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**PAPER**

# Diagenesis of settling seston: identity and transformations of organic phosphorus

**Kasper Reitzel,*[a] Joakim Ahlgren,[b] Emil Rydin,[c] Sara Egemose,[a] Benjamin L. Turner[d] and Michael Hupfer[e]**

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Solution $^{31}$phosphorus NMR spectroscopy and sequential fractionation were used to follow diagenetic changes in phosphorus forms during decomposition of settling seston in Lake Nordborg, a shallow eutrophic lake in Denmark. In a decomposition experiment, seston released >60% of their total phosphorus during ~50 days incubation, although seston collected during summer contained more phosphorus and released it over a longer period compared to seston collected during spring. Seston decomposition increased concentrations of potentially bioavailable polyphosphate and phosphodiesters, but also promoted the formation of refractory phosphorus forms that might be buried permanently in the sediment. Combining these results with *in situ* measurements of phosphorus concentrations in lake water and sediment traps revealed that the release from settling seston plays only a minor role in the accumulation of phosphorus in the hypolimnion of Lake Nordborg.

## 1. Introduction

Identifying the sources and forms of bioavailable phosphorus (P) in lakes has been a focus of aquatic research for several decades, but progress has been limited by a lack of analytical techniques for identifying different P forms at the molecular level. A key process is diagenetic transformation of particulate P, both during and after sedimentation. Analysis of sediment cores has been used to demonstrate changes in P pools over decadal periods[1–3] and to predict P release under changing environmental conditions.[4,5] However, few studies have investigated the impact of settling seston on P cycling in the water column or on sediment P composition. Pettersson[6] followed P transformations in settling seston using sequential fractionation and showed a gradual decrease in total P (TP) from suspended matter to settling particles and surface sediment, while more recent studies demonstrated that settling seston can contribute significantly to P accumulation in the hypolimnion of stratified deep lakes.[4,7,8] The accumulation of P in the hypolimnion is therefore the sum of P released from both the sediment and settling seston.

Solution $^{31}$P nuclear magnetic resonance (NMR) spectroscopy has been widely applied to aquatic systems.[9–12] This technique allows the detailed study of P turnover by identifying P compounds or functional groups, including orthophosphate (ortho-P), orthophosphate monoesters (monoester-P), orthophosphate diesters (diester-P), pyrophosphate (pyro-P)[13] and polyphosphates (poly-P).[14–17]

Here we use solution $^{31}$P NMR spectroscopy in combination with a sequential fractionation procedure[18] to follow the fate of organic P during decomposition of settling seston in a shallow eutrophic Danish Lake. This involved a combination of *in vitro* measurements of changes in P forms during seston decomposition with *in situ* measurements of P in lake water and sediment traps. The results allowed us to follow the transport and forms of

[a]*Institute of Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark. E-mail: reitzel@biology.sdu.dk; Fax: +4565930457; Tel: +4565502774*
[b]*Department of Aquatic Science and Assessment, Swedish University of Agricultural Sciences, P.O. Box 7050, SE-75007 Uppsala, Sweden*
[c]*Department of Ecology and Genetics, Uppsala University, Norbyvägen 18 D, SE-75 236 Uppsala, Sweden*
[d]*Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Republic of Panama*
[e]*Leibniz Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 301, D-12587 Berlin, Germany*

### Environmental impact

Limited knowledge exists on organic phosphorus turnover in lakes, and how the organic P contributes to the P concentration in the lake. This study focuses on release and transformations of inorganic/organic P forms from seston. We demonstrate that mineralization of seston leads to a release of mainly inorganic P from labile P species, characterized by sequential P extraction. In addition, diagenesis leads to formation of recalcitrant P compounds such as *e.g.* humic-bound P. During diagenesis, P compounds such as orthophosphate diesters and polyphosphates increase, and most likely reflect the growing microbial community.


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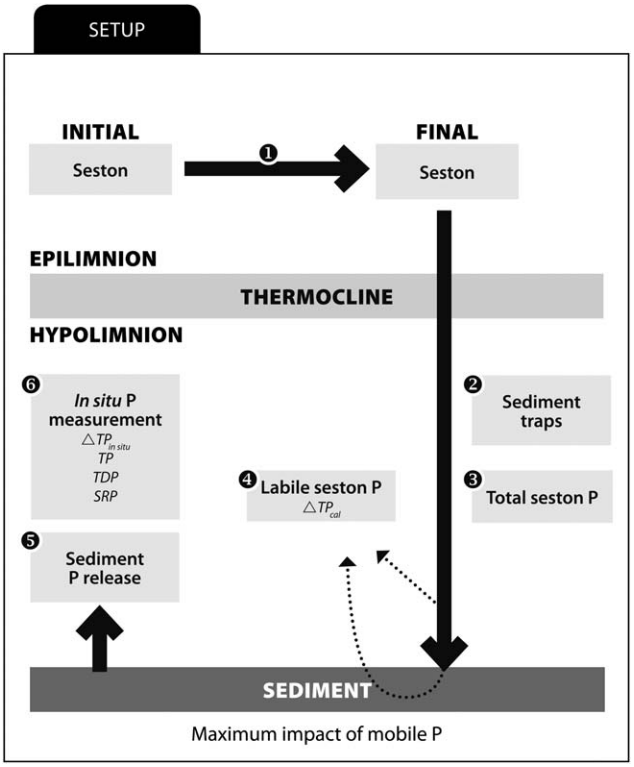


