The impact of climate change and eutrophication on phosphorus forms in sediment: Results from a long-term lake mesocosm experiment

Katrin Saar [a,*], Peeter Nõges [a], Martin Søndergaard [b], Maria Jensen [c], Charlotte Jørgensen [c], Kasper Reitzel [c], Erik Jeppesen [b,d,e], Torben L. Lauridsen [b], Henning S. Jensen [c]

[a] Centre for Limnology, Estonian University of Life Sciences, Kreutzwaldi 5, 51006 Tartu, Estonia

[b] Institute of Ecoscience, Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark

[c] Department of Biology, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark

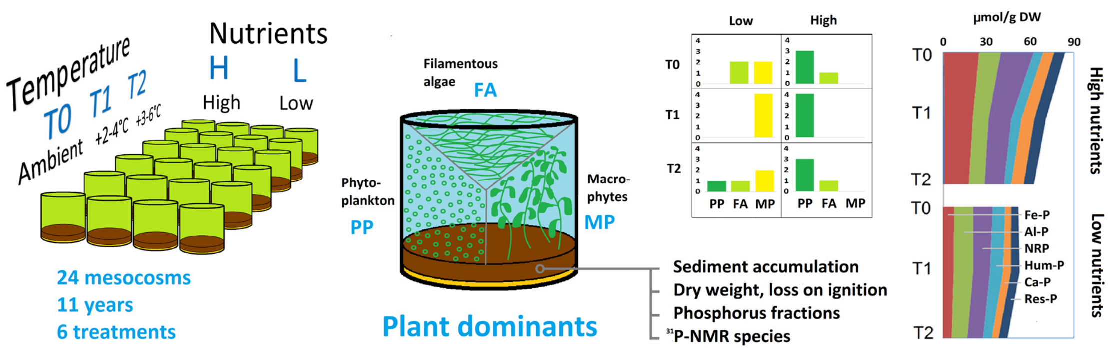
[d] Limnology Laboratory, Department of Biological Sciences and Centre for Ecosystem Research and Implementation, Middle East Technical University, Ankara 06800, Turkey

[e] Institute of Marine Sciences, Middle East Technical University, Erdemli-Mersin 33731, Turkey

HIGHLIGHTS

- Higher nutrient loading led to higher mobile P pool in the sediment.
- Nutrient addition increased sediment accumulation rate at ambient temperature.
- With warming, sediment thickness decreased in high nutrient mesocosms.
- Vegetation type affected vertical profiles of P fractions and metals in sediment.
- In macrophyte-rich lakes, plants can be important modifiers of early P diagenesis.

GRAPHICAL ABSTRACT



Abstract

Characteristics of bottom sediments in lake mesocosms 11 years after starting the experiment were studied in order to determine the effects of nutrient loading, temperature increase and vegetation type on concentration and vertical distribution of phosphorus (P) forms. The experimental setup consisted of 24 outdoor flow-through mesocosms with two nutrient treatments – low (L) and high (H) and 3 temperature levels – ambient (T0), heated by 2-4 °C (T1) and 3-6 °C (T2) in four replicates. Thickness of the organic sediment was measured and the sediment analysed for dry weight, organic matter, and P fractions (according to a sequential extraction scheme) and organic P compounds (by ^{31}P nuclear magnetic resonance spectroscopy). Higher nutrient loading led to increased sediment accumulation and higher concentration of total P and most P fractions, except P bound to aluminium and humic matter. The dominant vegetation type covaried with nutrient levels. Vertical gradients in Ca bound P and mobile P in low nutrient mesocosms was perhaps a result of P coprecipitation with calcite on macrophytes and P uptake by roots indicating that in macrophyte-rich lakes, plants can be important modifyers of early P diagenesis. Temperature alone did not significantly affect sediment accumulation rate but the interaction effect between nutrient and temperature treatments was significant. At high nutrient loading, sediment thickness decreased with increasing temperature, but at low nutrient loading, it increased with warming. The effect of warming on sediment composition became obvious only in nutrient enriched mesocosms showing that eutrophication makes shallow lake ecosystems more susceptible to climate change.

Key words: shallow lake, sediment accumulation, temperature gradient, nutrient load, ^{31}P NMR, sequential extraction, P-pools

1. Introduction

The effects of eutrophication and climate change, largely interacting with each other, represent a major threat for ecosystem integrity and services provided by fresh waters already now. The global mean surface temperature is predicted to increase by 1.0 – 5.7°C by the end of the 21st century, relative to 1850-1900, depending on forcing scenarios (IPCC, 2021) increasing the likelyhood of further stress on freshwater ecosystems in the decades to come (Jeppesen et al., 2010; Moss et al., 2011; Nazari-Sharabian et al. 2018). Several studies have shown a negative effect of temperature on net ecosystem production (NEP) resulting from a stronger stimulating effect of temperature on ecosystem respiration than on gross primary production (Kosten et al., 2010; Laas et al., 2012; Scharfenberger et al., 2019). This shift towards more heterotrophy in lakes with increasing temperatures (Staehr and Sand-Jensen., 2006: Laas et al., 2012) implies that more organic matter is mineralised and, hence, less is accumulated in lake sediments (Tammeorg et al., 2018).

With climate change, also the precipitation and frequency of extreme weather events are predicted to increase in Northern Europe (IPPC, 2014). These changes are expected to have diverse impacts on lake ecosystems (Blenckner et al., 2007; Kosten et al., 2011; Alsterberg et al., 2012; Jeppesen et al., 2014), including increased water inflow and higher nutrient loading from the catchment (Feuchtmayr et al., 2009; Jeppesen et al., 2014). Although much progress has been made to reduce the external loading from catchments, it is expected that the changes caused by climate change will exacerbate the eutrophication problem (Mooij et al., 2007; Feuchtmayr et al., 2009; Jeppesen et al., 2014, 2021). Contrary to the temperature effect, increased nutrient load and lake trophy has been shown to increase NEP and sediment accumulation rate (Hanson et al., 2003; Trolle et al., 2009; Battarbee et al., 2012).

The top sediment is an important contributor to the present day status of lakes as it, depending on the physical and chemical properties and environmental conditions, may become an internal source or sink of nutrients, especially phosphorus (P) (Søndergaard et al., 2003; Nürnberg, 2009; Horppila et al., 2017). Changing climate may increase the internal P loading in several ways: (1) Increased surface water temperatures has been shown to prolong stratification and lead to extended periods with oxygen depletion at the sediment-water interface (Boros et al., 2011, Adrian et al., 2009, Wilhelm and Adrian, 2008) and P leakage from sediments (Liboriussen et al., 2005; Mooij et al., 2007; Feuchtmayr et al., 2009; Jeppesen et al., 2014) due to release of redox sensitive P pools. (2) Lake ecosystem respiration has a higher temperature gain compared to gross primary production turning lake ecosystems more heterotrophic with increasing temperature (Scharfenberger et al., 2019). In shallow lakes, the increased water temperature leads to increased mineralization rates in the surface sediment and reduced thickness of the oxidized surface sediment layer (Jensen and Andersen 1992). Thus, mineralization of the significant pool of organic P (Reitzel et al. 2007) is increased and at the same time the redox sensitive P-binding to iron (Jensen and Andersen, 1992; Kosten et al., 2011; Søndergaard et al., 2003; Tammeorg et al., 2020), as well as by polyphosphate accumulating bacteria, is reduced (Hupfer et al., 2004). These two P fractions are typically released to the overlying water when the sediment becomes anoxic. (3) With increasing storm frequencies, intensities and duration (Stockwell et al., 2019) the importance of resuspension related aerobic P release may increase (Tammeorg et al., 2013). This could be due to ligand exchange reactions of phosphate with hydroxyl ions on the surface of ferric iron or aluminum hydroxides in shallow lakes at high pH (Boström et al. 1982; Holmroos et al., 2009). Thus, knowing the composition of sediment P compounds is essential for assessing the potential P release in lakes (e.g. Psenner et al. 1984, Nürnberg 1988, Hupfer et al. 1995, Jørgensen et al., 2011).

