

Fractionation of sediment phosphorus revisited: II. Changes in phosphorus fractions during sampling and storing in the presence or absence of oxygen

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Abstract

The purpose of this study was to evaluate the sensitivity of a phosphorus (P) fractionation procedure on sampling practices and other sources of error in the characterization of the chemical status of P in noncalcareous sediments rich in humic compounds and iron (Fe). We investigated the effects of nitrogen gas (N₂) shielding on the stability of various P fractions during sampling and various periods of sample storage on both oxic and reduced sediments. In addition, we studied storage-induced changes in preserved or unpreserved extracts from different fractionation steps. Sampling and storage conditions were critical factors in P fractionation studies. During sampling, sample storage, and extraction, the N₂ atmosphere (atm) protected samples from oxidation and consequent changes in P forms, especially in the redox-sensitive form. Long-term storage without N₂-shielding caused the most drastic changes in oxic sediment, whereas the reduced sediment protected with N₂ experienced only minor changes. Extracts preserved with acid can be stored for at least 1 week without significant changes. The extraction results of P and other elements obtained with both oxic and reduced sediment samples supported the biogeochemical basis of the fractionation procedure, demonstrated by the behavior of Fe and Fe-bound P in sediment originating from and treated in different oxygen (O₂) conditions. Despite a few shortcomings, with careful laboratory work and avoidance of sample oxidation during sampling and storage, the fractionation procedure and working practices used seem to provide reliable results and valuable information on the chemical character of sediment P.

One reason for the accelerated eutrophication of the stratified Baltic Sea may be the phosphorus (P) release from the recent bottom deposits. The release of P is traditionally attributed to oxygen (O₂) depletion and the consequent reduction of

oxidized iron (Fe) compounds, resulting in the liberation of Fe-bound P (Einsele 1938; Mortimer 1941, 1942). Even though P release is more pronounced during O₂ depletion, researchers have also found P liberation to occur during oxic conditions in the sediment water interface (e.g., Nriagu and Dell 1974; Boström et al. 1982; Hupfer et al. 2004). In the presence of O₂, the P release has been attributed to microbial degradation of organic material or to the increased degree of saturation of P-binding sites in the sediment. In the latter case, the sorption affinity of phosphate (PO₄-P) diffusing from the reduced layers to the oxic surface will decrease with the increasing saturation of hydrated oxides of Fe or aluminum (Al) due to an abundance of PO₄-P or other anions competing from the same sites. In addition, physical mixing of the sediment by burrowing animals can result in plumes of porewater PO₄-P from the deeper layers. The oxidized surface layer is incapable of retaining sorption of all P in this mass flow. Drake and Heaney (1987) proposed that when the surface water of high pH (especially during high algal production) mixes with the fluffy surface sediment of lower pH, the elevated hydroxyl ion (OH⁻) concentration can induce release of PO₄-P. pH can also affect the

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binding of $\text{PO}_4\text{-P}$ to humic complexes via Fe (Gerke and Hermann 1992). In addition, Jensen and Andersen (1992) have shown temperature to affect P release in oxic lake sediment surfaces.

Oxygen depletion is a major concern in the shallow areas of the Baltic Sea, such as the Archipelago Sea (AS) and the Gulf of Finland (GoF). The GoF possesses deep sedimentation areas that have long remained under anoxic conditions, as well as areas where the O_2 concentration varies because of degradation of organic matter or bottom morphology. In the coastal area with small semi-isolated basins (Voipio 1981), the morphological patchiness weakens the exchange of water. In the central GoF, inflows of heavy anoxic near-bottom water from the Baltic proper often keep the sediment-water interface anoxic.

A commonly used method to characterize and quantify sediment P is chemical fractionation that separates sequentially different forms of P in the order of decreasing bioavailability and increasing binding strength. Distribution of P into various forms allows evaluation of the part of sediment P that may contribute to the primary production in the aquatic system and the part that will be permanently buried with sediment. The literature describes numerous P fractionation procedures for sediments (e.g., Boström et al. 1982; Van Eck 1982; Pettersson et al. 1988; Ruban et al. 1999). The suitability of a certain procedure to characterize P forms depends not only on the environment and on the type of the material to be studied, but also on the type of material for which the procedure was originally designed. For instance, the content of clay minerals, organic matter, and calcareous minerals can affect the ability of various schemes with different extractants to distinguish between different P forms.

Many of the P fractionation studies are made using dried sediment (e.g., Williams et al. 1971; Ruttenberg 1992; Filippelli and Delaney 1996). This, however, affects results and leads to loss of important information, because exposure to air oxidizes reduced components (e.g., Fe compounds) that consequently bind dissolved P from porewater (e.g., Krom and Berner 1980). In addition, drying and freezing may result in disruption of cells and hydrolyzation of organic matter containing P and, thus, increase the labile P fraction (e.g., Goedkoop and Pettersson 2000). Several studies describe the specificity of extraction procedures for pure phases of commonly occurring P compounds in the marine environment (e.g., the precise work by Ruttenberg 1992), but only a few articles report the effects of treatment practices on P fractions in anaerobic samples (e.g., Wiltshire 1991). A common practice to avoid oxidation during sampling and extraction is to shield the sample with N_2 . This is an important precautionary measure while working with anoxic sediment samples, because oxidation of the sediment enhances the binding of $\text{PO}_4\text{-P}$ via ferri-iron (Fe^{3+}) compounds that are formed (Einsele 1938; Mortimer 1941, 1942) and also affects P-binding properties via oxidation-induced changes in pH. Furthermore, enhanced microbial degradation of organic matter due to elevated temperature (and O_2

content) or increased exposure to light may result in marked changes in samples during storage.

Generally, major changes in the sample properties are avoided by storing the sample at in situ temperature and analyzing it as soon as possible after sampling. For practical reasons, this is not always possible when working on a research vessel. P fractionation is time consuming and involves an unavoidable time lag in sample treatment. Therefore, it is important to know which kinds of changes can occur in separate P pools during long-term storage of the sediment samples and how to avoid such changes.

Recently, we tested (Lukkari et al. 2007) the relevancy and reproducibility of the sediment P fractionation procedure proposed by Psenner et al. (1984) and later modified by Jensen and Thamdrup (1993) with a calcareous commercial reference sediment. The aim of this present work was to study further the suitability of this procedure for non-calcareous GoF sediments rich in humic material and Fe. We focused special attention on how sediment samples should be treated and stored when extracted fresh, i.e., on the impact of O_2 conditions and N_2 shielding during sampling and long-term storage on the stability of P pools in anoxic and oxic sediment samples. Furthermore, we carried out an additional study related to the performance of the procedure: the effect of storage on dissolved and total P in extracts.

Materials and procedures

Sampling and sediment properties—We collected sediment samples with a Gemax-gravity corer (two polycarbonate cylinders, 9 cm in diameter and about 60 cm in length), during two cruises on R/V *Aranda*. The oxic surface sediment came from site AS5 in Paimionlahti Bay, AS (August 2003; Fig. 1). The water depth at this site was 33 m, the concentration of dissolved O_2 in the near bottom water was 1.7 mL L^{-1} , the salinity was 6.3 PSU, and the concentration of dissolved $\text{PO}_4\text{-P}$ was 0.9 $\mu\text{mol L}^{-1}$ about 5 cm above the sediment surface. The sediment was brown and oxic on the surface, followed by a reduced, black-colored layer below a depth of 2.5 cm. We collected the reduced sediment sample (dark gray colored) from site GF2B (in April 2004) in a deep sedimentation basin in the center of the GoF (Fig. 1) at a water depth of 85 m. Dissolved O_2 in the near bottom water was 0.8 mL L^{-1} , salinity was 9.5 PSU, and dissolved $\text{PO}_4\text{-P}$ was 3.4 $\mu\text{mol L}^{-1}$. We determined dissolved O_2 and $\text{PO}_4\text{-P}$ in the water column above the sediment surface using standard methods in seawater analyses: Winkler titration for O_2 (Grasshoff 1983) and a spectrophotometrical measurement (Lachat autoanalyser) for $\text{PO}_4\text{-P}$ (Koroleff 1983). Total digestion with aqua regia/fluoric acid/boric acid and the following inductively coupled plasma atomic emission spectrophotometry (ICP-AES) analysis revealed that the total P (TP_{sed}) content in the reduced sample was 47.9 $\mu\text{mol g}^{-1}$ dry weight (DW). In the oxic sample, it was 73.8 $\mu\text{mol g}^{-1}$ DW. Total Fe in the reduced sample was 950 $\mu\text{mol g}^{-1}$ DW, and total manganese (Mn) was 29.7 μmol