**Fig. 1** Experimental setup, demonstrating the *in situ* and *in vitro* experiments in this study. (1) Laboratory decomposition experiment to determine the amount of mineralizable phosphorus (P) in seston. Sequential P fractionation and solution $^{31}$P NMR spectroscopy were performed at the start and end to follow changes in P pools and chemical forms. (2) Sediment traps deployed just below the thermocline to determine the content of P transported to the hypolimnion. (3) Total P flux to the hypolimnion, calculated from the P content in the sediment traps. (4) Potential P released from the seston entering the hypolimnion. This is termed labile P and is calculated as the difference between P in the sediment trap and seston P at the end of the decomposition experiment. Labile P can be either released during sedimentation or after sediment deposition. Labile P was related to the area and volume of the lake (depending on stratification) and the duration of the P release experiment, to convert the flux into a concentration (termed $\Delta TP_{cal}$). (5) Laboratory incubation experiment with undisturbed sediment cores to calculate the contribution of the sediment P release to P accumulation in the hypolimnion. (6) *In situ* measurements of changes in the P concentration in the hypolimnion, conducted in the same period as the decomposition experiment (part 1). This allows a comparison with the potential P release (part 4) and the actual (*in situ*) P release ($\Delta TP_{in\ situ}$) calculated as the difference in lake water P concentration between the beginning and the end of the decomposition experiment. The maximum impact of the mobile P pool denotes the increase in lake water TP if all the mobile P in the sediment was released.

P in Lake Nordborg, from the presence in seston, through the release during sedimentation, to burial in the sediment (Fig. 1). Consequently, we were able to partition the P accumulation in the hypolimnion into P derived from either seston or sediment. This has significance for lake management, since information on P sources allows appropriate selection of measures aimed at remediating eutrophication.

## 2. Materials and methods

### 2.1. Lake Nordborg

**2.1.1. Study sites.** Lake Nordborg is a relatively small (55 ha), eutrophic, hard water lake (2.9 meq. $L^{-1}$), situated in the southern part of Denmark (55°3′27″ N, 9°45′43″ E). The catchment area is 1183 ha and is dominated by agriculture (63%), urban activities (28%), and undeveloped land (mainly forest) (9%). The lake has a mean depth of 5 m and a maximum depth of 8.5 m.

In autumn 2006 the lake was treated with poly-aluminium chloride (52 g Al $m^{-2}$) to reduce the internal P loading. Summer average TP concentration in the years prior to the Al treatment (May–Sep, since 2002) varied between 292 and 1176 μg P $L^{-1}$, but was reduced to 26 μg P $L^{-1}$ the year after treatment.[19]

**2.1.2. Phosphorus release experiments.** Seston was collected from vertical tows in the upper 1 m of the water column with a 40 μm plankton net in spring 2006 (42 μg $L^{-1}$ Chl *a*; dominated by *Fragilaria* and *Tabellaria*), summer 2006 (41 μg $L^{-1}$ Chl *a*; dominated by *Aphanizomenon*), and after Al addition in spring 2007 (13 μg $L^{-1}$ Chl *a*; dominated by *Fragilaria* and *Tabellaria*). Sufficient seston (~50 g wet weight) was collected to allow for $^{31}$P NMR analysis, sequential P fractionation, and decomposition experiments.

Immediately after sampling, 5 g of mixed seston was added to 250 ml of filtered (1.2 μm GF/C filter; Whatman, Maidestone, UK) lake water in a PVC beaker and incubated at 14 °C (Fig. 1, part 1). Each sample was replicated five times. The beakers were placed on a rotating table in complete darkness, and P release was monitored until a constant concentration of total dissolved P (TDP) in the water was reached. The beakers were sealed loosely with lids to ensure oxic conditions and reduce evaporation. Evaporated water was replaced by MilliQ water. In the incubation with summer seston an additional sample was collected at day 91, ~20 days after the cessation of P release, to assess any further diagenetic changes in the seston.

**2.1.3. Hypolimnetic phosphorus accumulation from settling seston in Lake Nordborg.** Based on results from the decomposition experiment and *in situ* data from Lake Nordborg,[19] we calculated the theoretical impact of settling seston on P accumulation in the hypolimnion during summer 2006. Vertical profiles of TP, TDP and soluble reactive P (SRP) were collected every second week at the deepest station during the summer period (May–September).[20] Phosphorus accumulation was not assessed during spring, since there was no stratification. We collected the seston entering the hypolimnion by placing sediment traps (Fig. 1, part 2) just below the thermocline (4 m depth, emptied every 2 weeks). As no inhibitors were added to the traps, the P contents integrate the effects of aging, chemical degradation, and microbial mineralization during the two week interval.

The maximum possible flux of seston P to the hypolimnion was calculated from the TP content in the sediment trap and related to the influx of seston to the sediment traps (g dry seston $m^{-2}$ $d^{-1}$) to obtain the influx of P to the hypolimnion (mg P $m^{-2}$ $d^{-1}$; Fig. 1, part 3). In addition, we calculated the flux of releasable (labile) seston TP (mg P $m^{-2}$ $d^{-1}$; Fig. 1, part 4) as

the difference between the TP content in the trap material (Fig. 1, part 2) and the final P content obtained from the laboratory incubations (Fig. 1, part 1). This difference was related to the areal influx of seston to the sediment traps to obtain the influx of P to the hypolimnion (mg P $m^{-2}$ $d^{-1}$).

The labile TP flux was converted into a concentration using the area and volume of the lake (depending on stratification) and the duration of the decomposition experiment. This was done to determine the potential P accumulation ($TP_{cal}$) in the hypolimnion (over the same period as the decomposition experiment) resulting from the labile TP influx alone (disregarding sediment P release); $TP_{cal}$ was calculated for the same time period as the decomposition experiment to compare the laboratory results with *in situ* data. Thus, $TP_{cal}$ was compared to the actual increase in the TP concentration in the water column ($\Delta TP_{in\ situ}$, Fig. 1, part 6) during the course of the decomposition experiment. Finally, $\Delta TP_{in\ situ}$ was calculated as the difference in TP concentration between the date of seston sampling and the date when P release from the seston ceased (based on the decomposition experiment; Fig. 2).

