Ecosystems (2017) 20: 1483–1493
DOI: 10.1007/s10021-017-0126-z

ECOSYSTEMS

Perpetual Phosphorus Cycling: Eutrophication Amplifies Biological Control on Internal Phosphorus Loading in Agricultural Reservoirs

Keunyea Song* and Amy J. Burgin

University of Kansas and Kansas Biological Survey, 2101 Constant Ave., Higuchi Hall, Lawrence, Kansas 66047, USA

Abstract

Nearly half of US lakes are impaired, primarily resulting from excessive nutrients and resultant eutrophication. The stability and recycling of sediment P results in differing degrees of internal P loading, which can alter lake water quality. In this study, we asked: (1) What are the underlying mechanisms controlling internal loading (net release) and retention of P? and (2) How does trophic state, specifically a hypereutrophic condition, affect internal P loading in agricultural reservoirs? We show that shifts in internal P loading are related to trophic-level indicators, including total P (TP) and chl-*a* concentrations. All study reservoirs were classified as hypereutrophic, and we grouped them as ''less eutrophic'' or ''more eutrophic'' based on TP and chl-*a* concentrations. In less eutrophic lakes, chemical variables (for example, oxygen) and sediment iron-bound P primarily controlled internal P loading under anaerobic conditions. However, in the more eutrophic lakes, biological variables, including phytoplankton biomass (as indicated by chl-*a* concentrations) and extracellular enzyme activity, drove internal P loading or reduced P retention under aerobic conditions. Biologically controlled aerobic internal P cycling was related to higher sediment organic P pools being broken down by enzymatic hydrolysis. Therefore, we theorize that as lakes become hypereutrophic, biological mechanisms begin to amplify internal P release by acting under both anaerobic and aerobic conditions, thus creating a perpetual cycle of internal P loading. Thus, the role of biological processes and oxygen availability should be considered in water quality management strategies aimed at alleviating eutrophication in lakes.

Key words: eutrophication; internal phosphorus cycling; oxygen availability; biological activity; sediment P fractionation; chemical precipitation.

Introduction

Agricultural landscapes are characterized by large phosphorus (P) exports (Tilman and others 2001). Phosphorus enters agricultural watersheds during high runoff (Carpenter and others 2015) and is transported downstream to lakes or reservoirs. Lake phosphorus concentrations in the USA continue to increase, a trend that results in nearly half of US lakes listed as impaired due to cultural eutrophi-

Received 4 October 2016; accepted 28 January 2017;
published online 13 March 2017
Electronic supplementary material: The online version of this article (doi:10.1007/s10021-017-0126-z) contains supplementary material, which is available to authorized users.
KS designed the study, conducted the research, data analysis, interpretation, and wrote the manuscript. AB designed the study, contributed to interpretation of the data, and wrote the manuscript.
Corresponding author; e-mail: keunyea@gmail.com

cation (Carpenter and others 1998; Stoddard and others 2016). Once P enters an aquatic ecosystem, it cycles internally; some P is retained into sediment layers, whereas other fractions (for example, labile P or loosely bound P) can be remobilized to the water column, a phenomenon termed internal P loading (Pettersson 1998). Although external P loading is the main driver of eutrophication, internal P loading can exacerbate eutrophication as large amounts of P from the sediment can be recycled for extended periods (Carpenter and others 1999; Hamilton 2012).

Internal P flux (or loading) is the balance between P retention and release, both of which are determined by a combination of physicochemical and biological processes. Physicochemical processes such as sedimentation, physical adsorption, and co-precipitation with minerals retain P in lake sediments (Reddy and others 1999; Wetzel 2001). In particular, redox-sensitive Fe–P interactions (for example, adsorption to iron-oxides, co-precipitation, and dissolution) are regarded as the dominant mechanism regulating internal P fluxes (Petticrew and Arocena 2001; Azzoni and others 2005; Ding and others 2015, 2016). This chemical control on P dynamics suggests that oxygen is a primary controller of internal P loading (Nürnberg 1987, 2009b; Genkai-Kato and Carpenter 2005). Under aerobic conditions, ferric hydroxide stabilizes P in the sediments; in contrast, anaerobic conditions reduce Fe and remobilize Fe and P from sediments (chemical reduction and dissolution). Thus, even a thin (mm scale) oxygenated sediment layer can prevent P release, whereas persistent stratification and anoxic hypolimnetic waters facilitate P and Fe release (Mortimer 1971; Nürnberg 1987; Sinke and others 1990; Song and others 2013).