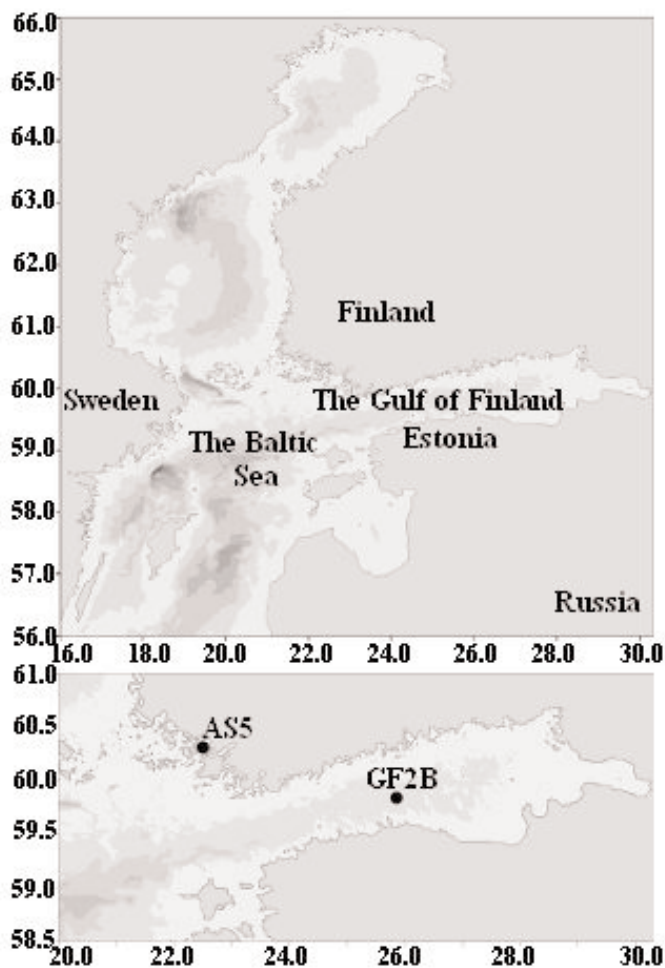


Fig. 1. Sampling sites for oxic (AS5) and reduced (GF2B) sediments

g^{-1} DW. In the oxic sample, total Fe was $1062 \mu\text{mol g}^{-1}$ DW, and total Mn was $15.4 \mu\text{mol g}^{-1}$ DW.

Sample cores were immediately set on a slicing table and covered with a large glove bag connected tightly to another glove bag. Glove bags were sealed and filled with N_2 (purity of 99.5%) until the O_2 content in the bags fell below 5% to 10% (detected using Gas Alert detectors calibrated against fresh air containing 20% to 21% O_2). We sampled the water column above the sediment surface and vacuum filtered (Nuclepore polycarbonate [PC] membranes, pore size $0.40 \mu\text{m}$) the

water sample for $\text{PO}_4\text{-P}$ analysis. The rest of the water above the sediment was removed. The sediment was immediately sliced and sealed into plastic containers in the adjacent glove bag, which was cooled with ice and kept anaerobic ($\text{O}_2 < 5\%$). Sediment containers were immediately vacuum packed (Tecla s.n.c Vacum 33) into gas-tight plastic bags using N_2 as a shielding gas and stored at $+5^\circ\text{C}$ (in dark) until analyzed.

Determination of sediment dry matter content—The amount of sediment sample extracted in this study was based on DW rather than on fresh weight (FW) (commonly used in P fractionation studies) to obtain a constant solid-to-solution ratio irrespective of the varying water content of different samples. Dry matter (DM) content was determined using a moisture analyzer: a balance equipped with a halogen lamp dryer (Ohaus MB45) that heats a small amount (0.5–1.0 g) of fresh sample at 140°C until no more weight loss occurs. We tested the accuracy of this method by comparing the DM results of two sediment samples of different water contents with those obtained using the standard method (ISO 11465; drying at 105°C for 24 h) and found the results to be similar.

P fractionation—The extraction procedure we used closely follows the five-step procedure (Table 1) described in Jensen and Thamdrup (1993), which is a modification of the procedure presented by Psenner et al. (1984). We made slight additional modifications to the procedure as well as to the treatment of the sample extracts. The procedure and working practices are described in detail in the previous work by the authors (Lukkari et al. 2007). Briefly, a fresh sediment sample (corresponding to 0.500 g DW) is extracted first with sodium chloride (NaCl) to remove loosely adsorbed and porewater P (NaCl-iP), then with sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) in sodium bicarbonate (NaHCO_3) buffer (pH 7.0) to separate any redox-sensitive fraction of P bound to hydrated oxides of reducible metals—mainly those of Fe (NaBD-iP). The next extraction with sodium hydroxide (NaOH) removes P bound to oxides of non-reducible Fe and Al (NaOH-iP). NaOH also extracts a marked part of alkali-soluble organic P compounds (nonreactive P [NRP]). In the fourth step, hydrochloric acid (HCl) serves to extract calcium (Ca)-bound P, mainly from apatite minerals (HCl-iP). Steps I–IV included one or more rinsing steps (15 min) with 0.46 M NaCl, or with the extraction solution used in each particular step, to complete the extraction and to minimize tailing. After the fourth step, refractory, mainly organic P, is determined by extracting the residue with

Table 1. Principle of the P fractionation procedure (modified from Jensen and Thamdrup 1993)

Step	Extractant	Separated P fraction
I	0.46 M NaCl (1 h)	Loosely sorbed P, porewater P
II	0.11 M NaBD (1 h)	Redox sensitive (Fe, Mn-bound) P
III	0.1 M NaOH (18 h)	P from Al oxides, non-reducible Fe oxides, and organic P (mobile)
IV	0.5 M HCl (1 h)	Apatite and other inorganic P
Ignition of the sediment residue, 2 h at 550°C .		
V	1 M HCl (16 h)	Refractory P, mainly organic P (immobile)

HCl after combustion. When these P fractions are roughly classified into immobile and mobile forms, the mobile pool is considered to include NaCl-iP, NaBD-iP, and NRP, while NaOH-iP, HCl-iP, and Res-P form the immobile, potentially buried P pool (Jensen et al. 1995).

The volume of the extractants and rinsing solutions was 50.0 mL, yielding a sediment DW-to-solution ratio of 1:100. All extraction steps were carried out using 100 mL polypropylene (PP) tubes and an orbital shaker table (400 rpm, at room temperature). Sample homogenization, sub-sampling for DM determination, weighing, and the first two extraction steps were carried out in a glove box in N₂ atm (O₂ content < 5%), except for the samples treated in normal atm after sampling (see below). In steps I-II, extraction tubes of the samples treated in N₂ atm (flushed with N₂ before sealing and caps tightened with laboratory parafilm) were sealed into gas-tight plastic bags filled with N₂ in the glove box. After each extraction and rinsing step, the samples were centrifuged (4000 rpm for 15 min at room temperature), and the supernatant was poured immediately into PP sample bottles. All plastic equipment used was rinsed with 10-fold diluted 65% nitric acid (HNO₃) and a couple of times with MilliQ-water before use. All reagents were of p.a. grade. Blank samples (two or three replicates) were run in each set of samples to detect possible contamination originating from chemicals, equipment, or working practices, but PO₄-P concentrations in these were usually negligible. We included commercial reference material BCR-684 in each set of samples to check the comparability of separate fractionations.

Pretreatment and analysis of extracts—We determined dissolved inorganic P (DIP, referred later as -iP) from filtered (Nuclepore PC-membranes, pore size 0.4 µm) and acid-preserved (sulphuric acid, H₂SO₄) samples with the molybdate-blue method of Murphy and Riley (1962), modified by Koroleff (1983). Acid was not added to extracts containing HCl. The final concentration of H₂SO₄ (0.1 M) in the mixed extract was chosen to be that recommended for phosphate determination to optimize color development of phosphomolybdate complex and to keep FE in ferro-form (and to decrease interference of silicate; Koroleff 1983). We made determinations with a Genesys 10uv Thermo Spectronic UV-VIS spectrophotometer (with flow injection cuvette of 50 mm) and prepared PO₄-P standards and controls in acidified MilliQ-water. We determined total P (TP) from nonfiltered extract as DIP, after acid persulphate digestion (Koroleff 1983; autoclaving time 1 h) and calculated nonreactive P (NRP) as the difference between TP and DIP. Differently from that presented in Jensen and Thamdrup (1993) and Paludan and Jensen (1995), we analyzed filtered NaOH extracts for TP to yield NRP in the dissolved (low molecular weight, LMW) fraction. As a result, we were able to separate NRP that had remained in particulate form (high molecular weight, HMW) after 18 h NaOH extraction. We assumed that NaOH extracts organic P compounds that vary highly in their degradability, and that the LMW part

of the NaOH-extractable NRP is more available than the HMW part. Consequently, the LMW portion of the NRP extracted with NaOH, as well as NRP in the NaCl and NaBD extracts (that together form the total NRP fraction), are assumed to represent the mobile fraction of P together with NaCl-iP and NaBD-iP. Particulate, HMW NRP in the NaOH extract and the remaining inorganic (NaOH-iP and HCl-iP) and residual P are considered immobile P.

We separated part of the filtered and acidified extracts from steps I-IV to determine total dissolved (T_{diss}) Fe, Mn, Al, Ca, magnesium (Mg), silicon (Si), and P. Measurements were made with ICP-OES in the laboratory of the Institute for Environmental Research at the University of Jyväskylä. Analytical limits were 10.0 mg L⁻¹ for Fe and Mn, and 20.0 mg L⁻¹ for the other elements. Standards for ICP-OES determinations were prepared in NaCl acidified to pH 2.