Aquatic macrophytes have diverse effects on sediment accumulation and properties depending on macrophyte types and abundance. By reducing water current velocities, submerged macrophyte stands increase sedimentation and suppress sediment resuspension (Madsen et al., 2001), thereby reducing disturbance related sediment P release. However, as macrophytes often acquire nutrients from the sediments via the roots, their biomass turnover may enrich lake water with dissolved P and organic matter that may become an important internal loading mechanism in eutrophic lakes (Carpenter, 1983). Furthermore, by creating an anoxic layer over the sediment in dense macrophyte stands, the senescent biomass may facilitate redox-sensitive sediment P release in late autumn and winter (Welch and Kelly, 1990).

The complexity of chemical, physical, and biological factors affecting sediment accumulation rate and composition in lakes (e.g. Tammeorg et al. 2018) makes it difficult to clearly distinguish the effect of external (catchment) and internal (lake) factors (e.g. Welch and Jacoby 2009). The complexity can be reduced by studying long term processes in mesocosms where e.g. morphometry and physical forcing are the same for all treatments.

The aim of this study was to assess the potential mobility and accumulation patterns of sediment P forms under different climate scenarios and nutrient loading levels in a long-term mesocosm study. Using a mesocosm system in which stochastic catchment effects as variable water discharge and loads of allochthonous substances depending on weather are replaced by controlled water exchange and nutrient additions, and where a heating system allows modifying the ambient temperature, provides an ideal opportunity to study internal processes in water bodies and sediments.

Twenty-four mesocosms of the present study represented different nutrient states and water clarity, with some dominated by submerged macrophytes and others by phytoplankton or filmentous algae. We hypothesized that (1) higher nutrient loading will lead to increased sediment accumulation and higher P concentration in sediment and (2) higher temperatures stimulate decomposition rates more than primary production rates thus leading to higher mineralization and lower accumulation of organic matter in the sediment.

2. Materials

2.1. Experimental set-up

The long-term mesocosm experiment has been running since 2003 in Lemming, Central Jutland, Denmark. The experiment consists of 24 outdoor flow-through mesocosms arranged in a 2 (nutrients) x 3 (temperatures) x 4 (replicates) factorial design according to Liboriussen et al. (2005).

Mesocosms made of stainless steel have a diameter of 1.9 m and are 1.5 m deep. The water column in the mesocosms was kept 1 m deep and was continuously stirred by moving paddles during the ice-free period. The stirring by paddle was gentle and did not create visible resuspension of sediment. However, it did create a pattern of accumulation areas which was similar in all enclosures, and the sampling atrategy accounted for that.

All the mesocosms were equipped with a flow-through system and were added groundwater. Water residence time in the mesocosms was approximately 2.5 months. A more detailed description of the experimental setup is provided by Liboriussen et al. (2005). The experiment consisted of two nutrient treatments – high (H) and low (L) where phosphorus and nitrogen (N) were added, and three different temperature treatments (Table 1). All mescosms received nutrients from the groundwater input in which total P (TP) and total N (TN) concentrations have varied with time, in 2014 averaging 0.01 (range 0-2) mg TP L^{-1} and 3.2 (range 2-3.8) mg TN L^{-1}, equivalent to 0.15 mg P m^{-2} d^{-1} and 43 mg N m^{-2} d^{-1} on average. The nutrient loading with groundwater was nearly constant over the years. Half of the 24 mesocosms had high nutrient treatment with an additional weekly loading of 2.7 mg P m^{-2} d^{-1} (Na_2HPO_4) and 27 mg N m^{-2} d^{-1} ($Ca(NO_3)_2$).

Eight mesocosms were unheated and acted as control (T0). They followed the seasonality in ambient temperature (Table 1). Another eight mesocosms had low heating according to the IPCC A2 emissions scenario (Houghton et al., 2001) scaled to local conditions for the period 2071-2100 (T1) and the remaining eight mesocosms had high heating according to scenario A2 + 50% (T2).

Table 1. Nutrient and temperature settings used in mesocosm treatments

Treatment	Nutrients	Temperature	N of replicates
HT0	high nutrient loading	ambient temperature	4
HT1		IPCC A2 scenario (2-4 °C)	4
HT2		IPCC A2 + 50% scenario (3-6 °C)	4
LT0	low nutrient loading	ambient temperature	4
LT1		IPCC A2 scenario (2-4 °C)	4
LT2		IPCC A2 + 50% scenario (3-6 °C)	4

In the beginning of the experiment in 2003, 0.2 m of sediment was placed into each mesocosm. This sediment consisted of a lower 0.1 m thick rinsed sand layer and a 0.1 m thick nutrient-rich lake sediment layer. Most of the nutrient-rich sediment originated from a nearby freshwater pond while smaller portions came from a plankton-dominated nutrient-rich lake and a macrophyte-dominated lake with lower trophic status. Before adding the sediment to the mesocosm, it was sieved through a net in order to remove larger plant fragments and to prevent vertebrates to be introduced to the mesocosms. Still the sediment contained invertebrates and plant propagules. The sediment was homogenized before addition.

Male *Gasterosteus aculeatus* (three-spined sticklebacks) captured from natural populations were initially stocked in each mesocosm in near to natural densities according to nutrient concentrations: one individual in L and twelve in the H mesocosms. Later, female three-spined sticklebacks were added to the H to allow breeding.he mesocosms have been uncovered since the start of the experiment (Suppl. Fig. 1)

During the sediment sampling, macrophytes (*Elodea canadensis* Michx. and *Potamogeton crispus* L.) were present in all the L mesocosms, whereas most of the H mesocosms were dominated by phytoplankton and filamentous algae and the macrophyte vegetation was sparse or absent. In this paper, the mesocosms were qualitatively categorized by the dominant primary producers at the sampling time (May 2014) into macrophyte (MP), filamentous algae (FA) and phytoplankton (PP) dominated ones. Neither biomass nor abundance of the primary producers was determined for this study, but unpublished results from August 2014 are presented in Suppl. Table 1. Further, the overall picture of the distribution of dominat primary producers has not changed even if there has been some year to year variation over the 11 years period: The high nutrient mesocosms are dominated by PP or FA and the low nutrient ones by MP and FA at times, with FA being most important in T2 treatments (T.L. Lauridsen, unpubl. data).

The structure and function of the mesocosms have been described in several recent papers, including metbolism (Jeppesen et al, 2021), greenhouse gas exchange (Davidson et al, 2018), phytoplankton (Filiz et al, 2020) and periphyton (Pacheco et al, 2022). In these papers, references to earlier studies in the mescosms can be found.

2.2. Sediment sampling

Three sediment samples were collected from each mesocosm in May 2014 (11 years following the beginning) by coring with a clear plastic tube (inner diameter 5.2 cm). The samples were taken in the same positions relative to the heating elements in all mesocosms. The cores were stored in darkness at 4 °C until slicing to three layers: top 2 cm (top), rest of the organic sediment (mid), and 2 cm of the sand layer (sand). The boundary between organic-rich sediment and sand layer was visually clearly distinguishable. In each mesocosm, similar layers from the three cores were pooled and homogenized before analysis.

2.3. Sediment analysis

Sediment dry weight (DW) was determined by freeze-drying until constant weight. Loss on ignition (LOI) was analyzed by combusting the freeze-dried samples at 520 °C for 5 h. Combusted sediment residues were extracted in hot nitric acid in microwave for analyses of total P (TP), total Fe (TFe), and total Al (TAl). Sediment P fractionation was performed according to Psenner et al. (1984) extraction scheme modified by Paludan and Jensen (1995). P was extracted from 1 g fresh wet sediment in consecutive steps by using distilled water for loosely bound and porewater P (Wat-P), 0.11 M bicarbonate-buffered dithionite (BD) for iron bound P (Fe-P), 0.1 M NaOH for aluminium bound (Al-P, Reitzel, 2005), and 0.5 M HCl for calcium bound P (Ca-P). Organic or nonreactive P (NRP) was determined as the sum of differences between total and soluble reactive P (SRP) in the first three extraction steps (H_2O, BD, NaOH). P bound to humic substances (Hum-P) was analysed on precipitate from the acidified NaOH extract and, as for residual P (Res-P), it was measured after digestion with 1

M HCl. SRP concentration was measured in the supernatant according to Hansen and Koroleff (1999). The mobile P pool was quantified as the sum of three fractions (Wat-P, Fe-P and NRP).