Although redox-controlled internal P loading is widely accepted as the dominant control on internal P cycling, many other mechanisms contribute to sediment P release. Some lakes exhibited continuous internal P release under well-mixed aerobic conditions (Sinke and others 1990) or released P during both aerobic and anaerobic periods (Hupfer and Lewandowski 2008). Additionally, oxygenation of the hypolimnion, a common restoration technique, does not always reduce internal P loading (Gächter and Wehrli 1998). These observations suggest that other mechanisms can play a major role in internal P loadings. Possible additional mechanisms controlling internal P loading include: (1) pH-dependent precipitation and dissolution of mineral bound P, (2) polyphosphate-accumulating bacteria, and (3) organic matter mineralization (Wetzel 2001). However, we still lack a comprehensive understanding of the factors that control P dynamics, and specifically which factors determine the degree of internal P loadings under highly eutrophic conditions.

In this study, we ask: (1) What are the underlying mechanisms controlling internal loading (net release) and retention of P? and (2) How does trophic state, specifically a hypereutrophic condition, affect internal P loading in agricultural reservoirs? We hypothesized that internal P dynamics and associated mechanisms would vary among reservoirs depending on lake trophic status, as well as water and sediment characteristics. We tested our hypothesis using a sediment core flow-through experiment to measure internal loading under a range of environmentally relevant conditions (for example, oxic vs. anoxic). We also related internal loading to sediment chemistry, microbial activities, and lake characteristics.

Materials and Methods

Site Description and General Sampling

We selected four hypereutrophic reservoirs in Eastern Nebraska (Table 1). July TP and chl-*a* contents in the epilimnion were compared with a trophic classification scheme (Nürnberg 1996; Galvez-Cloutier and Sanchez 2007). All study reservoirs have similar morphological characteristics including size, water depth, age, and land use

Table 1. Characteristics of the Study Reservoirs, Including Averaged TP and Chl-a Concentrations from the Epilimnion in July

Reservoirs	Construction year	Surface area (km^2)	Max. depth (m)	Land use	TP (μg/L)/ Chl-a(μg/L)	Trophic Status
Bluestem	1963	3.3	4.6	75 % Agri.	112/102	Hypereutrophic
Conestoga	1963	2.3	4.9	50 % Agri./ 50 % Prairie	71/256	Hypereutrophic
WagonTrain	1963	3.2	4.9	75 % Agri.	398/355	Hypereutrophic
YankeeHill	1965	2.1	4.3	75 % Agri.	332/436	Hypereutrophic

types (Table 1). Our study lakes (<10 km^2) represent the globally abundant small size impoundments (Downing and others 2006) and the increasing concerns about their susceptibility to eutrophication (Smith and Schindler 2009; Stoddard and others 2016). These reservoirs are also representative for the majority of Nebraska agricultural reservoirs that are listed as eutrophic (62%) or hypereutrophic (33%) (Nebraska Department of Environmental Quality 2016).

Water samples were collected from the deepest location of each reservoir; previous work documented that the deepest point was representative of the rest of the reservoir (Thomas and Chandrakiran 2013). To better explain the spatial and temporal variations (for example, with depth and seasons), water samples were collected from both surface (ca. 10 cm below the surface water: epilimnion) and near the sediment–water interface (ca. 50 cm above the sediment surface: hypolimnion) on a biweekly basis from June to November, 2014. A Garmin Legend CX-GPS was used to ensure samples were consistently collected at the same location. Water samples were preserved with acid for subsequent chemical analysis for total phosphorus (TP). Temperature and dissolved oxygen were measured in situ using an YSI 6920 V2 Multiparameter Water Quality Sonde. Triplicate 30 mL of surface water samples was filtered through GF/F filters for chlorophyll *a* (chl-*a*) analysis.

Internal P Loading: Sediment Core Flow-Through Design

To measure internal P loading, seven to nine sediment cores were collected using a suction coring device and acryl cylinder corers (8 cm × 40 cm) from each reservoir. The sediment cores were collected from the deepest location in each reservoir twice (July and October 2014). We isolated the surface 20 cm-deep sediment where water–sediment interactions mainly occur and discarded the remaining sediment. Three cores were broken down immediately to quantify sediment P fractions using the sequential fractionation process (details below). The remaining six cores (July) or four cores (October) were used for the flow-through experiments. Large volumes (120 L) of surface water were collected from each reservoir prior to the sediment collection; this served as the inflow source water for the flow-through experiments.