Assessment

Sample treatment during the experiment—To assess the effects of sample treatment and storage on the P fractions of a reduced sediment, a homogenous 4-cm section (from depth 3-7 cm) was taken from site GF2B in a single sediment core and sealed in a plastic container in N₂ atm. This sample was carefully homogenized (with plastic spoon), sub-sampled for DM determination, and divided into two portions. One portion was treated always in N₂ atm (O₂ content kept carefully below 5%), and the container was sealed in a gas-tight plastic bag filled with N₂. The other portion was allowed to react with atmospheric O₂ (normal atm treatment) for 30 min by mixing the sample gently before sealing the container. We chose a reaction time of 30 min, because it was the estimated time the sample would be subjected to O₂ while sampling and storing on board. Both samples were cooled with ice during handling and stored at +5°C (in the dark).

We carried out a corresponding treatment and storing experiment with oxic surface sediment. For that experiment, we took two parallel sediment cores (from site AS5), and the topmost 3-cm oxic, light-brown colored sediment layer was sliced and pooled in N₂ atm into one sample. After careful homogenization, the sample was split into two portions and treated in the same manner as the reduced sediment above.

To assess how long-term storage affects various P forms and to see if sampling and storing in N₂ atm protects the sample from oxidation, both sub-samples (the N₂-shielded and the one allowed to react with O₂) of the reduced sediment (site GF2B) were fractionated four times at different time intervals. We started the first extraction 30 min after sampling on board, and the last one after almost 13 weeks of storage (either with or without N₂-shielding) at +5°C. Correspondingly, both sub-samples from the oxic sediment (site AS5) were fractionated for the first time 2 weeks after sampling, and the extractions were repeated five times; the last extraction was made after a 40-week storage period.

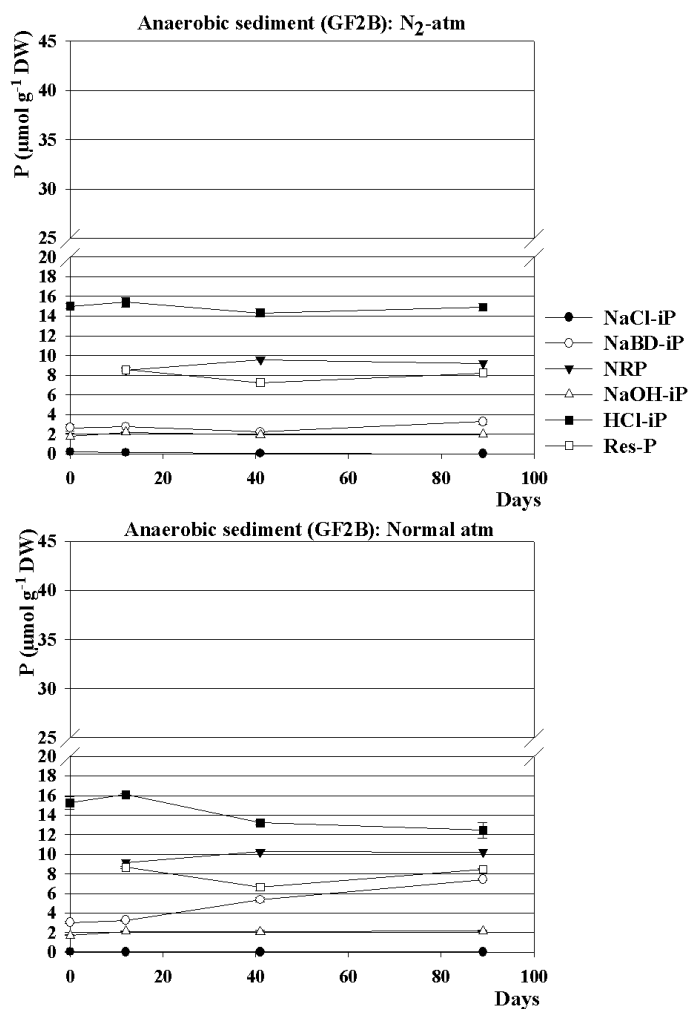


Fig. 2. P fractions of the reduced (GF2B) sediment during long-term storage, either with (above) or without (below) N_2 shielding

We tested the statistical significance of differences in P fractions resulting from the long-term storage using the t test for paired samples, and the differences between the two practices of sample treatment and storage using the t test for unpaired samples (allowing unequal variances). Differences of $P < 0.050$ between average values were considered significant.

Impact of N_2 -shielding on P fractions in the reduced sediment—When the reduced sediment (GF2B) was extracted immediately after sampling without N_2 -shielding, NaCl-iP was lower (Fig. 2); on the contrary, NaBD-iP was somewhat higher (though not statistically significant) than when not subjected to O_2 . This difference also occurred after 12 d of storage: NaCl-iP was lower and NaBD-iP, higher (both differences significant) in the sample treated in normal atm. In addition, NRP was higher under normal atm treatment, though not significantly. From day 41 onward, we extracted only two replicates of the sample shielded with N_2 and thus could not calculate statistical differences

between the treatments. Nevertheless, some differences are obvious. NaBD-iP was clearly higher (by $3.2 \mu\text{mol P g}^{-1} \text{ DW}$) in the sample subjected to O_2 than in the N_2 -shielded one, and after 89 d of storage, the difference became even more obvious ($4.1 \mu\text{mol P g}^{-1} \text{ DW}$). After day 41, no further increase occurred in NRP. Res-P and HCl-iP were higher in the sample shielded with N_2 . On day 89, the sample subjected to O_2 exhibited a clearly lower HCl-iP, but a slightly higher NRP.

We studied changes in the P fractions in the reduced sample during long-term storage under contrasting O_2 conditions by comparing the fractionation results obtained on day 0 with those obtained after 12, 41, and 89 d of storage (results are unavailable for Res-P and NRP on day 0). As a general trend, storage affected various P forms more severely if the reduced sample was treated and stored in contact with O_2 (Fig. 2). The most pronounced changes occurred by day 41. If only trends in each P fraction are considered, NaCl-iP, HCl-iP, and Res-P were almost stable or tended slightly to decrease irrespective of N_2 -shielding. On the other hand, HCl-iP tended to decrease strongly in the sample subjected to O_2 . This sample also showed a strong increasing trend in NaBD-iP and an increasing trend in NRP. NaOH-iP, in turn, remained rather constant or showed only a slightly increasing trend with time under both storing conditions.

We found statistically significant storage-induced changes on days 12 and 41: NaCl-iP decreased under both storing conditions, and HCl-iP in normal atm. Furthermore, in the presence of O_2 , NaOH-iP increased on day 12, and NaBD-iP, on day 41. A shortage of sample material allowed only two replicates on day 89.

Impact of N_2 -shielding on other elements in the reduced sediment—In addition to P, the sampling and storing conditions also affected the other elements in the reduced sediment (Table 2) measured at steps I-IV. While we could not test statistical significance (only two replicate determinations), we did observe certain trends.

$T_{\text{diss}} \text{Fe}$ in the NaCl extracts of the reduced sediment was generally very low, except for one higher concentration (day 89) in the sample subjected to O_2 . In NaBD, $T_{\text{diss}} \text{Fe}$ increased during storage, and was from two to eight times higher in the N_2 -shielded sample. In NaOH, $T_{\text{diss}} \text{Fe}$ fluctuated in both samples, with greater variation in the O_2 -affected one. The highest $T_{\text{diss}} \text{Fe}$ concentrations were in HCl, and were about 1.5 times higher in the N_2 -shielded sample.

From day 12 onwards, subsection to O_2 increased $T_{\text{diss}} \text{Mn}$ about 2- to 3-fold in NaCl, while it began to diminish markedly in NaBD. Contact with O_2 seemed not to affect $T_{\text{diss}} \text{Mn}$ in NaOH, but caused a clear decreasing trend in HCl with prolonged storing time.

In NaCl and NaBD, $T_{\text{diss}} \text{Al}$ was generally low, but was highest in HCl, showing a slightly increasing trend with prolonged storing time, while the storing conditions seemed to have no clear effect. Storing conditions did not affect $T_{\text{diss}} \text{Al}$ in NaOH, either. In NaCl, $T_{\text{diss}} \text{Ca}$ remained almost constant if the sample was shielded with N_2 , but if unshielded, increased with

Table 2. Dissolved total elements in various fractions of the reduced sediment during long-term storage, either with or without N₂ shielding