Iron contents in the HCl extract for TP (TFe), BD-, HCl-, and residual fractions (correspondingly, BD-Fe, HCl-Fe and Res-Fe) were measured spectrophotometrically according to Gibbs (1979). Aluminium was analysed in NaOH fraction (NaOH-Al) and TP and TAl fractions by inductively coupled plasma optical emission spectroscopy (ICP-OES). With this sequential extraction procedure BD-Fe is fairly specific for amorphous ferric hydroxides and NaOH-Al for gibbsite or amorphous $Al(OH)_3$ (Jensen and Thamdrup 1993, Reitzel 2005).

2.4. ^{31}P NMR

Various organic and condensed inorganic P compounds in the sediment were determined with ^{31}P nuclear magnetic resonance (^{31}P NMR) spectroscopy following the protocol of Jørgensen et al. (2011). Sediment from 18 enclosures were used for ^{31}P NMR analysis. Three enclosures were randomly selected from each treatment. The reason for this was limited run-time on the NMR facility. Sediment was mixed from top and mid layer in order to get enough sediment for the analysis. Fourteen g of wet sediment was extracted with 42 ml 0.05 M EDTA for 1 h in order to remove paramagnetic metals (mainly iron) and afterwards with 0.1 M NaOH for 18 h. After both steps the samples were centrifuged and the supernatant decanted from the sediment pellet. A 5 ml subsample of the decanted NaOH supernatant was collected for TP analysis, and the remaining supernatant was frozen until the ^{31}P NMR analysis.

Before the ^{31}P NMR analysis, the NaOH extract was concentrated approximately 10 times by rotary evaporation at 35 °C and centrifuged for 10 min at 22000 g to remove any inorganic P .

precipitated with $CaCO_3$ if not all Ca was removed by EDTA. For the analysis, 630 ml of extract was mixed with 70 ml D_2O and transferred to the NMR tube.

^{31}P NMR spectra were measured at ambient temperature using 80.9 MHz with Varian Mercury NMR spectrometer. The spectra were recorded by using 90° observe pulse, 1 s acquisition time, 5 s relaxation delay, acquiring app. 14000 transients.

MestReNova transform software was used to determine the peak area. The spectrum was processed with 20 Hz line broadening. Different groups of P compounds, such as ortho-, poly- and pyrophosphates (Ortho-P, Poly-P, Pyro-P), mono- and diesters (Mono-P, Di-P) were calculated from peak areas relative to TP content in the NMR samples (NMR-TP) determined by ICP. The different peaks were identified according to the reports from the literature (e.g. Turner et al., 2003).

2.5. Statistical analysis

STATISTICA 64 Version 13 (Dell. Inc. 1984-2016) was used to preform the statistical analysis.

Pearson correlation coefficient was used to identify relationships between P fractions, metals, LOI and DW in sediment.

For cluster analysis, 13 variables measured in the upper two organic sediment layers (top and mid layer) of all 24 mesocosms (DW, LOI, Wat-P, Fe-P, Al-P, Hum-P, Ca-P, Res-P, NRP, TP, TFe, NaOH-Al and TAl) were standardised by subtracting the mean of each variable and dividing the residuals by the standard deviation. Using the k-means clustering procedure, the sediment samples were grouped into three clusters. The number of clusters was optimised using the Elbow method (Kodinariya and Makwana, 2013).

Principal Components & Classification Analysis (PCCA) and two-way ANOVA were applied to test the effects of different treatments and their potential coeffects. The PCCA computes Principal Components for selected variables and applies the resultant factor structure to map observations into the same space (see Jambu, 1991 for more detail).

To interpret the results from two-way ANOVA, Fischer LSD post-hoc test was applied.

3. Results

3.1. Sediment thickness

We measured the organic sediment layer thickness in mesocosms 11 years after filling and compared it between the treatments. The organic sediment thickness (average ± standard deviation of 3 cores from each mesocosm) ranged from 7.2±5.7 to 19±3.0 cm. Compared to the initial status at filling when the mesocosms were added 10 cm of sand and 10 cm of organic sediment, the average organic sediment thickness increased in 20 of them and decreased in 4. When averaging sediment thickness for the four replicates in each treatment the largest difference of 4.9 cm was found between LT0 and HT0 treatments. If estimating total areal masses of dry sediment from bulk density numbers (Suppl. Table 2) this difference is equivalent to a higher sedimentation rate in HT0 than in LT0 of 591 g m^{-2} yr^{-1} (Suppl. Table 2).

We found no statistically significant differences between temperature treatments, however, nutrient treatment ($f(1, 65) = 4.98$; $p = 0.029$) and its coeffect with temperature ($f(2, 65) = 3.79$; $p = 0.028$) had significant effects on sediment thickness (Suppl. Table 3).

The post-hoc test showed that nutrient addition had a significant effect on sediment thickness at ambient temperature disappearing at higher temperatures because sediment thickness showed a tendency to decrease at high nutrient loading, and increase at low nutrient loading (Fig. 1).

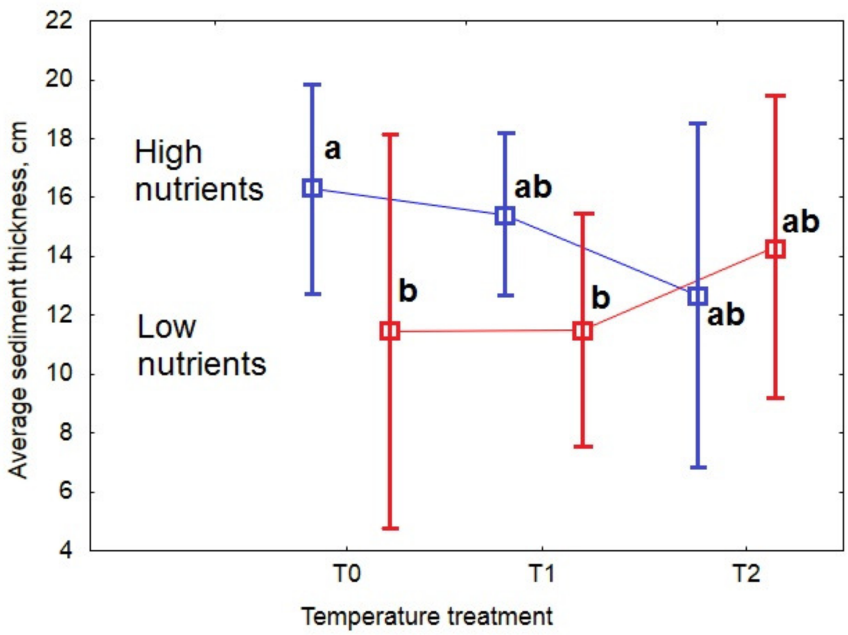


Figure 1. Effect of nutrient and temperature treatments on average thickness of the organic sediment layer (initial 10 cm + accumulation) in mesocosms (n=24). Letters a and b denote significant differences revealed by the Fischer LSD-test. Vertical bars denote 95% confidence interval.

3.2. Sediment properties and P fractions

In all treatments, sediment DW was lowest in the top layer and highest in the sand layer, while LOI showed the opposite gradient (Suppl. Table 4). Total P content (TP, Fig. 2A) as well as all measured P fractions and mobile P followed the pattern of LOI being highest in the top layer, somewhat lower in the mid layer and more than 10 times lower in the sand layer when calculated on a dry weight basis.