In the laboratory, we adjusted the sediment cores to have 10 cm of overlying water and sealed with a cap connected to inflow and outflow tubes. Replicate cores were then placed in the water bath filled with tap water to buffer the cores from temperature variations. Inflow water was kept either anaerobic (July; degassed with N_2) or aerobic (October; bubbled with air) to mimic in situ conditions of summer stratification or fall post-turnover of study reservoirs (confirmed by DO profiles, Supplementary Figure 1). Inflow water was circulated over the cores at a rate of 1 ml/min. During the flow-through experiments, temperature (°C), dissolved oxygen (DO, mg/L) and pH were measured in the inflow and outflow waters of each core using an YSI-556 MPS and pH probe. Inflow and outflow water samples were collected at 3–24-h intervals for 14–16 days. Water samples were filtered immediately through 0.45-μm filters and stored for subsequent chemical analysis.

Chemical and Microbial Analysis of Sediment and Water Samples

Acidified in situ water samples collected from each reservoir were digested using potassium persulfate. Filtered water samples from the flow-through experiment and digested lake water samples were analyzed via Flow Injection Analyzer (Astoria Pacific A2) for dissolved inorganic P and TP analysis (USEPA 1993, Method 365.1). Chlorophyll *a* (chl-*a*) was extracted in ethanol for 24 h and analyzed on a fluorometer (Nusch 1980) (Turner 10-AU) and used as a rapid indicator for phytoplankton biomass (Kasprzak and others 2008).

Subsamples of the initial three sediment cores were analyzed for P fractions and extracellular enzyme activities. Fractionation of different P forms in the sediment was determined using 2 g of fresh sediment by chemical extractions method (Diaz and others 2006). Inorganic P fractions including labile P, Fe (or Al-)-binding P, and Ca (or Mg-)-binding P fractions were extracted using 1M KCl, 0.1M NaOH, 0.5M HCl solutions in sequence. The residue from this sequential extraction was then combusted at 550 °C and digested in 6M HCl solution, which determines the residual organic P fraction. Each extracted solution was then filtered through 0.45-μm filters. All extracted P in the solution was determined by colorimetric method using a spectrophotometer (Cary 50 Bio, Varian Inc). Total P (TP) contents in the sediments were estimated by summation of all fractions.

Two extracellular enzyme activities related to P cycling, β-D-glucosidase and alkaline phosphatase, were determined by a fluorescence method (Freeman and others 1995). Five gram of fresh sediment subsampled from the initial three sediment cores was mixed with 40 mL distilled water to separate

extracellular enzymes into the supernatant. Four replicated, 250 µL supernatant per core were mixed with 50 µL of fluorescence labeled model substrates, methylumbelliferyl-β-D-glucoside and methylumbelliferyl phosphate in microplate wells. The solution was incubated for 60 min, and final fluorescence was measured in a plate reader (Synergy H4, Biotek).

Data Analysis

Phosphorus retention rate (%) was calculated based on the concentration difference between dissolved inorganic P (DIP) in inflow and outflow water samples from each core (Eq. 1):

$$R(\%) = \frac{[\mathrm{P}]\mathrm{in} - [\mathrm{P}]\mathrm{out}}{[\mathrm{P}]\mathrm{in}} \times 100 \quad (1)$$

where R represents the P retention rates (%), [P]in and [P]out are the DIP concentrations in inflow and outflow water samples, respectively. Positive retention rates (%) indicate that P is retained in the reservoir sediment while negative values signify that the reservoir releases more P into the outflow water than the amount in inflow water samples, acting as an internal P source. Rates were averaged using the measurements at every sampling time (Supplementary Figure 2). We applied a linear mixed effects model (LME) to identify the differences of P retention (%) between seasons (that is, oxygen availability) measured by the flow-through experiment for each reservoir, with the sampling time (hours) as a random factor. A Mann–Whitney U test was used for soil P fraction differences between seasons. Statistical analysis was performed in R.3.3.1.