Element content ($\mu\text{mol g}^{-1}$ DW)	Day 0		Day 12		Day 41		Day 89	
	Normal	N ₂	Normal	N ₂	Normal	N ₂	Normal	N ₂
NaCl T _{diss} Mg	102 ± 6	107 ± 0	115 ± 2	104 ± 1	118 ± 2	98.0 ± 0.5	121 ± 1	101 ± 0
NaCl T _{diss} Si	4.3 ± 0.1	7.0 ± 0.5	3.1 ± 0.2	6.0 ± 0.2	5.3 ± 0.2	5.4 ± 0.1	11.8 ± 1.8	3.7 ± 0.1
NaCl T _{diss} Al	0.1 ± 0	0.2 ± 0	0.3 ± 0.2	0.2 ± 0	0.3 ± 0	0 ± 0	17.3 ± 0.3	0.3 ± 0.1
NaCl T _{diss} Ca	35.9 ± 1.6	37.9 ± 0.2	45.7 ± 0.9	36.8 ± 0.6	54.9 ± 0.9	35.5 ± 0.2	60.5 ± 0.1	39.5 ± 1.3
NaCl T _{diss} P	0 ± 0	0.2 ± 0	0 ± 0	0.2 ± 0	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0
NaCl T _{diss} Mn	5.2 ± 0.2	6.7 ± 0.3	15.4 ± 0.3	6.1 ± 0.1	17.5 ± 0.4	6.4 ± 0.1	18.6 ± 0.2	13.6 ± 0.7
NaCl T _{diss} Fe	0.2 ± 0	0.3 ± 0	0.2 ± 0.2	0 ± 0	0.5 ± 0	0.1 ± 0.2	5.7 ± 0.1	0.2 ± 0.1
NaBD T _{diss} Mg	4.1 ± 0	3.9 ± 0.2	2.6 ± 0	3.9 ± 0.3	2.0 ± 0.3	3.8 ± 0	1.6 ± 0.1	3.7 ± 0
NaBD T _{diss} Si	14.1 ± 0.3	11.2 ± 0	11.0 ± 0.4	10.7 ± 0	12.6 ± 0.9	12.8 ± 0.6	17.9 ± 0.1	10.7 ± 1.3
NaBD T _{diss} Al	0.6 ± 0	0.3 ± 0.3	1.4 ± 0.6	0.8 ± 0.1	2.5 ± 0.6	1.2 ± 0.2	1.6 ± 0	0.8 ± 0.4
NaBD T _{diss} Ca	5.5 ± 0.1	5.2 ± 0	3.3 ± 0	4.2 ± 0.5	4.1 ± 0.1	4.4 ± 0	2.0 ± 0	4.7 ± 0.5
NaBD T _{diss} P	2.4 ± 0.2	2.4 ± 0.2	2.5 ± 0.1	1.6 ± 0.6	3.4 ± 0.4	2.0 ± 0.1	6.0 ± 0	3.2 ± 1.0
NaBD T _{diss} Mn	10.4 ± 0.5	10.3 ± 1.0	2.5 ± 0.1	9.6 ± 0	1.1 ± 0	8.9 ± 0	0.5 ± 0	3.1 ± 0.6
NaBD T _{diss} Fe	89.3 ± 2.0	16.5 ± 1.0	95.0 ± 1.5	39.8 ± 3.9	144 ± 1	18.1 ± 0.1	157 ± 4	74.4 ± 7.2
NaOH T _{diss} Mg	0.5 ± 0	4.5 ± 5.3	1.6 ± 1.3	0.4 ± 0.4	2.8 ± 2.7	2.1 ± 0.2	0.9 ± 0.2	1.6 ± 1.1
NaOH T _{diss} Si	558 ± 19	575 ± 53	612 ± 2	548 ± 3	581 ± 1	521 ± 25	642 ± 17	560 ± 17
NaOH T _{diss} Al	53.4 ± 1.5	64.1 ± 11.4	59.0 ± 1.0	55.2 ± 3.5	95.2 ± 48.7	81.4 ± 25.3	48.7 ± 2.7	56.7 ± 0.8
NaOH T _{diss} Ca	0.1 ± 0.1	0.6 ± 0.3	0 ± 0	0.1 ± 0.1	0.4 ± 0.5	0.4 ± 0	0 ± 0	0.1 ± 0.2
NaOH T _{diss} P	5.9 ± 0.2	6.4 ± 0.9	5.6 ± 0.2	6.1 ± 0.1	7.4 ± 1.1	6.8 ± 0	7.0 ± 0.6	6.8 ± 1.0
NaOH T _{diss} Mn	0.2 ± 0	0.3 ± 0.2	0.2 ± 0	0.2 ± 0	0.3 ± 0.2	0.3 ± 0	0.1 ± 0	0.2 ± 0
NaOH T _{diss} Fe	3.8 ± 0.1	13.1 ± 13.4	6.4 ± 1.3	4.3 ± 1.4	10.1 ± 7.6	10.1 ± 0.9	3.5 ± 0.2	5.4 ± 3.0
HCl T _{diss} Mg	42.4 ± 0.6	40.4 ± 0.6	43.3 ± 2.7	42.3 ± 2.7	49.0 ± 2.5	43.9 ± 2.5	42.7 ± 2.8	40.5 ± 2.8
HCl T _{diss} Si	92.0 ± 0	89.7 ± 0	107 ± 7	97.9 ± 7.4	124 ± 7	104 ± 7	98.1 ± 7.6	93.8 ± 7.6
HCl T _{diss} Al	93.6 ± 0.2	89.0 ± 0.2	98.6 ± 5.0	89.1 ± 5.0	114 ± 5	95.0 ± 4.6	98.9 ± 5.4	90.7 ± 5.4
HCl T _{diss} Ca	41.5 ± 0.2	41.8 ± 0.2	37.9 ± 2.4	41.0 ± 2.4	31.6 ± 1.5	41.2 ± 1.5	23.9 ± 1.1	32.9 ± 1.1
HCl T _{diss} P	16.0 ± 0.2	15.1 ± 0.2	16.0 ± 1.4	15.2 ± 1.4	14.5 ± 0.8	15.9 ± 0.8	12.2 ± 0.5	14.8 ± 0.5
HCl T _{diss} Mn	2.6 ± 0.1	3.1 ± 0.1	2.3 ± 0.2	3.0 ± 0.2	2.0 ± 0.1	3.1 ± 0.1	1.4 ± 0.1	2.2 ± 0.1
HCl T _{diss} Fe	110 ± 0	162 ± 0	122 ± 7	152 ± 7	103 ± 7	171 ± 7	94.4 ± 4.3	126 ± 4

All results appear as $\mu\text{mol g}^{-1}$ DW. 0 ± 0 = calculated result < 0.05 $\mu\text{mol g}^{-1}$ DW.

prolonged storing time, probably at the expense of the NaBD fraction, which showed a slightly decreasing trend. In NaOH, T_{diss}Ca was very low and showed no clear changes. On the contrary, in HCl, the effect of storing conditions was clear: if the sample was shielded with N₂, T_{diss}Ca remained constant until it dropped on day 89; in the presence of O₂ it decreased almost linearly. Storing conditions had no clear effect on T_{diss}Mg in NaBD, NaOH, or HCl. In NaCl, however, T_{diss}Mg decreased only slightly in the N₂-shielded sample, whereas the decrease was more pronounced in the unshielded sample. The same trend occurred for T_{diss}Si in NaCl. In NaBD, NaOH, and HCl, T_{diss}Si fluctuated and was slightly higher in the unshielded sample.

Interpretation of fractionation results of the reduced sediment—Sample treatment and storage experiments on the reduced sediment sample showed the oxidation reaction of Fe to be very rapid: in the sample subjected to O₂, soluble NaCl-iP was bound to sediment and transferred to the NaBD-iP fraction immediately after sampling. On the contrary, when contact with O₂ was avoided, NaCl-iP remained in the porewater or in

loosely bound form. Also, Wiltshire (1991) noticed a higher concentration of NaCl-extractable P in reduced sediment treated under anaerobic conditions than under aerobic conditions. Even though Wiltshire's (1991) results are not well suited for comparison to the results in this study because of the different fractionation procedure, they demonstrate the need for anaerobic treatment when studying P forms in reduced sediments. Because ferro-iron (Fe²⁺) oxidizes to ferric iron (Fe³⁺) in several minutes (e.g., Stumm and Morgan 1996), the 30-min reaction time with atmospheric O₂ in this experiment was sufficient for oxidation of Fe²⁺, as evinced by the 5-fold higher T_{diss}Fe in NaBD in the sample subjected to O₂ than in that shielded with N₂. If the sample was protected from O₂, NaCl was higher while NaBD was lower in T_{diss}Si and T_{diss}Mn, suggesting the release of Fe-bound Si and the reduction and dissolution of Mn.

A further increase in NaBD-iP and T_{diss}Fe in NaBD in the unshielded sample after day 12 indicates the continuing oxidation of Fe²⁺ as O₂ diffuses to the inner parts of the sediment

sample (always mixed, when sub-sampled), and the entrapment of released P to Fe^{3+} . The simultaneous disappearance of $T_{\text{diss}}\text{Mn}$ from NaBD may result from its extraction already in the NaCl step, because the reduction of Mn^{4+} takes place at a higher redox-potential than does that of Fe^{3+} . $T_{\text{diss}}\text{Fe}$ extracted in NaOH differed less between the treatments than did $T_{\text{diss}}\text{Fe}$ extracted in NaBD (except on day 0), which suggests that $T_{\text{diss}}\text{Fe}$ was not in a redox-sensitive form in NaOH.

When the originally reduced sediment was allowed to react with O_2 , NRP increased during the 12 d of storage. This may indicate the formation of microbial polyphosphates that are NaOH-extractable and defined as NRP (Hupfer et al. 1995). Uhlmann et al. (1990) reported polyphosphate formation taking place preferentially in aerobic conditions. During long-term storage, P was slowly released from the Res-P pool irrespective of the storing conditions, which may indicate anaerobic and aerobic microbial degradation or the hydrolyzation of refractory organic material. For example, Vandenbergue et al. (1985) suggested microbial degradation of refractory organic material.