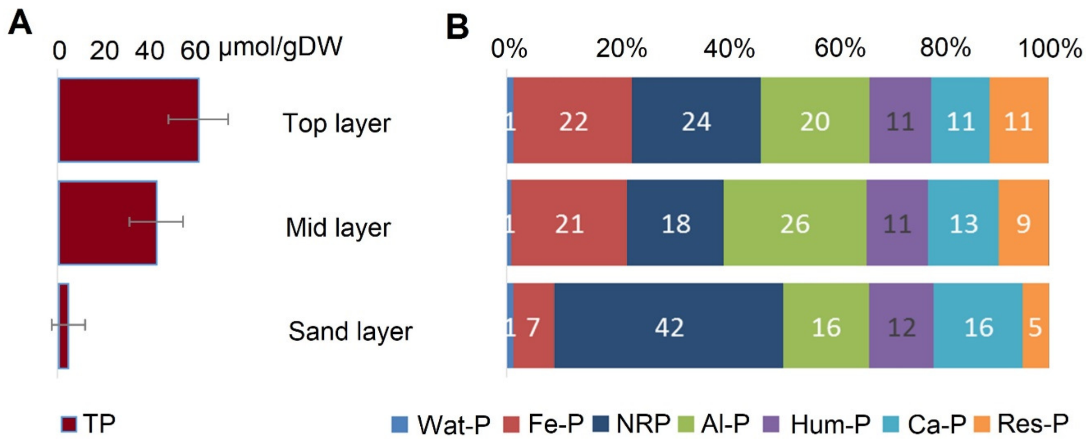


Figure 2. Phosphorus distribution in the top, mid and sand layer of the sediment in the 24 mesocosms. A – average total phosphorus (TP) ± standard deviation, B – average percentages of P fractions in TP

In the two organic sediment layers (top and mid layer), Fe-P, Al-P and NRP were the major fractions, each forming 18-26% of TP (Fig. 2B). They were followed by twice smaller Hum-P, Ca-P and Res-P fractions. The proportions of Al-P and NRP differed slightly between the two organic layers, but the differences were statistically non-significant.

In the sand layer, NRP was the dominant P fraction making up 42% of TP, significantly more than in the mid layer ($p<0.05$). Wat-P was the smallest fraction in all sediment layers forming 1% of TP.

Similarly to P fractions, Fe and Al concentrations increased with sediment depth (Suppl. Table 5). TFe and TAl were strongly positively correlated with TP ($r=0.74$ and $r=0.85$, $p<0.001$, Suppl. Table 6). TP and Res-P correlated with all metal fractions, whereas Hum-P and Al-P showed only a small number of significant correlations. Still Hum-P correlated with TFe and NaOH-Al ($r = 0.37$ and $r = 0.53$, correspondingly) and Al-P with BD-Fe, NaOH-Al, and TAl (corresponding r values 0.33, 0.80, and 0.38).

The total concentration of P species extracted and identified by the ^{31}P-NMR analysis from the two combined upper sediment layers), constituted on average 59% of the sediment TP. Among them, Ortho-P, reflecting P dissolved from various inorganic P phases, constituted 59% of the NMR-TP followed by Mono-P (average 28%), Di-P (6%), Pyro-P, and Poly-P (both 3%) (Fig. 3). The three latter species together were termed as microbial P (Micr-P), according to the findings by Ahlgren et al. (2011) and Staal et al. (2019). Among 18 samples, the two major P species were found in all samples, Di-P in 17, Pyro-P in 15, and Poly-P in 6 samples.

Several significant correlations were found between P species determined by the sequential fractionation method and the ^{31}P-NMR analysis (Suppl. Table 7). Wat-P and Ca-P correlated positively with Micr-P and Poly-P. In addition, Wat-P was negatively correlated with Ortho-P. Al-P correlated positively with NMR-TP and Ortho-P, and NRP with Pyro-P. Fe-P and Hum-P showed no significant correlations with the ^{31}P-NMR species.

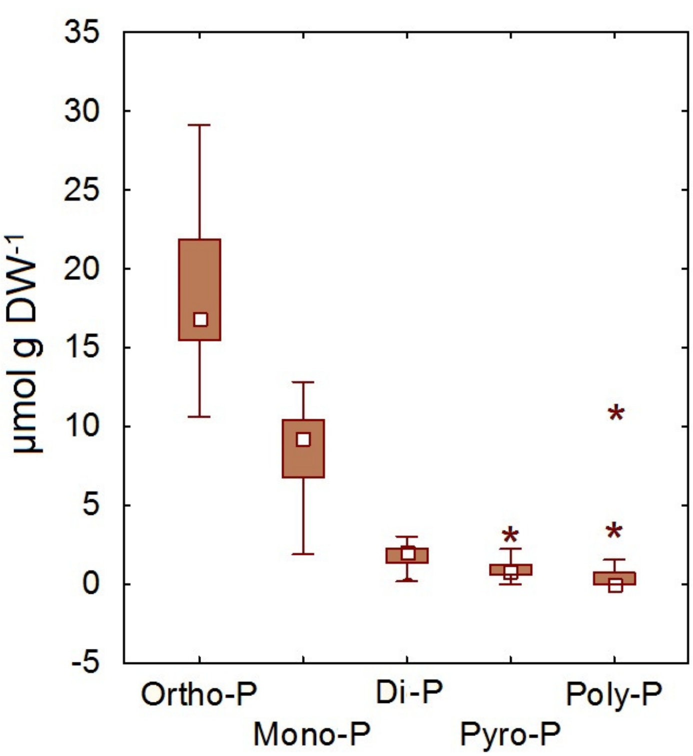


Figure 3. Phosphorus species identified by ^{31}P-NMR analysis in the upper two sediment layers of the 24 mesocosms (ordered by their median values). Outliers denoted by asterisks.

3.3. Cluster analysis

Thirteen sediment variables including DW, LOI, seven P fractions, TP, TFe, TAl and NaOH-Al were used to cluster the 48 sediment samples taken from the top and mid layers of the mesocosms. K-means clustering revealed three distinctive clusters (Fig. 4, Table 2, Suppl. Fig. 2) that were characterised by the following features:

Cluster 1 (20 samples) had the lowest mean DW and highest values of most P-fractions except NaOH-P and Hum-P (Fig. 4). It also showed the highest values of TP and TFe. All 20 samples originated from high nutrient mesocosms and 18 of them had phytoplankton as the vegetation dominant (Table 2). The three temperature treatments and two sediment layers were almost equally represented.

Cluster 2 (17 samples) had the highest average LOI, Al-P, Hum-P, and NaOH-Al, while Wat-P and Fe-P were the lowest (Fig. 4). All samples originated from low nutrient mesocosms and more than half of them from macrophyte dominated ones (Table 2). Similarly to Cluster 1, the three temperature treatments and two sediment layers were almost equally represented.

Cluster 3 (11 samples) had the highest DW and lowest LOI, most P-fractions, TP, TFe, and NaOH-Al (Fig. 4). The samples represented slightly more low than high nutrient mesocosms with mostly macrophyte dominance (Table 2). Temperature treatments and sediment layers were almost equally represented.

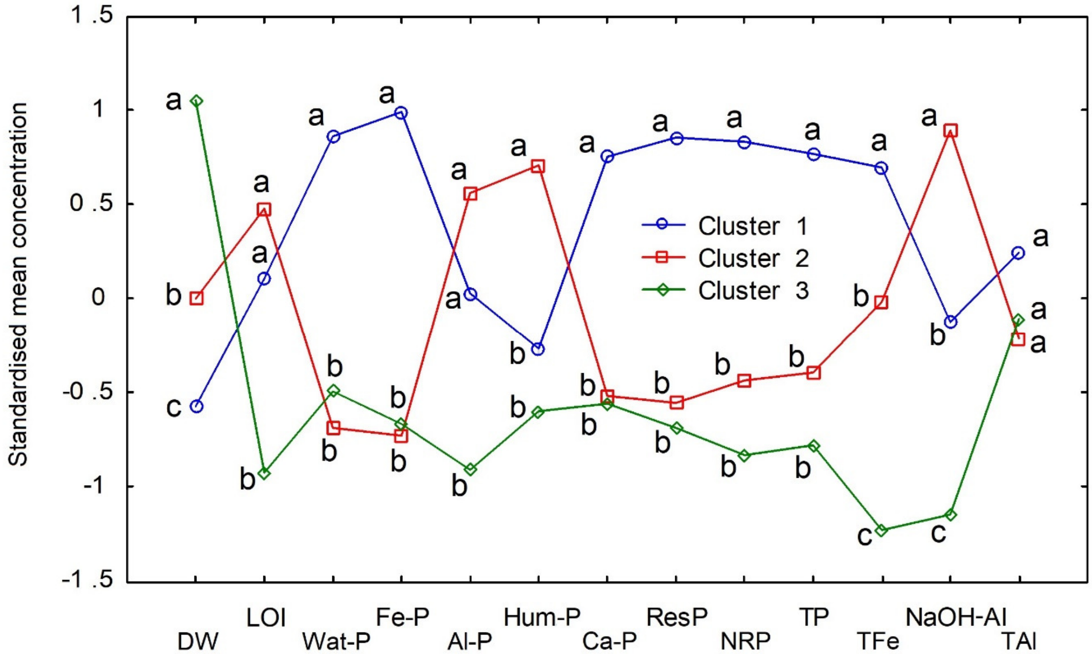


Figure 4. Standardised mean values of sediment variables used for clustering. At each variable, different letters denote significant differences between groups distinguished by the Fischer LSD test at 95% confidence level. See the text for cluster information. Variables in arbitrary order are connected with lines to better visualise the contrastive patterns.