In addition to the parameters measured in the water column, undisturbed sediment cores were collected during the period of the decomposition experiment and incubated for 24 h at 14 °C to determine the efflux of TP from the sediment (Fig. 1 part 5) during spring 2006/2007 and summer 2006.

**2.1.4. Solution $^{31}$P NMR spectroscopy.** Ten grams of wet seston was pre-treated to remove iron (Fe) by extraction in 0.11 M sodium bicarbonate–sodium dithionite (BD) for 1 h in a 1 : 3 (w/v) sample to solution ratio. The mixture was centrifuged (59 860*g*, 10 min) and the supernatant discarded. The remaining solid sample was extracted in 0.1 M NaOH for 16 h in a 1 : 3 (w/v) sample to solution ratio at room temperature (~21 °C). Following extraction, the supernatant was isolated by centrifugation (59 860*g*, 10 min) and an aliquot of the NaOH extract was analyzed for TP by inductively coupled plasma optical-emission spectrometry (ICP-OES) on an Optima 2100 DV (Perkin Elmer, Shelton, CT). The remaining sample was pre-concentrated ~20 times by lyophilization and dissolution in 80% (v/v) 0.1 M NaOH, 10% BD solution, and 10% $D_2O$.

Solution $^{31}$P NMR spectra were recorded at 80.9 MHz on a Varian 200 MHz NMR spectrometer at ambient laboratory temperature (21 °C). Spectra were recorded using a 90° pulse, 0.4 s acquisition time, and 1.5 s relaxation delay, and ~32 000 scans (17 h). Chemical shifts were indirectly referenced to external 85% $H_3PO_4$ ($\delta = 0.0$) *via* the lock signal. Spectra were plotted with a line broadening of 5 Hz. Peaks were assigned to P compounds or functional groups based on literature values,[21] integrated to obtain peak areas, and converted to P concentrations using the TP concentration determined by ICP-OES. Spectral processing was done using NMR utility transform software (NUTS) (Acorn NMR, Livermore, CA).

**2.1.5. Sequential phosphorus fractionation.** Seston P was analyzed by sequential fractionation and solution $^{31}$P NMR spectroscopy both prior to and after the P release experiment. Due to an accidental loss of material in spring 2006, the sequential P fractionation was not conducted. A modified P fractionation scheme proposed by Paludan and Jensen[18] was followed, except for a minor modification of extraction times.[22] Seston P was divided into the following operationally defined pools: water extractable P ($H_2O$–P), redox sensitive P extracted in bicarbonate-buffered dithionite (BD–P), sodium hydroxide (NaOH) extractable P (NaOH–P), humic bound P (Hum-P), hydrochloric acid (HCl) P (HCl–P), and residual P (Res-P). Total dissolved P and SRP were measured in the water, BD, and

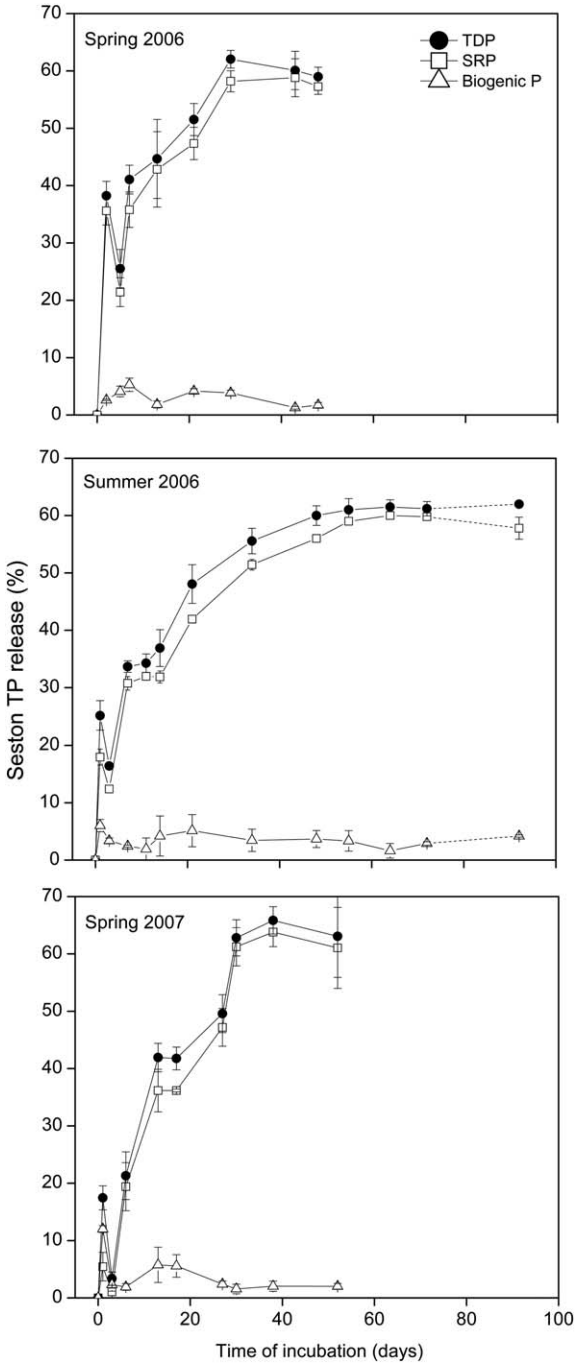


**Fig. 2** Percentage of total phosphorus (TP) released from the seston during decomposition. Release of total dissolved P (TDP), soluble reactive P (SRP) and biogenic P is shown. Upper graphs are seston from spring 2006 and seston from summer 2006, whereas the lower graph is seston from spring 2007. Standard errors of the mean ($n = 5$) are shown.