Long-term storage diminished the HCl-iP pool more when the sample was subjected to O_2 . The presence of O_2 likely allowed the oxidation of pyrite (FeS_2) that may have been present (dark gray color of the sediment), resulting in the formation of H_2SO_4 . The acid is strong enough to dissolve some apatitic P on particle surfaces. This response coincided with the increase of $T_{\text{diss}}\text{Ca}$ in the NaCl extract and its simultaneous decrease in HCl. The lower $T_{\text{diss}}\text{Fe}$ in HCl throughout the experiment in the presence of O_2 supports this conclusion. Fe^{3+} produced in oxidation of FeS_2 was likely transferred to a redox-sensitive form and observed as an increase in $T_{\text{diss}}\text{Fe}$ in NaBD (see above). In addition, some increase of $T_{\text{diss}}\text{Ca}$ in NaCl may have resulted from acids produced by sediment bacteria, as Harrison et al. (1972) showed in laboratory experiments with several P precipitates. Changes in $T_{\text{diss}}\text{Fe}$, $T_{\text{diss}}\text{Al}$, $T_{\text{diss}}\text{Si}$, or $T_{\text{diss}}\text{Mg}$ extracted with HCl were not marked and probably attributable to the heterogeneity of the samples.

Impact of sampling and storing conditions on P fractions in the oxic sediment—In the oxic surface sediment (site AS5) extracted 13 d after sampling (Fig. 3), if the sample was subjected to O_2 , NaCl-iP was lower, whereas NaBD-iP and NRP were slightly (though not statistically significantly) higher. We observed the same differences between the storing conditions on day 26 and found the most drastic changes in various P forms on the 119th day of storage: NRP, and especially NaBD-iP, were clearly higher when unshielded with N_2 . The obvious difference in NaBD-iP between the storing treatments proved insignificant with the *t* test, possibly because of differences in variances. After the 260th day of storage, the differences between the two storing treatments remained the same (now statistically significantly) as in the previous extraction: in the sample that was allowed to react with O_2 , NaBD-iP and NRP were higher, and NaCl-iP and Res-P fractions were lower. At the end of this experiment (day

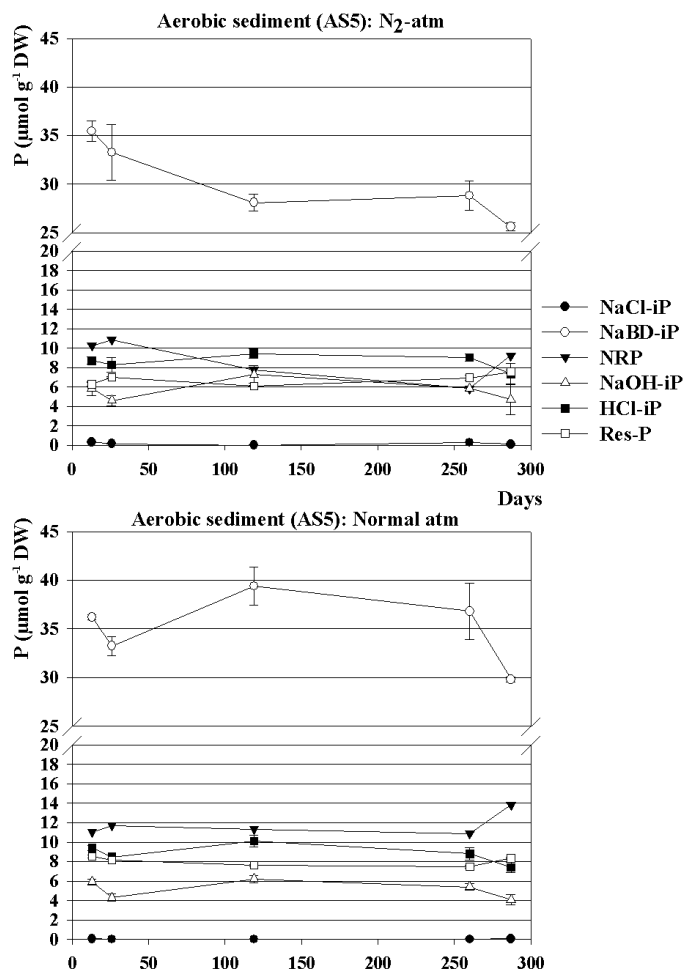


Fig. 3. P fractions of the oxic (AS5) sediment during long-term storage, either with (above) or without (below) N_2 shielding

287), we observed the same reaction pattern, even though we did not test for it statistically (only two replicate samples).

We studied changes in the various P fractions in the oxic sample during long-term storage under contrasting O_2 conditions by comparing the fractionation results obtained on day 13 to those obtained after days 26, 119, 260, and 287 of storage (Fig. 3). Generally, the fraction the storage most severely affected was NaBD-iP, which showed a clearly decreasing trend irrespective of storing conditions, even though it varied in the unshielded sample. NRP showed a slightly increasing trend in the O_2 -affected sample, whereas it showed a mainly decreasing trend in the N_2 -shielded sample. Even though HCl-iP remained relatively constant, it decreased slightly in the unshielded sample, and Res-P increased slightly in the N_2 -shielded one. NaCl-iP and NaOH-iP remained almost stable with prolonged storage.

The only statistically significant change in the N_2 -shielded sample by day 26 was a decrease of NaCl-iP. In the sample

Table 3. Dissolved total elements in various fractions of the oxic sediment during long-term storing, either with or without N₂-shielding

Element content ($\mu\text{mol g}^{-1}$ DW)	Day 13		Day 26		Day 119		Day 260		Day 287	
	Normal	N ₂	Normal	N ₂	Normal	N ₂	Normal	N ₂	Normal	N ₂
NaCl T _{diss} Mg	*	*	*	*	128 ± 1	113 ± 3	114 ± 2	117 ± 4	129 ± 5	112 ± 1
NaCl T _{diss} Si	3.5 ± 0.1	3.4 ± 0.2	4.2 ± 0.2	3.2 ± 0	4.4 ± 0.1	3.5 ± 0.1	5.8 ± 0.2	3.3 ± 0.1	6.4 ± 0.1	3.9 ± 0.2
NaCl T _{diss} Al	0.2 ± 0	0.3 ± 0.1	0 ± 0	0 ± 0	0.6 ± 0.1	0.3 ± 0	3.3 ± 0.1	0.4 ± 0.1	2.1 ± 0.1	0.4 ± 0.1
NaCl T _{diss} Ca	44.7 ± 1.6	39.9 ± 1.3	53.3 ± 1.7	38.2 ± 0.3	54.8 ± 0.1	45.8 ± 0.7	60.4 ± 1.3	42.1 ± 1.8	52.9 ± 2.1	43.4 ± 0.3
NaCl T _{diss} P	0.1 ± 0.1	0.5 ± 0	0 ± 0	0.3 ± 0	0 ± 0	0 ± 0	0 ± 0	0.4 ± 0	0 ± 0	0 ± 0
NaCl T _{diss} Mn	6.3 ± 0.2	2.0 ± 0.1	7.4 ± 0.5	2.4 ± 0	8.2 ± 0.1	3.1 ± 0.2	9.2 ± 0.3	1.1 ± 0.1	8.1 ± 0.2	3.8 ± 0.1
NaCl T _{diss} Fe	7.9 ± 0.8	0.3 ± 0	6.5 ± 0.1	0 ± 0	0.2 ± 0	0.2 ± 0	0.3 ± 0	0.3 ± 0	0.7 ± 0.1	0.7 ± 0
NaBD T _{diss} Mg	*	*	*	*	4.0 ± 0.4	8.1 ± 0.6	3.5 ± 0.3	11.0 ± 1.2	2.8 ± 0.1	5.2 ± 0.6
NaBD T _{diss} Si	*	*	9.3 ± 0	8.6 ± 1.1	15.2 ± 1.0	10.2 ± 0.8	16.8 ± 0.7	11.5 ± 0.9	15.6 ± 0.6	11.6 ± 0.8
NaBD T _{diss} Al	1.3 ± 0.2	0.7 ± 0.2	1.6 ± 0.1	0.7 ± 0	5.2 ± 0.4	0.6 ± 0.1	5.5 ± 0.4	0.8 ± 0.2	4.6 ± 0.1	1.4 ± 0
NaBD T _{diss} Ca	5.7 ± 0.5	8.4 ± 1.1	2.3 ± 0.1	6.1 ± 1.4	2.0 ± 0.4	4.4 ± 0.4	1.3 ± 0.2	6.6 ± 0.4	1.6 ± 0.3	4.5 ± 0.2
NaBD T _{diss} P	40.3 ± 0.7	39.7 ± 0.9	35.7 ± 0.9	35.5 ± 3.3	47.3 ± 5.0	37.7 ± 1.9	40.6 ± 4.5	32.0 ± 2.9	29.8 ± 2.1	27.8 ± 0
NaBD T _{diss} Mn	2.4 ± 0.1	5.4 ± 0.3	1.4 ± 0.1	4.9 ± 0.6	1.5 ± 0.1	5.3 ± 0.2	1.1 ± 0.1	6.1 ± 0.7	0.9 ± 0	3.4 ± 0.1
NaBD T _{diss} Fe	247 ± 6	78.6 ± 5.1	314 ± 6	147 ± 10	365 ± 21	44.4 ± 5.4	341 ± 19	41 ± 42	305 ± 18	113 ± 3
NaOH T _{diss} Mg	*	*	*	*	0.1 ± 0.2	0 ± 0	4.6 ± 0.7	1.9 ± 0	0.7 ± 0.6	0.1 ± 0.2
NaOH T _{diss} Si	365 ± 1	341 ± 5	362 ± 22	330 ± 6	350 ± 7	341 ± 12	370 ± 28	377 ± 12	285 ± 2	268 ± 21
NaOH T _{diss} Al	98.0 ± 1.5	87.9 ± 1.3	101 ± 8	97.3 ± 5.9	105 ± 2	99.4 ± 3.9	102 ± 8	98.9 ± 1.3	81.8 ± 4.1	75.6 ± 5.1
NaOH T _{diss} Ca	0 ± 0	0 ± 0	0 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
NaOH T _{diss} P	10.6 ± 0.7	11.4 ± 0.8	10.2 ± 1.5	10.2 ± 1.3	12.8 ± 0.6	15.0 ± 0.6	10.1 ± 1.3	12.0 ± 0.9	8.9 ± 0.5	8.6 ± 1.4
NaOH T _{diss} Mn	0.1 ± 0.1	0 ± 0	0.4 ± 0.7	0 ± 0	0 ± 0	0 ± 0	0.1 ± 0	0.1 ± 0	0 ± 0	0 ± 0
NaOH T _{diss} Fe	7.5 ± 5.6	2.5 ± 0.5	5.7 ± 4.9	2.6 ± 2.2	1.8 ± 0.2	1.9 ± 0.1	9.7 ± 1.8	7.2 ± 0.6	1.5 ± 0	1.4 ± 0
HCl T _{diss} Mg	*	*	*	*	64.8 ± 4.1	56.0 ± 1.2	55.2 ± 3.2	49.2 ± 2.5	50.6 ± 4.2	49.9 ± 4.4
HCl T _{diss} Si	106 ± 2	106 ± 5	135 ± 14	116 ± 11	121 ± 5	118 ± 6	124 ± 8	121 ± 113	112 ± 8	113 ± 12
HCl T _{diss} Al	129 ± 2	131 ± 7	147 ± 15	141 ± 15	162 ± 9	157 ± 5	138 ± 7	144 ± 8	119 ± 9	126 ± 13
HCl T _{diss} Ca	21.6 ± 5.0	18.8 ± 0.8	18.1 ± 2.0	18.0 ± 2.2	17.8 ± 1.2	19.7 ± 1.6	18.0 ± 1.4	21.1 ± 0.3	15.3 ± 1.4	16.7 ± 2.2
HCl T _{diss} P	9.4 ± 0.3	8.5 ± 0.4	9.6 ± 1.2	8.7 ± 0.9	11.0 ± 0.8	9.8 ± 0.5	9.9 ± 0.7	10.3 ± 0.3	7.8 ± 0.7	7.9 ± 1.1
HCl T _{diss} Mn	1.4 ± 0	2.0 ± 0.1	1.4 ± 0.1	1.9 ± 0.3	1.5 ± 0.1	2.8 ± 0.1	1.2 ± 0.1	2.7 ± 0.2	1.0 ± 0.1	1.8 ± 0.3
HCl T _{diss} Fe	124 ± 4	256 ± 8	121 ± 13	223 ± 40	116 ± 8	302 ± 4	103 ± 8	294 ± 16	87.8 ± 7.8	199 ± 31

