc.* No. 100.
- HUPFER, M., R. GÄCHTER, AND H. RÜEGGER. 1995. Polyphosphate in lake sediments: ³¹P NMR spectroscopy as a tool for its identification. *Limnol. Oceanogr.* **40**: 610–617.
- , B. RUBE, AND P. SCHMIEDER. 2004. Origin and diagenesis of polyphosphate in lake sediments: A ³¹P-NMR study. *Limnol. Oceanogr.* **49**: 1–10.
- LANG, W. K., K. GLASSEY, AND A. R. ARCHIBALD. 1982. Influence of phosphate supply on teichoic acid and teichuronic acid content of *Bacillus subtilis* cell walls. *J. Bacteriol.* **151**: 367–375.
- LARSSON, U., R. ELMGREN, AND F. WULFF. 1985. Eutrophication and the Baltic Sea: Causes and consequences. *Ambio* **14**: 9–14.
- MAKAROV, M. I., L. HAUMAIER, AND W. ZECH. 2002a. The nature and origins of diester phosphates in soils: A P-31-NMR study. *Biol. Fertil. Soils* **35**: 136–146.
- , ———, AND ———. 2002b. Nature of soil organic phosphorus: An assessment of peak assignment in the diester region of ³¹P NMR spectra. *Soil. Biol. Biochem.* **34**: 1467–1477.
- MATTHIENSEN, H., K.-C. EMEIS, AND B. T. JENSEN. 1998. Evidence for phosphate release from sediment in the Gotland Deep during oxic bottom water conditions. *Meyniana* **50**: 175–190.

- MEILI, M., P. JONSSON, AND R. CARMAN. 1998. Cs dating of laminated sediments in Swedish archipelago areas of the Baltic Sea. Sateilyturvakeskus, [Rapportti] STUK-A. (STUK-A145), 127–130.
- MORRIS, R. J., Å. NIEMI, L. NIEMISTÖ, AND E-L. POUTANEN. 1988. Sedimentary record of seasonal production and geochemical fluxes in a nearshore coastal embayment in the northern Baltic Sea. *Finn. Mar. Res.* **256**: 77–94.
- RÖNNBERG, C., AND E. BONSDORFF. 2004. Baltic Sea eutrophication: Area-specific ecological consequences. *Hydrobiologia* **154**: 227–241.
- STÄHLNACKE, P., A. GRIMVALL, K. SUNDBLAD, AND A. TONDESKI. 1999. Estimation of riverine loads of nitrogen and phosphorus to the Baltic Sea, 1970–1993. *Environ. Monit. Assess.* **58**: 173–200.
- SUNDARESHWAR, P. V., J. T. MORRIS, P. J. PELLECHIA, H. J. COHEN, D. E. PORTER, AND B. C. JONES. 2001. Occurrence and ecological implications of pyrophosphate in estuaries. *Limnol. Oceanogr.* **46**: 1570–1577.
- SUZUMURA, M., AND A. KAMATANI. 1995. Mineralization of inositol hexaphosphate in aerobic and anaerobic marine-sediments—implications for the phosphorus cycle. *Geochim. Cosmochim. Acta* **59**: 1021–1026.
- TURNER, B. L., E. FROSSARD AND D. S. BALDWIN [EDS.]. 2005. Organic phosphorus in the environment. CABI.
- , N. MAHIEU, AND L. M. CONDRON. 2003a. Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. *Soil. Sci. Soc. Am. J.* **67**: 497–510.
- , ———, AND ———. 2003b. Quantification of myo-inositol hexakisphosphate in alkaline soil extracts by solution P-31 NMR spectroscopy and spectral deconvolution. *Soil Sci.* **168**: 469–478.

Received: 18 November 2005

Accepted: 18 March 2006

Amended: 30 March 2006