Results

Reservoirs were all classified as hypereutrophic due to TP concentrations ranging from 71 to 1090 µg/L (Table 1; Figure 1A/B). Although TP concentrations varied seasonally (Figure 1A), YankeeHill and WagonTrain had higher TP concentrations (for example, average TP of 398 and 332 µg/L in July) compared to Bluestem and Conestoga (for example, 112 and 71 µg/L in July, respectively; Figure 1D). Chl-*a* concentrations followed the similar pattern to water column TP with higher phytoplankton biomass in WagonTrain and YankeeHill compared to Bluestem and Conestoga in the summer (Figure 1F). For comparison of our results, we classify WagonTrain and YankeeHill as ''more eutrophic,'' whereas we term Bluestem and Conestoga ''less eutrophic'' in the following figures. This distinction is based on large differences in the average July epilimnetic and hypolimnetic TP concentrations (Figure 1D/E) and higher chl-*a* (Figure 1F) in WagonTrain and YankeeHill.

P retention and release rates measured in flow-through experiments showed two distinct and contrasting patterns among four reservoirs (Figure 2): (1) less eutrophic reservoirs (Bluestem and Conestoga) released P under anaerobic conditions, but retained it during aerobic conditions, whereas (2) more eutrophic–hypereutrophic reservoirs (WagonTrain and YankeeHill) released more P, leading to net release (WagonTrain) and decreased retention of P (YankeeHill) under aerobic conditions. Bluestem and Conestoga released P during anaerobic conditions (July) with an average P retention rate of −51 and −142%, respectively (Figure 2). Aerobic conditions (in October) resulted in positive P retention rates for both reservoirs (Bluestem = 16% and Conestoga = 49%). In contrast, the more eutrophic reservoirs (WagonTrain and YankeeHill) showed the opposite direction of changes in internal P fluxes depending on oxygen availability. The average P retention rate for WagonTrain was 8% with anaerobic conditions and −143% with aerobic conditions (Figure 2). YankeeHill sediments retained P under both anaerobic and aerobic conditions, but the retention rate decreased significantly from 34 to 16% between aerobic and anaerobic conditions (Figure 2). Dissolved oxygen (DO) in the cores during the experiments confirmed anaerobic conditions in July (DO ranged 1.0–1.3 mg/L) and aerobic conditions in October (DO ranged 8.6–9.8 mg/L; Table 2). Phosphorus (measured as dissolved inorganic P) concentrations in inflow water varied between reservoirs, which reflects the variation in reservoir water column P concentrations (Table 2). Water pH in YankeeHill was the highest relative to the other reservoirs during both anaerobic and aerobic experiments with pH 9.6 in anaerobic (July) and pH 8.4 in aerobic (October) conditions. WagonTrain showed the second-highest pH values with pH 9.2 and 8.4 in the water columns, respectively (Table 2).

The total amount of P and distribution of P in four major sediment fractions suggest that more eutrophic lakes gain sediment P, particularly in the organic fraction, under aerobic conditions that set up in October after lake turnover (Figure 3). In contrast, less eutrophic lakes lost total P from the sediment post-turnover (aerobic conditions), particularly in the iron-bound pool (Figure 3). In the more eutrophic lakes, the increased sediment P was mainly derived from higher organic P and Ca–P