Table 2. Representation of treatments, vegetation types and sediment layers in the revealed clusters.

Categorising factors	Levels	**Cluster 1 (n=20)**	**Cluster 2 (n=17)**	**Cluster 3 (n=11)**
Temperature	T0	7	5	4
	T1	8	6	2
	T2	5	6	5
Nutrients	Low	0	17	7

	High	20	0	4
Vegetation	MP	0	9	7
	FA	2	6	2
	PP	18	2	2
Layer	top	10	8	6
	mid	10	9	5

3.4. PCCA

Principal Components & Classification Analysis (PCCA), based on the same 13 sediment parameters as used for clustering, was conducted to detect possible relationship between the measured variables and different treatments. The two first factors described 60% of the total variance (Fig. 5).

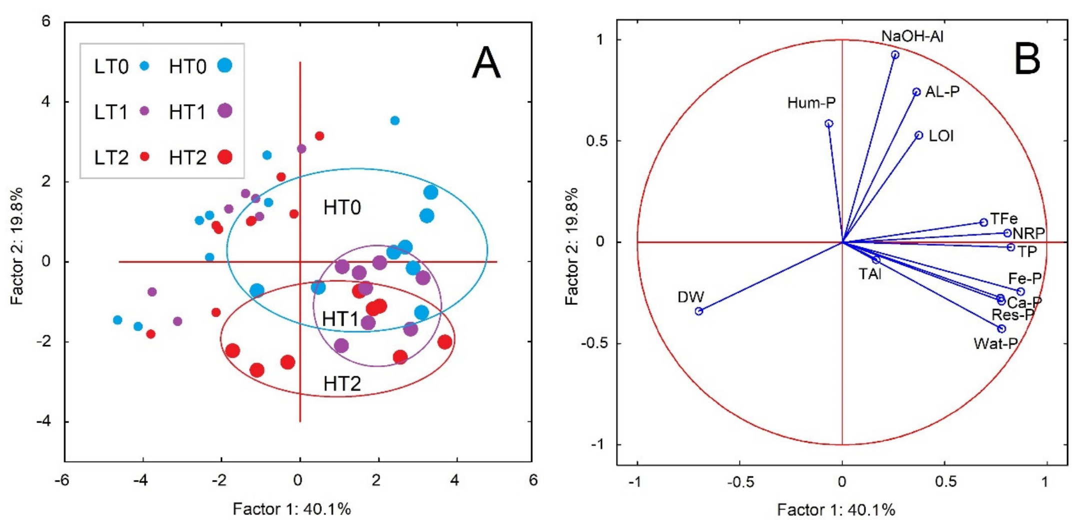


Figure 5. PCCA projection of sediment samples from differently treated mesocosms (A) and sediment properties (B) on the Factor 1 - Factor 2 plane.

PCCA Factor 1 described the nutrient effect. Twenty out of 24 samples with nutrient addition (virtually overlapping with Cluster 1) were located on its positive side. These sediments were characterised by low dry weight and higher TP, mobile P, Ca-P, and TFe. Twenty two out of 24 low nutrient samples (largely overlapping with Cluster 2) were located on the negative side of Factor 1 and were characterised by higher DW and low values of most P fractions.

PCCA Factor 2 described the effect of temperature treatment but only in nutrient enriched mesocosms. Samples were ordered along the axis according to temperature treatments from HT2 (most negative values) over HT1 to HT0.

Ambient temperature mesocosms had higher LOI, NaOH-Al, Al-P and Hum-P.

3.5. Effect of treatments

A summary of the two-way ANOVA results on treatment effects is given in the Suppl. Table 3.

Temperature and nutrient treatment separately had no significant effect on sediment DW and LOI, but a weak co-effect on DW occurred in the sediment top layer ($f(2,18) = 5.07$; $p = 0.018$). With warming, DW tended to increase at high nutrient treatment, but decreased at low nutrient treatment.

Among P fractions, nutrient addition had no effect on Al-P in any of the layers (Fig. 6c). High nutrient treatment resulted in lower Hum-P values in the upper two sediment layers, but the difference was significant only for the mid layer (Fig. 6d). Conversely, the contents of all other P fractions and also of TP, were significantly higher at high nutrient treatment in both upper sediment layers (Fig. 6).

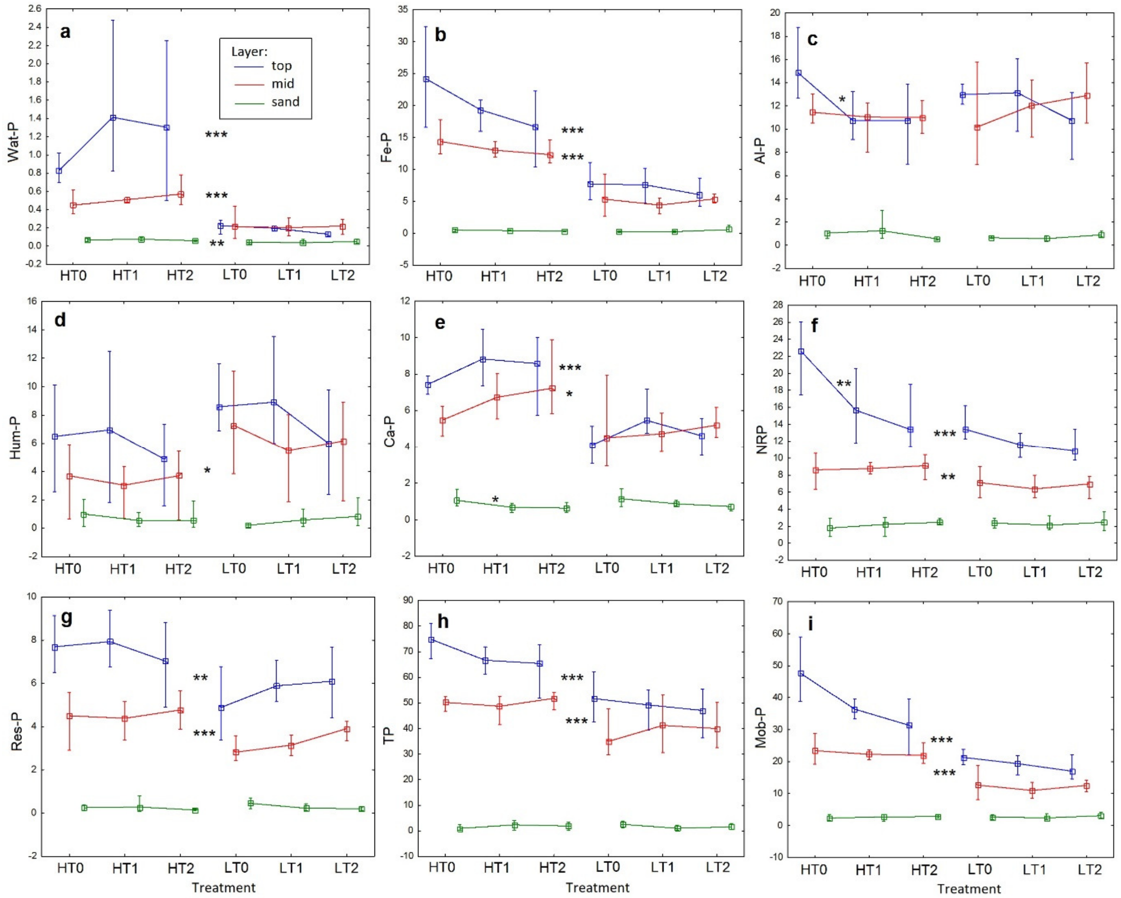


Figure 6. Distribution of phosphorus fractions in the three sediment layers (µmol/g DW) under different treatments (abbreviations explained in Table 1). Middle point – mean, spread – minimum and maximum of four replicates. Connected series show increasing temperature treatments (from left to right) under similar nutrient treatment. Asterisks in the middle field show significant effects of nutrient treatment (F-test), asterisks above a series show significant effects of temperature treatments (* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$).