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NaOH extracts, with biogenic P (here defined as P not detected as SRP *i.e.*, including organic P and inorganic polyphosphate) calculated as the difference between TDP and SRP. Only TDP was determined in the humic-bound, HCl, and residual P fractions. Hum-P was determined by precipitating organic matter in the NaOH extract by acidifying to pH ~1 with 2 M sulfuric acid, collecting the precipitate on a filter, and determining TDP in the precipitate by digestion in 1 M hot (120 °C) HCl. Soluble reactive P was analyzed according to the procedure mentioned in Section 2.1.6, whereas total Fe was determined by ICP-OES.

Results are expressed as μg P $g^{-1}$ initial dry weight (DW) to follow P changes during the experiment. Dry weight was measured by lyophilization to a constant weight on seston samples before and after the decomposition experiment to correct for changes in DW. Loss on ignition (LOI) was measured on the same lyophilized samples (550 °C, 6 h).

**2.1.6. Elemental analyses in the water column.** Triplicate subsamples of water (filtered on 1.2 μm GF/C filters) were collected during the decomposition experiment and analyzed for SRP and TDP by standard molybdate colorimetry.[23] Total dissolved P was analyzed by digesting the sample in 0.18 M potassium peroxydisulfate for 1 hour at 105 °C. Biogenic P was determined as the difference between TDP and SRP.

**2.1.7. Statistical analyses.** The difference in the maximum P release between summer 2006 and spring 2006/2007 was determined by comparing the average P release from the three sampling days when a constant concentration was reached (the plateau in Fig. 2) by a *t*-test performed in SigmaStat version 2.03. This *t*-test was also used to verify that there were no differences between P release calculated by sequential fractionation and the decomposition experiment. In addition, changes in P fractions during the decomposition experiment were evaluated by *t*-tests. In all cases a significance level of $\alpha = 0.05$ was used.

## 3. Results

### 3.1 Phosphorus release from seston

The pattern of P release was similar for decomposition of both summer and spring sestons, with an initial rapid release of TDP and SRP, a short period of P uptake (3–4 days), and then a gradual increase in SRP and TDP to a maximum of ~60% of the seston TP (Fig. 2). Only a small amount of biogenic P was released, mainly within the first 4 days. Phosphorus release was significantly greater from the summer seston compared to the spring seston (Table 1), and the period of P release was almost twice as long for the summer seston as for the spring seston (Fig. 2). The additional sample of summer seston collected from Lake Nordborg at day 91 showed a slight increase in TDP and biogenic P, but similar SRP, compared to the sample from day 72 (Fig. 2).

### 3.2. Hypolimnetic phosphorus accumulation from settling seston in Lake Nordborg

The final P content in the seston was lower than the initial P content (Fig. 3 and 4). The P content in the sediment traps was more than twice as high as the final P content in the seston from the spring experiment in 2006, but was only 5% higher in the summer experiment and slightly lower in spring 2007.

During spring 2006, 54% (15 mg P $m^{-2}$ $d^{-1}$) of the P flux to the hypolimnion was considered to be labile TP, whereas only 5% of the TP flux to the hypolimnion was labile in summer 2006. No labile TP was transported to the hypolimnion in spring 2007 (Fig. 3). The $\Delta TP_{in\,situ}$ decreased during the period of the decomposition experiment (spring 2006), whereas it remained unchanged during spring 2007. During summer 2006 (Fig. 4), the $TP_{in\ situ}$ was >7 times higher than the $TP_{cal}$ in the hypolimnion, and the sediment P release was 83 mg $m^{-2}$ $d^{-1}$ (and therefore ~4 times higher than the maximum possible P flux from the seston in summer 2006). During spring 2006/2007 there was no sediment P release. After the Al addition in autumn 2006, P concentrations (TP, TDP, and SRP) were lower during spring 2007 than they had been in spring 2006, resulting in a lower P flux to the hypolimnion in spring 2007.

### 3.3. Solution $^{31}$P NMR spectroscopy

Orthophosphate and monoester-P concentrations decreased during all decomposition experiments (Table 2 and Fig. 5). In contrast, diester-P and polyphosphate (including both pyrophosphate and long-chain polyphosphate) declined only during incubation of the summer seston: concentrations of both forms increased during incubation of the spring seston for both 2006 and 2007 samples (Table 2 and Fig. 5a–d). Notably, polyphosphate declined dramatically after 91 days of incubation in the summer seston (Fig. 5e).

### 3.4 Sequential phosphorus fractionation

Sequential fractionation revealed clear diagenetic changes in P pools (Fig. 6). Significant decreases in $H_2O$–P, BD–P and biogenic P were observed during decomposition of both spring

**Table 1** Maximum phosphorus (P) release from decomposing seston in Lake Nordborg. Values are the mean of five replicate samples, with standard deviations in parentheses. Different letters indicate statistically significant differences among different seston samples ($p < 0.01$). The final column shows the maximum P release (MPR) as a proportion (%) of particulate P and the duration (*D*) of the P release. TDP, total dissolved P; SRP, soluble reactive P; biogenic P; TP, total P

| | P released/mg $g^{-1}$ DW | | | | |
|---|---|---|---|---|---|
| | TDP | SRP | Biogenic P | TP initial/mg $g^{-1}$ DW | MPR (%)/*D* (days) |
| Spring seston 2006 | 2.71 (0.04)[a] | 2.61 (0.02)[a] | 0.1 (0.04)[d] | 4.49 | 59/29 |
| Summer seston 2006 | 5.13 (0.03)[b] | 4.97 (0.06)[b] | 0.16 (0.04)[d] | 7.78 | 64/60 |
| Spring seston 2007 | 2.98 (0.09)[c] | 2.93 (0.08)[c] | 0.05 (0.03)[d] | 4.52 | 65/38 |