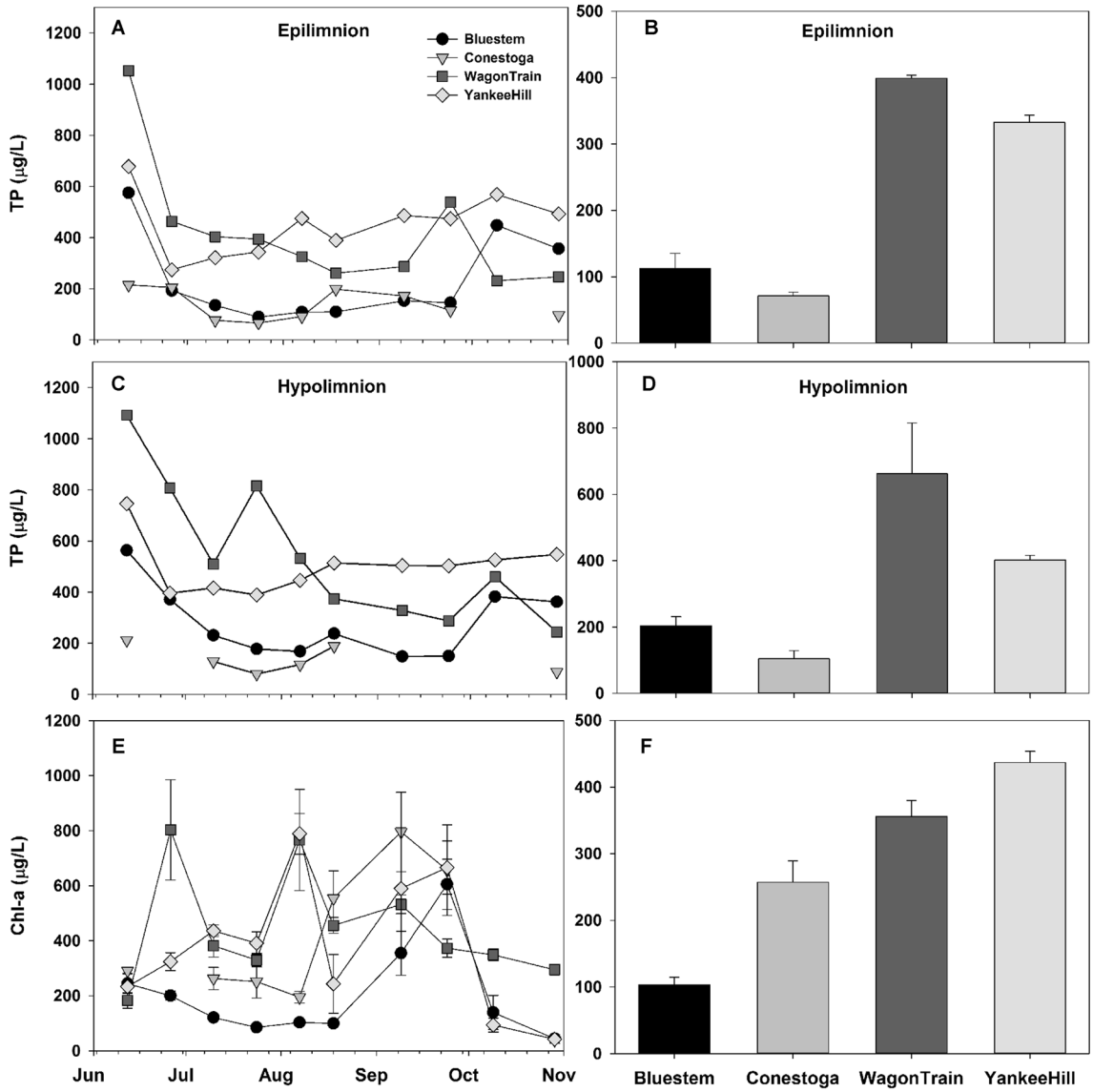

Figure 1. Temporal changes of water TP in the epilimnion (**A**), hypolimnion (**B**), and chlorophyll *a* concentrations (**C**) in the four hypereutrophic study reservoirs. Average (mean ±SE) July TP from the epilimnion (**D**), hypolimnion (**E**), and chl-*a* concentrations (**F**; from the epilimnion) comparing directly comparing the four lakes.

pools in the fall. Fall sediment organic P pools were substantially higher than summer sediment organic P in WagonTrain ($p = 0.057$) and YankeeHill ($p = 0.114$), but were not significantly different in the two less eutrophic lakes (Bluestem, $p = 0.400$; Conestoga, $p = 0.886$). Among P fractions, Fe–P was the most abundant form in 7 of 8 sampling periods, accounting for an average of 57% (range 28–72%) of sediment P across all four reservoirs, whereas labile inorganic P (for example, pore-water dissolved inorganic P; DIP) contributed less than 1% of sediment P.

Larger pools of organic P (Figure 3) in more eutrophic lake sediments correlated with higher extracellular enzyme activities compared to those in the less eutrophic reservoirs (July [summer] vs. October [fall]; Table 2; Figure 4). Moreover, phosphatase activities in the sediments of more eutrophic reservoirs increased significantly (~6×) after the hypolimnion was re-aerated post-turnover (Figure 4). Phosphatase activities in the more eutrophic reservoirs were negatively correlated with internal P retention and release patterns at the flow-through experiment (Figure 4A), though

there were large differences between phosphatase activity between July and October (anaerobic vs. aerobic conditions). Note that the relationship between phosphatase activity and P retention during the summer (anaerobic conditions) is not significant, but is negatively correlated (Figure 4B). No significant correlation was found between internal P loadings and extracellular enzyme activities for Bluestem and Conestoga reservoirs (Supplementary Figure 3).