All results appear as $\mu\text{mol g}^{-1}$ DW; 0 ± 0 = calculated result < 0.05 $\mu\text{mol g}^{-1}$ DW.

*Not determined.

subjected to O₂, in turn, NaCl-iP, NaOH-iP, and Res-P decreased significantly. Marked changes occurred in P forms by day 119: NaCl-iP and NaBD-iP diminished despite the N₂-shielding, whereas both NaCl-iP and Res-iP decreased significantly in the presence of O₂. The overall change in NaBD-iP by day 119 (3.2 $\mu\text{mol P g}^{-1}$ DW) in the unshielded sample remained statistically insignificant (possibly due in part to variable variances). On day 260, N₂ shielding had diminished NaBD-iP, whereas the presence of O₂ diminished NaCl-iP. When comparing the results of the first and the last extraction (day 287), N₂ shielding had protected the sample from changes other than a decrease in NaCl-iP and NaBD-iP. In the sample subjected to O₂, NaBD-iP-, NaOH-iP-, and HCl-iP had decreased significantly. The statistical significance of the 1.6 $\mu\text{mol P g}^{-1}$ DW increase noticed in NRP in the O₂-affected sample was impossible to assess (only two replicate samples).

Impact of sampling and storing conditions on other elements in the oxic sediment—The storage-induced changes in the other elements extracted from the oxic sediment sample at various

extraction steps appear in Table 3. The most pronounced differences occurred for T_{diss}Fe, which clearly showed higher concentrations in the NaBD and, again, clearly lower concentrations in the HCl of the O₂-affected sample. In addition, T_{diss}Fe increased in NaBD and decreased in HCl with prolonged storing time in the presence of O₂, while the effects of storage were less clear in the N₂-shielded sample. T_{diss}Fe was usually lower in NaOH, and especially in NaCl, than in NaBD and HCl. In NaOH, T_{diss}Fe showed no clear trend, but was somewhat higher in the O₂-affected sample.

We recorded the highest T_{diss}Mn concentrations in NaCl. The concentration was higher in the sample subjected to O₂ and increased with prolonged storing time. In NaBD, the situation was the opposite: the N₂-shielded sample contained a higher T_{diss}Mn concentration with no clear trend, whereas the sample subjected to O₂ showed a decreasing trend. T_{diss}Mn was almost undetectable in the NaOH and was also low in the HCl.

T_{diss}Al was generally very low in NaCl and NaBD, and even slightly lower in the N₂-shielded sample. NaOH and HCl,

instead, extracted high quantities of $T_{\text{diss}}\text{Al}$ that were only slightly affected by prolonged storage.

$T_{\text{diss}}\text{Ca}$ showed the highest concentrations in NaCl, and the O_2 -affected sample showed a clearly higher concentration than did the N_2 -shielded sample, and the former also showed an increasing trend with time. Rather, in NaBD, $T_{\text{diss}}\text{Ca}$ was higher in the N_2 -shielded sample, and prolonged storing time diminished it in both treatments. $T_{\text{diss}}\text{Ca}$ was undetectable in NaOH, and in HCl showed a decreasing trend with no clear influence of the N_2 shielding. Although $T_{\text{diss}}\text{Mg}$ results are unavailable from the first two extractions, it later showed the same trends as did $T_{\text{diss}}\text{Ca}$ and was highest in NaCl with somewhat higher concentrations in the O_2 -affected sample. In NaBD, $T_{\text{diss}}\text{Mg}$ was higher in the N_2 -shielded sample, while in NaOH or HCl the storing condition showed no clear effect. In NaCl, $T_{\text{diss}}\text{Si}$ was low and showed higher concentrations in the O_2 -affected sample which increased with time. We observed the same response to both storing condition and time for $T_{\text{diss}}\text{Si}$ in NaBD. Although we found the highest $T_{\text{diss}}\text{Si}$ concentrations in NaOH, the diverse storing conditions showed no clear effects and the prolonged storage caused only a slight decrease. In HCl, neither the treatment nor the prolonged storage seemed clearly to affect $T_{\text{diss}}\text{Si}$.

Interpretation of fractionation results of the oxic sediment—The results of the sample treatment and storage experiment with the oxic surface sediment were in accordance with those obtained with the reduced sediment. The first extraction after a 13-d storage period showed that under storage without N_2 -shielding, NaCl-iP diminished as a consequence of more effective P binding to sediment. That N_2 shielding showed little effect on NaBD-iP indicates that the amount of O_2 transported to the container with oxic sediment was sufficient to prevent the release of Fe-bound P for up to 26 d of storage. $T_{\text{diss}}\text{Fe}$ in NaBD on days 13 and 26 support this conclusion. Although the N_2 shielding diminished $T_{\text{diss}}\text{Fe}$ in NaBD, $T_{\text{diss}}\text{Fe}$ was decisively higher in NaBD in the originally oxic than in the anoxic sediment.

NaCl- $T_{\text{diss}}\text{Ca}$ was higher in the sample subjected to O_2 (the same was true for the reduced sediment) and increased with prolonged storing time at the expense of a concomitant decrease in $T_{\text{diss}}\text{Ca}$ in HCl. This suggests the chemically (or microbially) induced dissolution of Ca-bound P discussed earlier. The O_2 conditions had no effect on other HCl extractable elements. The increase of $T_{\text{diss}}\text{Si}$ in NaCl in the presence of O_2 with prolonged storing may indicate the dissolution of biogenic Si (e.g., diatoms) during long-term storage (see Tallberg and Koski-Vähälä 2001). If this was the case, the same phenomenon may also partly explain the increase in Ca content in the same sample.