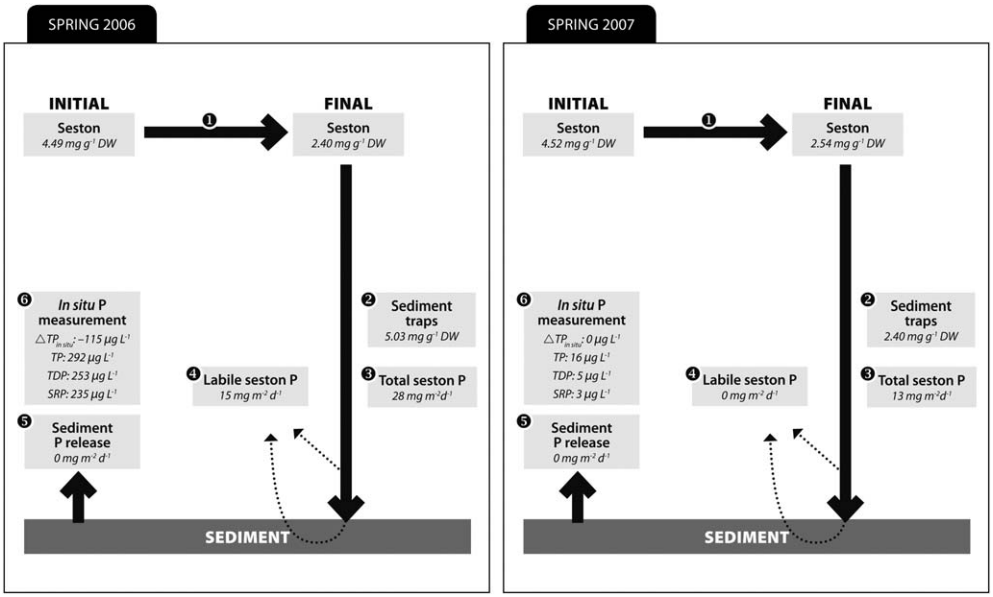


**Fig. 3** Impact of settling seston in Lake Nordborg during spring 2006 and 2007. (1) Changes in seston total phosphorus (TP) during the P release experiment. (2) Phosphorus content in the sediment trap. (3) Total P flux to the sediment. (4) Labile P from the seston. (5) Phosphorus release measured in intact sediment cores. (6) *In situ* measurements of changes in TP, as well as P concentrations at the time of seston sampling. See Fig. 1 for further information.

and summer sestons, whereas Hum-P and Res-P increased during incubation. There was no significant difference between the decline in TP in the seston samples measured by sequential P fractionation and the amount of P released to the water column measured in the decomposition experiment (data not shown). The amount of BD-extractable Fe increased during the decomposition of spring seston, but decreased during incubation of the summer seston (Table 3). The molar Fe : P ratios in the BD extracts were generally <0.07 in the initial seston and <0.3 in the final seston.

## 4. Discussion

This study demonstrates that diagenetic changes in P forms during decomposition of seston can be followed by sequential fractionation and $^{31}$P NMR spectroscopy. Seston decomposition initially increases the concentrations of potentially bioavailable P species such as poly-P and diester-P,[3,11,24,25] which are subsequently hydrolyzed and released as SRP. However, seston decomposition also leads to formation of refractory P forms (Hum-P and Res-P) that resist degradation and might be permanently buried in the sediment. The P budget for Lake Nordborg demonstrates that the release of P from settling seston plays only a minor role in the accumulation of P in the hypolimnion, with the majority of the P being derived from the sediment.

### 4.1 Phosphorus release and speciation

The main source of P released during decomposition of both spring and summer sestons was from $H_2O$–P, BD–P, and biogenic P pools (Fig. 6). Most of the increase in soluble P in the water after the first few days of incubation probably originated from the $H_2O$–P pool, although this alone cannot account for the P increase in the water. Thus, BD–P and biogenic P must have also contributed to the initial P release. The general findings of P release from the $H_2O$–P, BD–P, and biogenic P pools support findings from lake sediments where these P pools are known to constitute mobile forms of P.[1,22,26] We note that Fe : P ratios showed that the BD–P pool was not associated with Fe, indicating that the BD extraction is not always specific for Fe–P[17,27] However, since the pH of the BD solution is neutral, we expect that the BD–P consists of loosely sorbed P remaining after the previous $H_2O$ extraction. Consequently, BD–P should still be regarded as 'labile' P.[28]

The initial P increase was followed by a decline, indicating that live phytoplankton in the added seston continued to take up P for a short period after the start of the incubation.[29] The initial P uptake was followed by a longer period of P release through seston decomposition. This was expected, since the sediment matrix consists of a mixture of phytoplankton, external organic matter, and mineral particles.

Our results demonstrate that seston contains a large fraction (≥59%) of labile P, supporting previous findings.[6,30] However, seston decomposition also produced Hum-P and Res-P in both the summer and the spring incubations (Fig. 6). Reitzel *et al.*[3] demonstrated the presence of Hum-P in the precipitate from the sediment and seston in Lake Erken, but here we show that refractory P is formed by the mineralization process. To our knowledge, this is the first time that the production of refractory P forms has been linked directly to diagenetic processes, as suggested by Gächter and Meyer.[31] This means that the Hum-P and Res-P defined by the sequential fractionation procedure cannot be regarded solely as allochthonous P.