Warming had a significant effect on Al-P and NRP in the top layer ($f(2,18) = 3.56$; $p = 0.050$ and $f(2,18) = 8.11$; $p = 0.003$, respectively), both showing highest values in unheated enclusures (Fig. 6c,f). No interaction effects of nutrient and temperature treatments were observed in any sediment layer.

Among metals, nutrient treatment had significant effects on TAl, TFe and HCl-Fe in the sediment top layer and additionally for HCl-Fe and BD-Fe in the mid layer (Fig. 7). In all occasions, metal concentrations were higher under high nutrient treatment. Nutrient addition had no effect on Res-Fe and NaOH-Al.

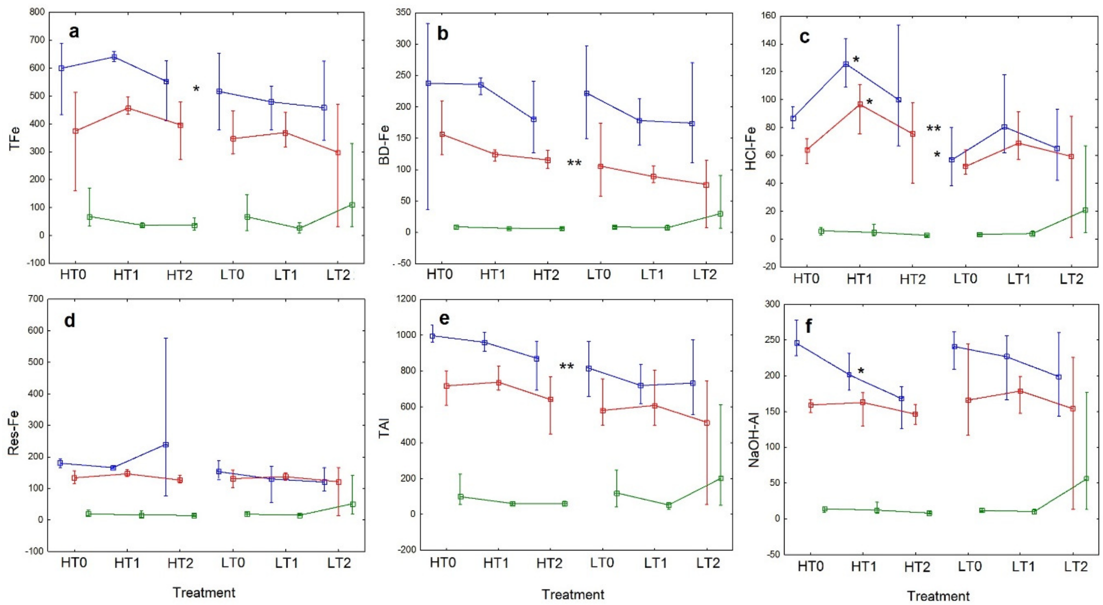


Figure 7. Distribution of iron and aluminium and their fractions in the three sediment layers (µmol/g DW) under different treatments. Middle point – mean, spread – minimum and maximum of four replicates. Connected series show increasing temperature treatments (from left to right) under similar nutrient treatment. Asterisks in the middle field show significant effects of nutrient treatment (F-test), asterisks above a series show significant effects of temperature treatments (* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$).

Temperature treatment had significant effects on HCl-Fe in both organic layers and for NaOH-Al in the top layer.

Among the P species measured in ^{31}P-NMR analysis, nutrient addition had no significant effect, but warming caused significant decline in Ortho-P and Mono-P (Fig. 8). The tendency of Poly-P to increase with higher temperature treatments remained non-significant due to large variability of the results under T2.

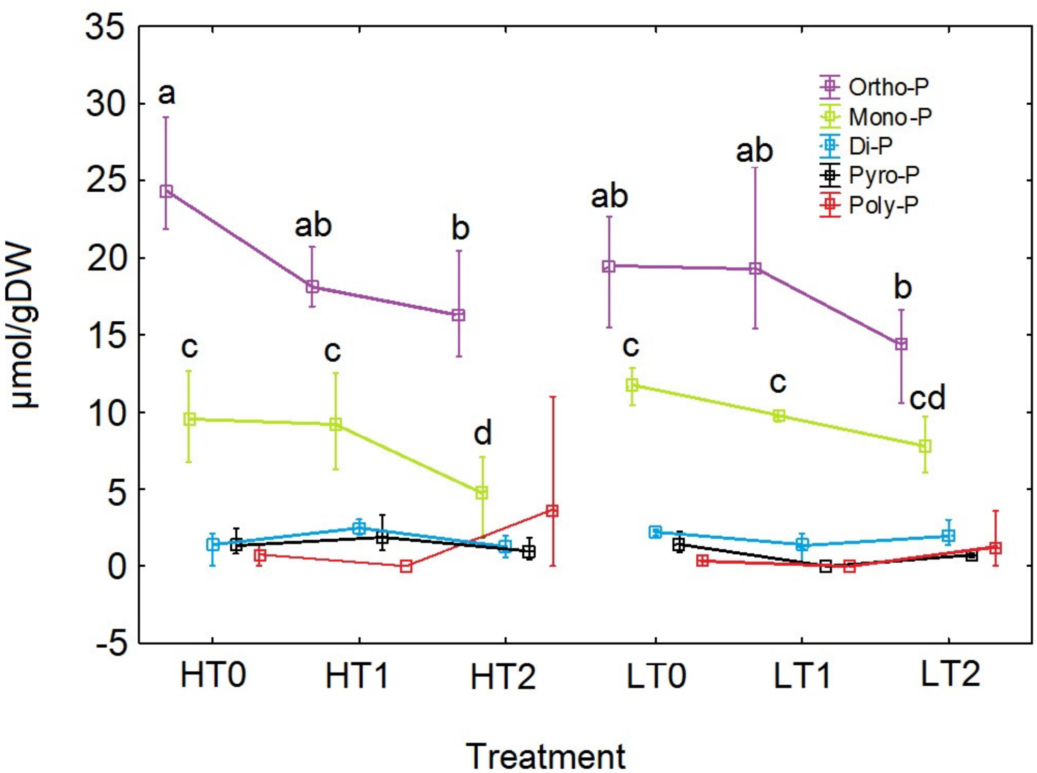


Figure 8. Distribution in the sediment of P species measured in ^{31}P-NMR analysis (µmol/g DW) under different treatments. Concept of the graph similar to that in Fig. 6. Different letters denote homogenous groups distinguished by LSD post-hoc test at 95% confidence level.