As expected on the basis of the classical binding theory of P in aquatic sediments (Einsele 1938; Mortimer 1941, 1942), the constant decrease in NaBD-iP was rather closely accompanied by a decreasing trend in $T_{\text{diss}}\text{Fe}$ in the N_2 -shielded sample. Even though the presence of O_2 tended to decrease NaBD-iP, its concentration level remained higher than that of the

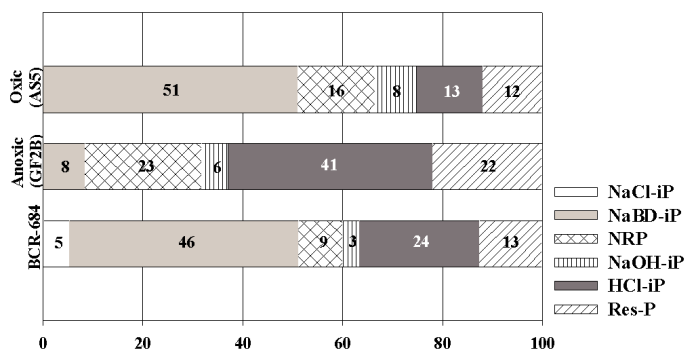


Fig. 4. Percent share of separated P fractions of total extractable P in reduced (GF2B) and oxic (AS5) sediment. P fractions of a calcareous reference material (BCR-684) are included for comparison.

N_2 -shielded sample. The presence of O_2 maintained NaBD-extractable $T_{\text{diss}}\text{Fe}$ high, and its concentration trend tended to increase. $T_{\text{diss}}\text{Al}$ extracted with NaBD followed the same trend as did $T_{\text{diss}}\text{Fe}$. Even though Al is an irreducible metal, this response indicates the co-occurrence of these metals in oxides and their concomitant release as a result of slow reduction in a closed container during prolonged storage. A higher NaBD- $T_{\text{diss}}\text{Si}$ in the sample subjected to O_2 and a higher NaCl- $T_{\text{diss}}\text{Mn}$ in the sample shielded with N_2 indicate the redox-dependent binding and desorption of these elements.

$T_{\text{diss}}\text{Al}$ or $T_{\text{diss}}\text{Si}$ extracted in NaOH changed only slightly during the experiment, except in the last extraction, and O_2 conditions seemed not to affect them. However, the organic P pool of the NaOH extract (the bulk of the total NRP) was higher in the sample subjected to O_2 during storage. This finding may be attributable to the microbial formation of polyphosphates (Hupfer et al. 1995). In the unshielded sample, the lower NRP coincided with slightly higher Res-P.

Comparison of P fractions in different sediment types—General comparison of the P fractions of the reduced and the oxic Baltic Sea sediment with those in the reference material (Fig. 4) reveals that the procedure successfully distinguished between various P forms in the different sediment types. The dominant portion of NaBD-iP in the oxic Baltic Sea sediment is obvious when compared with the reduced one. P bound to oxides of nonreducible metals (NaOH-iP) was also more abundant in the oxic sediment collected from an estuary receiving river-transported material. On the contrary, the portions of mobile organic P (NRP) and the immobile Res-P and HCl-iP were larger in the reduced open sea sediment. The calcareous reference material originating from the River Po, Italy (Ruban et al. 2001), contained a higher portion of HCl-iP than did the oxic Baltic Sea sediment; it also contained a considerable portion of NaCl-iP not observed in the Baltic Sea sediments. This may result in part from the drying of the reference material (see Ruban et al. 2001) where the predominant portion of NaBD-iP indicates that it was highly oxidized. Small portions of NRP and NaOH-iP, on the other hand,

suggest lower contents of organic and surface-bound P than in the two Baltic Sea sediments.

Effect of storage on P in preserved or unpreserved extracts—We investigated storage-induced changes in DIP and TP with extracts preserved with 4.5 M H₂SO₄. We made this test with a bulk sample obtained by combining the extracts of five replicates of reduced subsurface sediment. One portion of the bulk sample was filtered, preserved, and determined for DIP, and the other portion was preserved and then determined for TP two or three times during 1 week (stored at +5°C). We made an additional experiment to determine storage-induced changes in DIP and TP in unpreserved extracts. This test was made as described above, except that these bulk samples were stored unfiltered and unpreserved at +5°C, and only a portion of those were filtered, acidified, and determined after 0, 1, 4, 7, and 14 d of storage.

In unpreserved extracts, 1 d of storage decreased the TP in NaOH and in HCl (0.6 and 0.4 μmol P g⁻¹ DW, respectively) (Fig. 5). After 3 d, DIP had increased and TP had decreased in NaOH (0.4 and 0.3 μmol P g⁻¹ DW, respectively). Compared to day 0, TP also decreased in NaBD (by 0.6 μmol P g⁻¹ DW). On day 7, DIP had increased in NaOH and in HCl (0.6 and 0.4 μmol P g⁻¹ DW, respectively). TP, however, had decreased in NaBD and increased in NaOH (0.5 and 0.3 μmol P g⁻¹ DW, respectively). After 14 d, however, TP had increased in NaBD.

In preserved NaOH extracts, DIP had decreased (0.3 μmol P g⁻¹ DW) on day 7, while TP (unfiltered) had decreased (0.7 μmol P g⁻¹ DW) on day 4. The highest change occurred in HCl: 7 days of storage decreased both DIP and TP (1.1 and 0.6 μmol P g⁻¹ DW, respectively). When evaluating the changes in NRP in steps I-III (i.e., TP-DIP), the only notable change was the decrease (0.7 μmol P g⁻¹ DW) of HMW-NRP (unfiltered) part in the NaOH extract on day 4.

These results show that the preservation of extracts, if filtered (for DIP) and stored at +5°C, allows satisfactory P determinations even after a 1-week delay. Storage-induced changes were generally more obvious in unpreserved extracts than in the preserved ones and the organic NaOH fraction was among the most sensitive. The addition of acid probably causes some hydrolyzation of organic P to PO₄-P. We only used one sediment type where the concentration of organic matter was typical for the central GoF. The effect of acid-induced hydrolyzation could be more pronounced in samples higher in organic matter. NaOH-extractable NRP constitutes the bulk of total NRP, and thus its decrease with prolonged storage would affect the total NRP fraction. A decrease of TP in NaOH, however, might partly result from an uneven distribution of particulates in the sub-sample separated for TP determination. A decrease of DIP and TP in HCl is difficult to explain; growth of bacteria could be one explanation if the solution was not too acid.

Discussion

The classification of wide variety of naturally-occurring P compounds into a few pools makes chemical fractionation

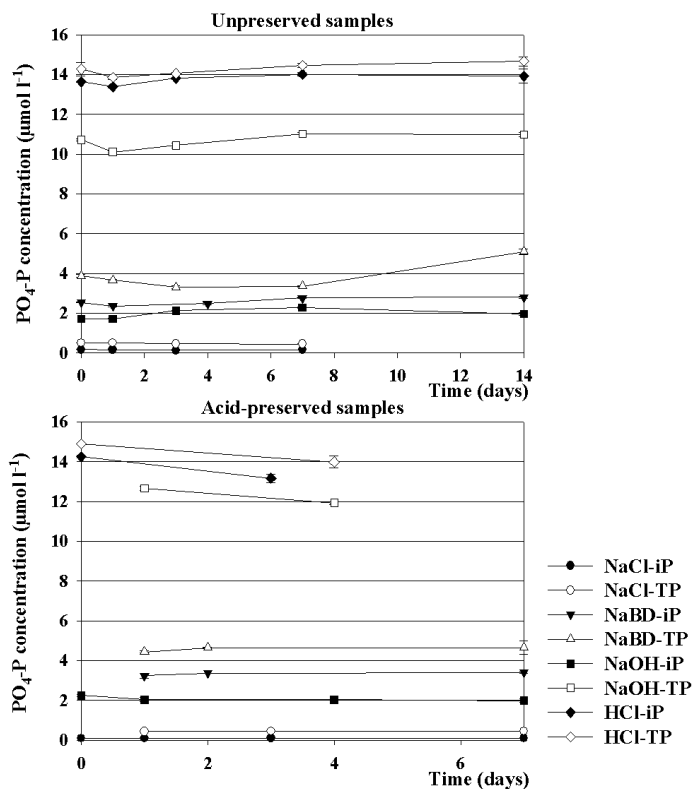


Fig. 5. Effect of storage on unpreserved (above) and acid-preserved (below) extracts from separate experiments.

methods rather coarse. Still, characterization of the chemical nature of sediment P allows us to assess the part of sediment P that can be, under certain environmental conditions, available for production in the marine environment. On the other hand, sequential extraction provides useful information on the share and forms of P that do not participate in the nutrient cycle, but are potentially buried with sediment, thus removing P from the marine ecosystem.

The sediment P fractionation procedure and the working practices used in this study provide reliable results in separating different forms of P in Baltic Sea sediments rich in humic matter and Fe, but poor in Ca-carbonates. The results of P and co-occurring elements obtained from the fractionation of oxic and anoxic sediment support the biogeochemical basis of the method as it was previously found for commercial reference material (Lukkari et al. 2007). This allows us to conclude that the procedure can adequately separate different P fractions not only in oxic and anoxic sediment with high humic and Fe content, but also in Ca-rich carbonate sediments.