Refractory P in the seston contributes a substantial P input to the sediment. For example, the summer seston in 2006

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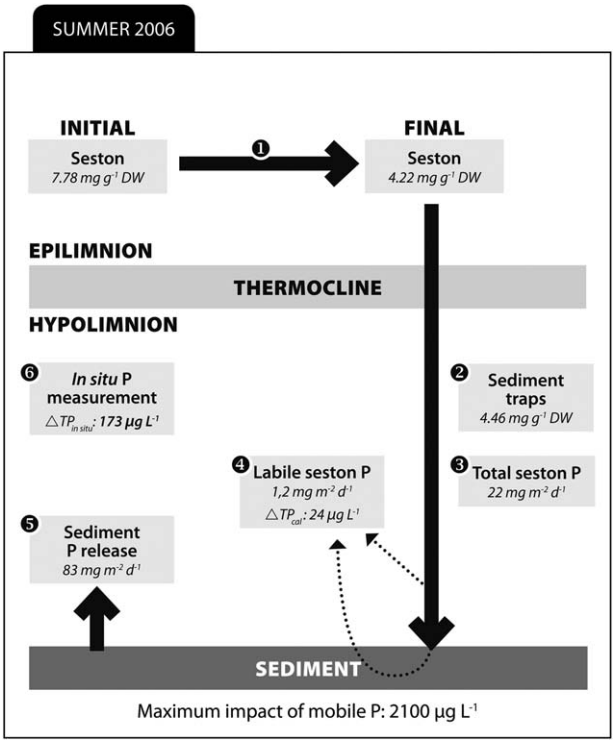


**Fig. 4** Impact of settling seston in the hypolimnion of Lake Nordborg during summer 2006. (1) Changes in seston total P (TP) during the P release experiment. (2) Phosphorus content in the sediment trap. (3) Total P flux to the sediment. (4) Labile P release from the seston in mg P $m^{-2}$ $d^{-1}$ and calculated as the increase in hypolimnion TP during the period of the P release experiment. (5) Phosphorus release measured in intact sediment cores. (6) *In situ* measurements of changes in TP, as well as P concentrations at the time of seston sampling. The maximum impact of the mobile P pool denotes the increase in lake water TP if all the mobile P in the sediment was released. For further information see Fig. 1.

potentially transported ~21 mg refractory P $m^{-2}$ $d^{-1}$ to the hypolimnion (Fig. 4). Whether this P will be further mineralized or be permanently buried in the sediment cannot be concluded from this experiment, but it seems likely that at least some of the compounds will contribute significantly to the future P release.

**Table 2** Amounts of phosphorus (P) groups (µg) in seston before and after ~50 days incubation in decomposition experiments. Contributions of the various P groups to the extracted P (%) are shown in parentheses. Loss of P is shown as a proportion (%) of the initial P form, with negative values indicating an increase in the respective seston P group during incubation

| | *Ortho*-P | Monoester-P | Diester-P | Polyphosphate |
|---|---|---|---|---|
| *Spring seston 2006* | | | | |
| Start | 1224 (47) | 925 (36) | 284 (11) | 147 (6) |
| End | 455 (26) | 823 (47) | 303 (17) | 176 (10) |
| Loss (%) | 63 | 11 | −7 | −20 |
| *Summer seston 2006* | | | | |
| Start | 1215 (36) | 1660 (50) | 256 (7) | 207 (6) |
| End (day 72) | 135 (27) | 177 (36) | 84 (17) | 96 (20) |
| End (day 91) | 198 (40) | 226 (46) | 60 (12) | 9 (2) |
| Loss (%) day 72 | 89 | 89 | 67 | 53 |
| Loss (%) day 91 | 83 | 86 | 76 | 96 |
| *Spring seston 2007* | | | | |
| Start | 1069 (47) | 876 (39) | 225 (10) | 87 (4) |
| End | 271 (18) | 665 (45) | 372 (26) | 160 (11) |
| Loss (%) | 75 | 24 | −148 | −84 |

Solution $^{31}$P NMR spectroscopy showed a clear tendency towards higher percentages of poly-P and diester-P at the end of the decomposition experiment, most likely reflecting changes in the microbial community.[3,25,32,33] In contrast, the amounts of ortho-P and monoester-P in decomposing seston declined during the incubation, indicating that these two P groups do not reflect the microbial community to the same extent as diester-P and poly-P. However, since we did not study the microbial community we can only draw indirect conclusions on the origin of these P compounds. Several studies have demonstrated that monoester-P declines more slowly with depth in sediment cores compared to diester-P or poly-P, suggesting different degradation rates.[3,11,24] Our study indicates that the seston is a source of monoester-P to the sediment and that some of the monoester-P is likely to be associated with humic complexes.[3] In addition, the degradability of monoester-P seems to depend on the source, because while 90% of the monoester-P was lost from the seston during summer, only 11–24% was lost during the spring. The decomposition experiment indicated that these monoesters were mineralized to SRP, since there were no corresponding increases in biogenic P.

Interestingly, we found that 96% of the poly-P and a 76% of the diester-P were lost when summer seston was incubated for 91 days, while ortho-P and monoester-P increased. This could be explained by mineralization of diester-P and poly-P to monoester-P and ortho-P, respectively, after mineralization ceased. No final conclusion can be drawn since we only studied one pooled seston sample, but the low standard errors of the replicate seston samples (seen from the sequential fractionation and the P release experiment) combined with small standard errors involved in $^{31}$P NMR analysis[2,34] indicate this finding to be robust. However further studies are needed to fully address this issue.

### 4.2. Hypolimnetic phosphorus accumulation from settling seston in Lake Nordborg

**4.2.1. Epilimnion.** Combining results from the laboratory P release experiment with *in situ* P concentrations, P release rates, and sediment trap experiments, allows us to propose some general patterns in the seasonal P cycle in Lake Nordborg. *In situ* sediment P release during spring 2006 was zero, whereas the TP input to the sediment from settling seston was ~28 mg $m^{-2}$ $d^{-1}$ (of which the labile P input was 15 mg $m^{-2}$ $d^{-1}$). This demonstrates clearly that settling seston in spring 2006 added a substantial amount of labile P to the sediment that was not immediately released, probably due to P immobilization by oxidized Fe in the sediment. In spring 2007, several months after Al treatment of the lake, all the labile P was released before seston reached the sediment traps, resulting in less P input to the sediment. This can be explained by the Al treatment reducing P concentrations in the water column. A possible explanation for the similar seston P contents in pre-and post-treatment years is that the Al-induced P reduction was not high enough to change the phytoplankton structure or induce P limitation of the phytoplankton.[20]