3.6. Coverage

The dominant vegetation type was strongly determined by nutrients: most of the nutrient enriched mesocosms were PP-dominated and non-enriched ones MP-dominated (Fig. 9, Suppl. Table 1). As an exception, PP dominated in one of the low nutrient mesocosms at the higher heating regime (T2). FA dominated nearly equally in both types and presented an intermediate affinity to nutrients

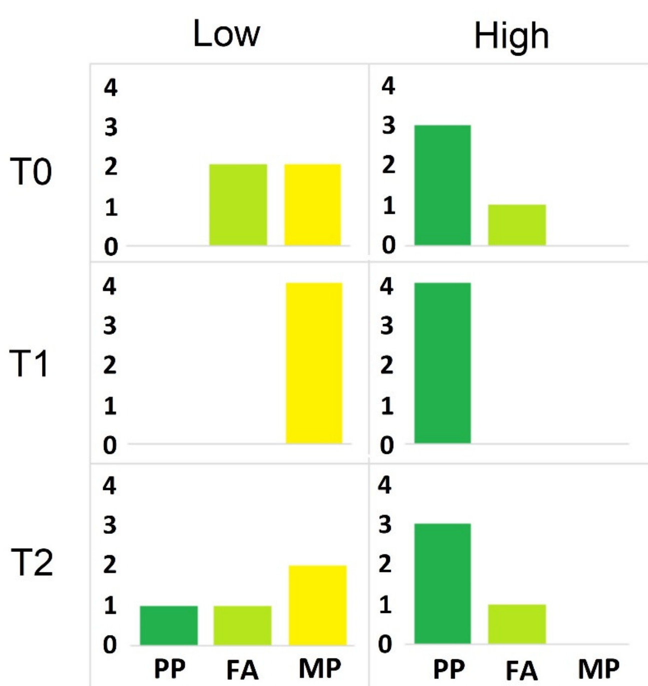


Figure 9 Distribution of dominant primary producers (PP – phytoplankton, FA – filamentous algae, and MP – macrophytes) by treatments. Height of the columns shows the number of mesocosms.

To identify the specific effects of the dominant vegetation type on sediment characteristics, the analysis was carried out separately for fertilized and unfertilized mesocosms. In addition to measured sediment characteristics in the top and mid layer, we tested the effect of the vegetation type on their vertical gradients (top/mid layer ratio) to follow the idea that macrophytes may have an active role in nutrient transport and sediment structure transformation. As the LSD post-hoc tests of the ANOVA analysis showed, in the low nutrient treatment, most of the significant differences occurred between MP and FA dominated mesocosms (Table 3). In the top layer of MP dominated mesocosms, there was more Ca-P than in FA mesocosms (p=0.037) and less TFe than in all other mesocosms causing steeper vertical gradients for Ca-P and gentler gradients for TFe. The mid layer of unfertilized MP dominated mesocosms was significantly poorer in Fe-P, Hum-P, and NRP than in the FA counterparts (p≈0.001). The DW gradient in unfertilized MP mesocosms was less than in FA mesocosms (p=0.025). Compared to unfertilized PP dominated mesocosms, the MP counterparts had less TFe in the top sediment layer (p=0.038) causing also a gentler vertical TFe gradient (p=0.022).

As none of the fertilized mesocosms were MP dominated, differences could be described only between PP and FA dominated ones. No significant differences were found in the sediment top layer. The mid layer of the fertilized PP dominated mesocosms had lower DW (p=0.040) but higher LOI (p=0.040) and TAl (p=0.002) than the FA dominated counterparts causing significantly gentler (corresponding p=0.033 and p=0.001) vertical gradients for the latter variables.

Among P species measured in ^{31}P-NMR analysis, Poly-P in low nutrient mesocosms dominated by PP was significantly (p<0.0001) higher than in the case of other plant dominants.

Table 3. Significant differences in sediment parameters and their vertical gradients between mesocosms dominated by macrophytes (MP), phytoplankton (PP) and filamentous algae (FA) as revealed by the LSD post-hoc test. For all other possible combinations of

parameter*layer*nutrient treatment, the differences caused by dominant vegetation type were statistically nonsignificant.

	Low nutrient treatment			High nutrient treatment		
Layer	Parameter	Difference	Significance (p)	Parameter	Difference	Significance (p)
Top	Ca-P	MP > FA	0.037			
	TFe	MP < FA	0.018			
		MP < PP	0.038			
Mid	Fe-P	MP < FA	0.010	DW	PP < FA	0.040
	Hum-P	MP < FA	0.010	LOI	PP > FA	0.040
	NRP	MP < FA	0.011	TAl	PP > FA	0.002
Top/mid ratio	DW	MP < FA	0.025	LOI	PP < FA	0.033
	Ca-P	MP > FA	0.049	TAl	PP < FA	0.001
	TFe	MP < PP	0.022			
Top+mid	Poly-P	MP < PP	0.000			
		FA < PP	0.000			

4. Discussion

Our analyses asserted the importance of the two factors – nutrient enrichment, temperature increase, but also of dominating plant community – on sediment accumulation rate, properties, distribution of metals (Fe, Al), and various P forms in shallow lake sediment, the latter having implicatons for P release and the persistence of internal loading. The study highlighted that higher nutrient load makes the system more susceptible to warming impacts.

The three statistical approaches (clustering, ordination and ANOVA) yielded coherent results each of them adding detail to the general picture.

Clustering of the 48 sediment samples from the two upper organic-rich layers clearly distinguished two larger clusters differing by nutrient treatment, although the treatments were not included as clustering parameters. The high nutrient Cluster 1 (20 samples) was characterised by the highest TP, TFe, and mobile P components (Wat-P, Fe-P, NRP), whereas the low nutrient Cluster 2 (17 samples) by the highest LOI, Al-P, Hum-P, and NaOH-Al and the lowest mobile P components. This suggest that nutrient addition was the leading factor determining the fractional composition of sediment P having strong implications for P mobility and internal loading potential (Søndergaard et al., 2003; Nürnberg, 2009). For the small cluster 3 (11 samples) with the highest DW and lowest LOI and TP in which both treatments sediment layers and vegetation types were more or less equally represented, we could not find a resonable explanation, except maybe enrichment with sand in the two organic layers. There was no clear pattern in the representation of different temperature treatments in any of the clusters.

Also the PCCA clearly showed the leading role of nutrient regime for most sediment P fractions and TFe. The effect of warming became visible only in nutrient enriched mesocosms and was negatively associated with parameters related to organic matter (LOI, Hum-P, Al-P, and NaOH-Al). A similar moderator effect of nutrients on the expression of temperature impact has been found in a lab study on phytoplankton (Staehr and Sand-Jensen, 2006) where the rates of photosynthesis, respiration and growth of phytoplankton generally increased with temperature, but this effect was further enhanced by high nutrient availability.

The ANOVA results showed that high nutrient loading led to higher accumulation of TP and most P fractions in the sediment, concuring with field observations in many shallow lakes subjected to large input of nutrients over decades (Granéli, 1999, Søndergaard et al., 2003). Higher P accumulation rates in fertilized enclosures could be expected and can be explained by higher net productivity in these encosures (Jeppesen et al., 2021). According to IPCC (2014) and simulation studies for Danish lakes (Jeppesen et al, 2009, 2011), we can expect a higher input of nutrients from the catchments in Danish lakes due to higher precipitation. Higher loading and higher retention increases the risk of problematic internal P-loading in the future. The Wat-P, Fe-P, and NRP fractions that increased with high nutrient loading treatment make up the mobile P pool and can potentially be released to the water column.

Hum-P was the only P-fraction that declined with high nutrient loading. This was in accordance with the decline in monoester-P and supports the finding by Reitzel et al. (2007), who analyzed the humic acid fraction from the sediment of Lake Erken by ^{31}P NMR, and found a large share of monoester P. The decline in Hum-P under high nutrient treatment could be a result of predominant phytoplankton production that is easily mineralizable leaving less recalcitrant humic matter compared to macrophytes. In a decomposition experiment with phytoplankton and macrophytes (Zhang et al., 2013), macrophytes produced at least twice the amount of chromophoric dissolved organic matter, CDOM (≈ humic matter) than did the phytoplankton. Humic substances can form complexes with Fe and P, making these essential elements less available for primary producers (reviewed by Maranger and Pullin 2003). Aluminum also forms complexes with humic acids and thereby competes with Fe for available complexing sites. The competition determins the relative abundances of the two metals in natural waters (Perdue et al. 1976, Jensen et al. 2005). In our data, Hum-P did not correlate with any other P fraction, but correlated positively with TFe and NaOH-Al. The adsorption strength of humic acids on iron oxides (Jones et al. 1993) and aluminium oxides (Tombácz et al. 2000) is pH-dependent and declines rapidly in the pH range of 6-10. An earlier study carried out in the same mesocosms (Ventura et al. 2008) showed that the nutrient enriched mesocosms had a higher pH, likely caused by enhanced photosynthesis (Jeppesen et al, 2021). Our results suggest that with higher external loading, the pH related desorption of Fe and Al releases also Hum-P indicating that release of all three substances is associated with dissolution of solid humic matter at high pH. Humic acids and complexed metals would thereby be lost from the system since higher nutrient loading to the mesocosms were not, as in natural lakes, associated with higher loading of metals and humic acids from a watershed.