While working with fresh sediment material, however, especially with reduced sediments common in the Baltic Sea, the prevailing conditions should be taken into account in sampling, storage, and further treatment of the samples. As an overall conclusion of this study, the most pronounced changes in the mobile P fractions take place during or soon after sampling and storing the samples. These initial steps of

the work are thus the most critical when characterizing P in the sediment. In this study, the smallest storage-induced changes in different P fractions occurred in the reduced sediment, if the sample was shielded with N₂ during sampling, storage, and fractionation (during the first two steps). If, however, the reduced sediment was subjected to atmospheric O₂, marked changes occurred, especially in the redox-sensitive, loosely bound, and organic P fractions. Generally, long-term storage caused more pronounced changes in P fractions in the oxic surface sediment. However, higher variation in P fractions of the oxic sediment might partly result from the longer time intervals between fractionations. N₂-shielding of the originally oxic sediment seemed to cause no marked changes in P fractions: only after about 4 weeks of storage did the portion of redox-sensitive NaBD-iP begin to decrease. The P fractions most severely affected by treatment or storage were NaCl-iP and NaBD-iP, whereas NaOH-iP proved rather stable. This fraction represents P bound to hydrated oxides of non-reducible metals with ligand exchange reactions and is considered permanently bound in sediments (Jensen and Thamdrup 1993; Kleeberg and Dudel 1997). From these results, we can conclude that sediment samples should be protected from oxidation during sampling and fractionated as soon as possible after sampling. However, if this is impossible, shielding with N₂ during storing is recommended.

The previous studies with the dry, homogenous reference material had revealed the good reproducibility of the procedure for different P fractions as well as for the co-occurring elements (Lukkari et al. 2007). Careful homogenization and DM-based weighing of samples with routine laboratory work enable well-reproducible results for fresh sediment material as well. Additional studies carried out in this work showed that the P concentration of the acid-preserved extracts could be determined reliably within a few days.

References

- Boström, B., M. Jansson, and C. Forsberg. 1982. Phosphorus release from lake sediments. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* 18:5-59.
- Drake, J. C., and S. I. Heaney. 1987. Occurrence of phosphorus and its potential remobilization in the littoral sediments of a productive English lake. *Freshw. Biol.* 17:513-523.
- Einsele, W. 1938. Über chemische und kolloidchemische Vorgänge in Eisen-Phosphat-Systemen unter limnochemischen und limnogeologischen Gesichtspunkten. *Arch. Hydrobiol. Bd.* 33:361-387.
- Filippelli, G. M., and M. L. Delaney. 1996. Phosphorus geochemistry of equatorial Pacific sediments. *Geochim. Cosmochim. Acta* 60(9):1479-1495.
- Gerke, J., and R. Hermann. 1992. Adsorption of orthophosphate to humic-Fe-complexes and to amorphous Fe-oxide. *Z. Pflanzenernähr. Bodenk.* 155:233-236.
- Goedkoop, W., and K. Pettersson. 2000. Seasonal changes in sediment phosphorus forms in relation to sedimentation and benthic bacterial biomass in Lake Erken. *Hydrobiologia* 431:41-50.
- Grasshoff, K. 1983. Determination of oxygen, p. 61-72. *In* Grasshoff, K., M. Ehrhardt, and K. Kremling. [eds.], *Methods of seawater analysis*, 2nd ed. Verlag Chemie GmbH.
- Harrison, M. J., R. E. Pacha, and R. Y. Morita. 1972. Solubilization of inorganic phosphates by bacteria isolated from Upper Klamath Lake sediment. *Limnol. Oceanogr.* 17(1):50-57.
- Hupfer, M., R. Gächter, and H. Rügger. 1995. Polyphosphate in lake sediments: ³¹P NMR spectroscopy as a tool for its identification. *Limnol. Oceanogr.* 40(3):610-617.
- , B. Rube, and P. Schmieder. 2004. Origin and diagenesis of polyphosphate in lake sediments: A ³¹P-NMR study. *Limnol. Oceanogr.* 49(1):1-10.
- Jensen, H. S., and F. Ø. Andersen. 1992. Importance of temperature, nitrate, and pH for phosphate release from aerobic sediments of four shallow, eutrophic lakes. *Limnol. Oceanogr.* 37(3):577-589.
- , P. B. Mortensen, F. Ø. Andersen, E. Rasmussen, and A. Jensen. 1995. Phosphorus cycling in a coastal marine sediment, Aarhus Bay, Denmark. *Limnol. Oceanogr.* 40(5):908-917.
- and B. Thamdrup. 1993. Iron-bound phosphorus in marine sediments as measured by bicarbonate-dithionite extraction. *Hydrobiologia* 253:47-59.
- Kleeberg, A., and G. E. Dudel. 1997. Changes in extent of phosphorus release in a shallow lake (Lake Großer Müggelsee; Germany, Berlin) due to climatic factors and load. *Mar. Geol.* 139:61-75.
- Koroleff, F. 1983. Determination of phosphorus, p. 125-142. *In* Grasshoff, K., M. Ehrhardt, and K. Kremling [eds.], *Methods of seawater analysis*, 2nd ed. Verlag Chemie GmbH.
- Krom, M. D., and R. A. Berner. 1980. The diffusion coefficients of sulfate, ammonium, and phosphate ions in anoxic marine sediments. *Limnol. Oceanogr.* 25(2):327-337.
- Lukkari, K., H. Hartikainen, and M. Leivuori. 2007. Fractionation of sediment phosphorus revisited: I. Fractionation steps and their biogeochemical basis. *Limnol. Oceanogr. Meth.* 5:433-444.
- Mortimer, C. H. 1941. The exchange of dissolved substances between mud and water in lakes. *J. Ecol.* 29:280-329.
- . 1942. The exchange of dissolved substances between mud and water in lakes. *J. Ecol.* 30:147-201.
- Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta.* 27:31-36.
- Nriagu, J. O., and C. I. Dell. 1974. Diagenetic formation of iron phosphates in recent lake sediments. *Am. Mineral.* 59:934-946.
- Paludan, C. and H. S. Jensen. 1995. Sequential extraction of phosphorus in freshwater wetland and lake sediment: significance of humic acids. *Wetlands* 15(4):365-373.

- Pettersson, K., B. Boström, and O.-S. Jacobsen. 1988. Phosphorus in sediments – speciation and analysis. *Hydrobiologia* 170:91-101.
- Psenner, R., R. Pucsko, and M. Sager. 1984. Die Fraktionierung organischer und anorganischer Phosphorverbindungen von Sedimenten. Versuch einer Definition ökologisch wichtiger Fraktionen. [Fractionation of organic and inorganic phosphorus compounds in lake sediments. An attempt to characterize ecologically important fractions.] *Arch. Hydrobiol. Suppl.* 70(1):111-155.
- Ruban, V., J. F. López-Sánchez, P. Pardo, G. Rauret, H. Muntau, and P. Quevauviller. 1999. Selection and evaluation of sequential extraction procedures for the determination of phosphorus forms in lake sediment. *J. Environ. Monit.* 1: 51-56.
- , G. Rauret, J. F. López-Sánchez, H. Muntau, and P. Quevauviller. 2001. EUR 19776 – The certification of the extractable contents (mass fractions) of phosphorus in freshwater sediment following a five-step extraction procedure, BCR-684. BCR information series. Luxemburg.
- Ruttenberg, K. C. 1992. Development of a sequential extraction method for different forms of phosphorus in marine sediments. *Limnol. Oceanogr.* 37(7):1460-1482.
- Stumm, W., and J. J. Morgan. 1996. *Aquatic chemistry. Chemical equilibria and rates in natural waters*, 3rd ed. Wiley.
- Tallberg, P., and J. Koski-Vähälä. 2001. Silicate-induced phosphate release from surface sediment in eutrophic lakes. *Arch. Hydrobiol.* 151(2):221-245.
- Uhlmann, D., I. Röske, M. Hupfer, and G. Ohms. 1990. A simple method to distinguish between polyphosphate and other phosphate fractions of activated sludge. *Wat. Res.* 24(11): 1355-1360.
- Vandenbroucke, M., R. Pelet, and Y. Debyser. 1985. Geochemistry of humic substances in marine sediments, p. 249-273. In G. R. Aiken, D. M. McKnight, R. L. Wershaw, and P. MacCarthy [eds.], *Humic substances in soil, sediment, and water. Geochemistry, isolation, and characterization*. Wiley.
- Van Eck, G. T. M. 1982. Forms of phosphorus in particulate matter from the Hollands Diep/Haringvliet, The Netherlands. *Hydrobiologia* 92:665-681.
- Voipio, A. [ed.]. 1981. *The Baltic Sea*. Elsevier Oceanography Series, 30. Elsevier Scientific Publishing Company.
- Williams, J. D. H., J. K. Syers, R. F. Harris, and D. E. Armstrong. 1971. *Soil Sci. Soc. Amer. Proc.* 35:250-255.
- Wiltshire, K. H. 1991. Experimental procedures for the fractionation of phosphorus in sediments with emphasis on anaerobic techniques. *Verh. Internat. Verein. Limnol.* 24: 3073-3078.

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