Another striking finding was that the P content in the sediment traps was reduced by only 50% by the Al treatment, whereas the

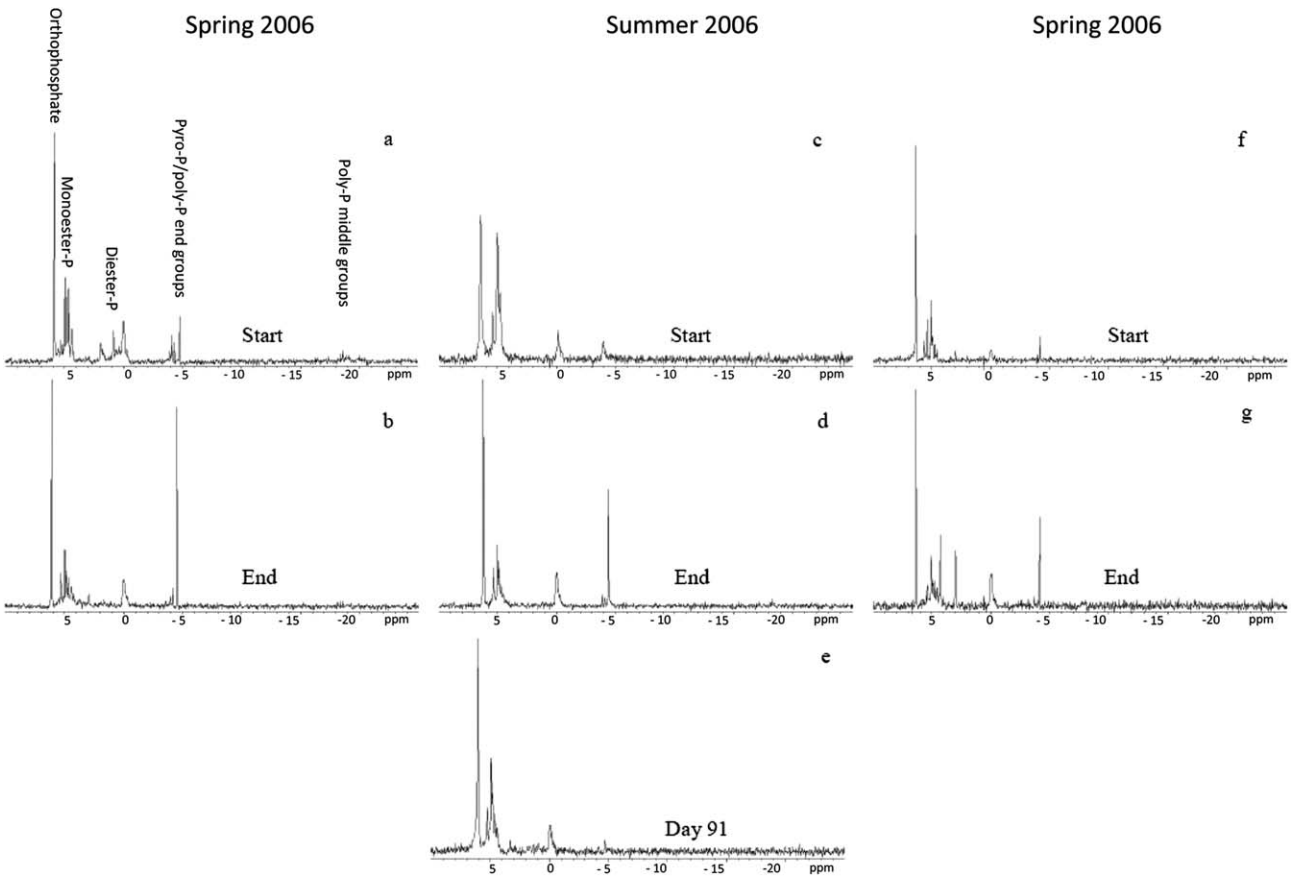


**Fig. 5** Solution $^{31}$P NMR spectra from the start and end of the seston decomposition experiment, showing spring seston in 2006 (a and b) and 2007 (f and g), and summer seston in 2006 (c and d). Summer 2007 includes an additional sample at day 91 (e).

TP concentration in the water was reduced by 95%. This lower P content in the 2007 sediment traps indicates more P regeneration in the epilimnion following the Al treatment. This is supported by several observations after the Al treatment. For example, Egemose *et al.*[20] found more large-bodied zooplankton in 2007 compared to 2006, indicating more grazing and, therefore, P mineralization in the epilimnion. This presence of large-bodied zooplankton was explained by a lower abundance of planktivorous fish in the epilimnion due to improved water transparency, and consequently greater predation pressure on the fish. In addition, Egemose *et al.*[20] reported higher average epilimnetic temperature in 2007 (14 °C) compared to 2006 (8 °C), probably leading to increased microbial activity.

**4.2.2. Hypolimnion.** During summer 2006, there was an influx of 22 mg TP $m^{-2}$ $d^{-1}$ to the hypolimnion, but only 1.2 mg $m^{-2}$ $d^{-1}$ of this was labile. Hence, the seston entering the hypolimnion in summer 2006 was more decomposed compared to the spring seston in 2006. This can be explained to some extent by differences in sedimentation rates between spring and summer. Thus, a generally higher sedimentation rate of diatoms[35] of up to 4 m $d^{-1}$ most likely resulted in an incorporation of the spring seston in the sediment matrix early in the diagenetic process. Furthermore, the diatoms are mineralized at a time of the year when the surface sediment is mainly oxic, resulting in no detectable P release after deposition. Hence, the immediate impact of spring seston on the P concentration in the water was less pronounced than the impact of cyanobacteria (dominating the summer seston), which are mineralized during summer when anoxic conditions prevail and higher temperatures enhance bacterial activity. Also, the settling velocity of cyanobacteria can be 10 times lower than for diatoms,[35] so cyanobacteria might remain in the epilimnion for many days with a consequently larger proportion of P released. Hence, in Lake Nordborg, it seems likely that the spring seston from 2006 is predominantly mineralized in the sediment, whereas a larger fraction of the summer seston might be mineralized in the epilimnion.