With increasing temperature, sediment thickness showed a tendency to decrease under high nutrient loading, probably due to a higher mineralization rate. Treatments with high nutrient loading (HT0-2) were dominated by PP. Opposite to this LT0 and LT1 with MP dominance had the same (lower) sediment height while it increased in LT2 and even became higher than in HT2. Differences were not significant but it may be speculated that the increased PP dominance in LT2 caused higher sediment accumulation than in LT0 and LT1, so that the shift in dominant primary producer here overruled the effect increased temperature and caused higher sediment accumulation. Temperature had an effect also on NRP and Al-P concentration in the sediment. Highest amount of these P fractions was accumulated in the lowest temperature treatment. Liboriussen et al. (2011) who studied sediment respiration in the same mesocosms concluded that sediment respiration was stimulated by higher temperature, which suggests that high mineralization rate is a possible reason for the lower NRP content at higher temperatures in the current study. This also support the general assumption that NRP should be considered a part of the mobile P pool (e.g. Reitzel, 2005). Several mechanisms affect the release of P with higher temperature (Søndergaard et al., 2003). Temperature can stimulate the mineralization of organic matter leading to release of inorganic P (Boström et al., 1982; Søndergaard et al., 2003). Moreover, enhanced oxygen and nitrate consumption by bacteria at higher temperature reduce the redox potential and increase P release from reducible iron-bound P pools and from polyphosphate accumulation bacteria

(Boström et al., 1982). In addition, temperature can have an indirect impact on P bound to metals (Fe and Al), since there are smaller metal pools in the sediment at higher temperatures available for P sorption.

The type of primary producer may have both positive and negative effects on P and metal content in the sediments (Stephen et al., 1997; Søndergaard et al., 2003), however, as our study revealed, the impact was primarily nutrient level specific.

Under low nutrient conditions, the effect of MP contrasted first of all with that of FA. The higher Ca-P content in the sediment top layer and the steeper vertical gradient in MP dominated mesocosms could be caused by the stimulated Ca and P co-precipitation by macrophytes. Elevated pH on leaf surface due to intensive photosynthesis has been shown for both *Elodea* and *Potamogeton* species (Prins et al. 1980; Liu et al. 2016) and can lead to calcite precipitation (Liu et al. 2016) with possible P coprecipitation (Otzuki and Wetzel 1972. We found only *Elodea* and *Potamogeton* species in the enclosures.

In the present study the most remarkable differences occurred in the sediment mid layer that in the MP dominated mesocosms contained significantly less mobile P components (Fe-P and NRP), but also less Hum-P, than the FA mesocosms giving evidence of macrophyte P-uptake by macrophyte roots, which is in accordance to Boström et al. (1982) and Stephen et al. (1997). In a study by Christiansen et al. (2016) roots of *Elodea* and *Potamogeton* were most abundant down to 10 cm depth which is more or less the depth covered by our „mid layer“.

Such P mobilization can be quite substantial (Boström et al., 1982) and the sediment P concentration in macrophyte beds is therefore often reduced (Horppila and Nurminen, 2005), and so is the P release from the sediment due to root and shoot oxidized surface sediment (Andersen and Ring, 1999; Horppila and Nurminen, 2005; Boros et al., 2011), thereby enhancing the redox potential and thus P sorption to iron compounds (Andersen and Ring, 1999; Aldridge and Ganf, 2003; Søndergaard et al., 2003; Boros et al., 2011). Shallow and nutrient-rich lakes with dense macrophyte coverage may, however, have increased respiratory activities leading to decreased redox potential (Stephen et al., 1997; Horppila and Nurminen, 2005; Søndergaard et al., 2003; Boros et al., 2011), and thus increase P release (Stephen et al., 1997; Horppila and Nurminen, 2005; Søndergaard et al., 2003). That was likely the cause for the observed low Hum-P values in the MP dominated mesocosms where an earlier study by Boros et al. (2011) showed low redox values at the sediment-water interface. Therefore, in macrophyte-rich lakes, plants can be important modifiers of early P diagenesis as well as activators of the internal P loading (Boström et al., 1982).

In general macrophytes did not thrive under high nutrient conditions. The only significant plant cover related differences at high nutrients were the lower DW and higher LOI in the mid layer of PP vs FA dominated mesocosms causing also gentler vertical gradients in the two latter parameters under PP dominance. This difference is not easy to interpret as the sedimentation of both FA and PP production should directly affect only the sediment top layer. The mid layer will be affected only if mixing occurs or if the accumulations rates are so high that the fresh sediment layer thickness exceeds the top layer thickness of 2 cm. The latter was likely the case in enclosures with 15-16 cm of organic sediment (HT0, HT1, LT2) that all had a significant share of PP.

The type of primary producers also had differential effects on sediment metal content. Highest TFe and TAl concentrations in the top sediment layer, and highest TAl concentrations in the mid layer were measured in the mesocosms dominated by PP. There are more possible explanations for that: (1) PP dominated mesocosms had higher accumulation rates and thereby higher retention rates of Fe and Al from the inflowing water, (2) the organic rich sediments of PP dominated mesocosms in combination with high redox in the surface sediment will favour upwards migration and reprecipitation in the surface of Fe, and (3) in

MP dominated mesocosms, part of the metals was taken up and incoorporated in the macrophytes at the time of sediment sampling. Boros et al. (2011), studying the redox potential in the same mesocosms, found the highest redox potential in PP dominated mesocosms and lowest in mesocosms with a dense submerged macrophyte coverage.

5. Conclusions

The use of mesocosms where only temperature and nutrients were varied, provided us with significant results on the long-term effect of the two factors that would have been very difficult to obtain from studies of natural lakes.

With continued nutrient addition to H-mesocosms we mimiced a climate change induced higher external loading of nutrients that led to increased sediment accumulation and higher nutrient concentration in the sediment. The concentration of TP and most P fractions, except Al-P and Hum-P, were significantly higher in nutrient enriched mesocosms. A predicted higher input of nutrients from the catchments due to higher precipitation in the north temporate zone (IPCC, 2014) implies that a higher mobile P pool is formed in the sediment that can eventually be released to the water column. This process might be modified by an accordingly higher erosional input of sequioxides of Fe and Al with binding sites for P. The formation of humic-metal-P complexes that bind part of P making it unavailable for bioproduction, is hampered by high pH caused by enhanced photosynthesis under high nutrient conditions.

We found that nutrient addition had a significant effect on sediment thickness at ambient temperature. With increasing temperature, sediment thickness showed a tendency to decrease at high nutrient loading, and increase at low nutrient loading, the first supporting our hypothesis of increased mineralisation in warmer conditions as warming caused significant declines in organic matter content (LOI), Hum-P and Mono-P, while the latter probably is a result of phytoplankton dominating primary production in some of the „high temperature and low nutrient“ mesocosms.

The dominant vegetation type was a strong covariant of nutrients. In macrophyte dominated mesocosms, there was more Ca-P in the top layer and less Fe-P, Hum-P and NRP in the deeper layer. The higher Ca-P content in the sediment top layer was likely caused by coprecipitation of P with calcite on macrophyte leaves. The low concentration of mobile P components (Fe-P and NRP) but also Hum-P in the deeper sediment layer provide evidence of an upward transport by macrophyte roots showing that plants can be important activators of the internal P loading.

Our study showed that eutrophication of shallow lakes through external nutrient loading is the primary factor increasing sediment accumulation rate and concentrations of mobile P forms in the sediment whereas the differences between high and low nutrient conditions decrease with warming as higher temperatures support mineralization of organic matter and P release especially under high nutrient conditions. The dominant vegetation type, although being a covariant of nutrient loading, can cause numerous differences in the vertical distribution of sediment P fractions and metals and possibly also in sediment accumulation rates. We can expect less dominance of submerged macrophytes and higher dominance of phytoplankton (and filamentous algae) with climate change in eutrophic lakes (Jeppesen et al., 2010;Moss et al., 2011) implying major changes in accummulation of organic matter and the forms of P in lake sediments in the future.

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