Some seasonal trends can be identified by considering the change in the $TP_{in\ situ}$ concentration in the water column during the periods when the respective decomposition experiments were conducted. During spring 2006 there was a 40% decline in $TP_{in\ situ}$ during the period of P release, indicating that the high sedimentation rate of the diatoms combined with an oxidized sediment surface resulted in a net removal of TP from the water column. Due to the Al treatment, no increase in $TP_{in\ situ}$ was observed in 2007, but P concentrations in the water column were dramatically reduced compared to spring 2006. Hence, a smaller bloom with less effect on the TP in the water column, along with a higher P regeneration, occurred in 2007.

No net increase in the TP concentration in the epilimnion was expected to occur during the relatively dry summer period, due to a low external P load. This low input of P should reinforce

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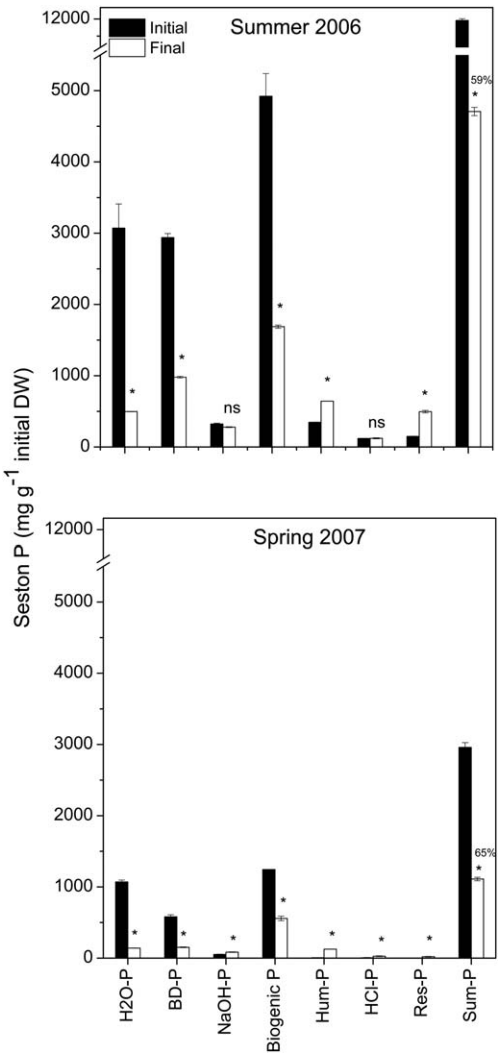


**Fig. 6** Phosphorus (P) analysis of seston from the decomposition experiment by sequential fractionation, showing the P content (mg P $g^{-1}$ initial dry weight) in the incubated beakers with standard errors of the mean ($n = 3$) at the start (black) and end (white) of the incubation. Total proportion of P released (%) is shown above the Sum-P column. Asterisks denote significant differences between the start and end ($p < 0.05$).

a rapid recycling between particulate and dissolved P and a net removal of P from the epilimnion by sedimentation. Hence, we only followed the changes in P in the hypolimnion, where the increase in $TP_{cal}$ was much lower than the increase in $TP_{in\ situ}$. This was explained by the four times higher P flux from the anoxic sediment, partly from mineralization of the settled spring seston, but also from redox sensitive Fe–P complexes.

**Table 3** Molar iron (Fe) to phosphorus (P) ratios and total contents (μg $g^{-1}$ initial dry weight) of Fe in the seston at the beginning and at the end of the release experiment in the bicarbonate–dithionite fraction

| | Initial content | Final content | Fe : P (molar) initial/final |
|---|---|---|---|
| BD fraction (spring 2007) | 80 | 227 | 0.02/0.13 |
| BD fraction (summer 2006) | 80 | 43 | 0.07/0.30 |

Specifically, the efflux from the sediment was 70 times higher than the input of labile P from settling seston. During summer, the presumably lower seston sedimentation rates, along with higher regeneration of P (*e.g.*, due to higher temperatures), led to a higher release and recycling of TP in the epilimnion. Consequently, there was a lower transport of particulate P to the hypolimnion (based on the sediment traps), where P release from the sediment explained the majority of the TP accumulation.

Hupfer and Lewandowski[4] demonstrated that settling seston was the major contributor to the accumulation of TP in the hypolimnion of Lake Arendsee. They concluded that potential P release from the sediment played a minor role in the P cycle of this lake, due to the small size of the mobile P pool in the sediment. In Lake Nordborg, the impact of settling spring seston and summer seston seems to play a different role than in Lake Arendsee. Although <10% of the mobile sediment P pool was released during summer 2006, its impact was still 70 times higher than the net influx of labile TP from settling seston. Hence, the shallow depth of Lake Nordborg ensures a high transport of labile particulate P to the sediment matrix during spring, where it indirectly influences the accumulation of P in the hypolimnion through subsequent mineralization during summer.

## 5. Conclusion

Solution $^{31}$P NMR spectroscopy and sequential fractionation were used to follow diagenetic changes in P forms during decomposition of settling seston in Lake Nordborg, a shallow eutrophic lake in Denmark. Seston decomposition increased concentrations of potentially bioavailable polyphosphate and phosphodiesters, but also promoted the formation of refractory P forms that might be buried permanently in the sediment. Combining these results with *in situ* measurements of P concentrations in lake water and sediment traps revealed that P release from settling seston plays only a minor role in the accumulation of P in the hypolimnion of Lake Nordborg.

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