Discussion

Agricultural reservoirs receive excessive external P from surrounding areas (Carpenter and others 1998; Withers and Jarvie 2008), leading to eutrophication and P enriched sediments. Stability and recycling of sediment P results in differing degrees of internal P loading and retention, which can exacerbate eutrophication. We show that shifts in internal P retention and release patterns from summer (anaerobic) to fall (aerobic) (Figure 2) are related to trophic-level indicators of lakes, including TP and chl-*a* concentrations (Table 1). We attribute this pattern to the higher P content of sediments post-turnover in more eutrophic lakes (Figure 3). The increased sediment organic P pool correlates with higher enzyme activity (Figure 4), which releases more P from the sediments thereby decreasing P retention (that is, net P release or reduced P retention) in more eutrophic lakes under aerobic conditions (Figure 2). Below, we discuss how the links between increased biomass (Chl *a*; Figure 1) create larger total and organic sediment P fractions (Figure 3) and increased enzyme activity (Figure 4), which in turn drive the shift from decreased sediment P retention measured in more eutrophic lakes (Figure 2). We postulate that, under these conditions, hypereutrophic lakes manifest a perpetual P cycle wherein biological and chemical factors support continual internal P loading under anaerobic *and* aerobic conditions.

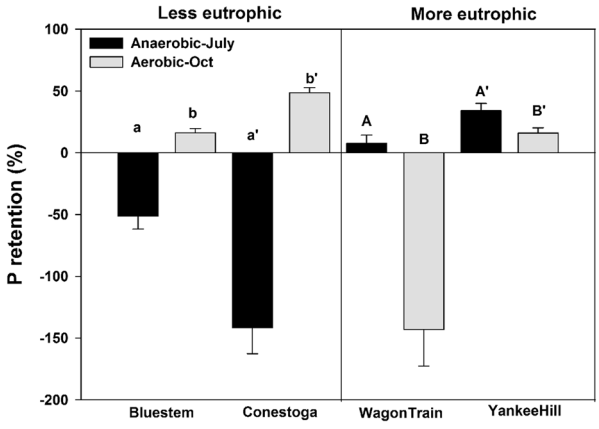

Figure 2. Averaged P retention (expressed as % of loading) during flow-through internal loading experiments conducted under anaerobic (July) and aerobic (October) conditions. Different letters represent significant difference ($p < 0.05$) between seasons for each reservoir.

The redox-sensitive Fe–P interaction, which we consider a predominantly chemical control, profoundly influences P availability (Mortimer 1942, 1971; Wetzel 2001) (Figure 5A). In oligotrophic and mesotrophic lakes, phosphate is largely bound by mineral oxides in the sediment. As lakes become eutrophic, P can be released from Fe-containing minerals under anaerobic conditions (Figure 5C). However, aeration of the hypolimnion post-turnover reestablishes the P-binding mechanism. Our

Table 2. Sediment Phosphorus and Extracellular Enzyme Activities (mean ± s.e.), and Inflow Water Properties During Flow-Through Incubation Experiments

Reservoirs	Month	Flow-through Experiment Inflow Water				Sediment		
		DIP (μg/L)	Temp (°C)	DO (mg/L)	pH	TP (μg/g)	G-ase ($nmol \cdot g^{-1} \cdot h^{-1}$)	P-ase ($nmol \cdot g^{-1} \cdot h^{-1}$)
Bluestem	July	30 (±3.9)	24.7 (±0.1)	1.0 (±0.1)	9.0 (±0.0)	1107 (±53)	39.4 (±1.8)	434.5 (±13.0)
	Oct	141 (±23.3)	21.7 (±0.2)	8.8 (±0.1)	8.3 (±0.0)	688 (±46)	76.2 (±9.8)	456.6 (±32.1)
Conestoga	July	13 (±1.5)	24.4 (±0.2)	1.3 (±0.2)	9.0 (±0.0)	949 (±47)[a]	22.7 (±0.5)	421.6 (±13.4)
	Oct	63 (±6.3)	21.8 (±0.2)	8.6 (±0.2)	8.3 (±0.0)	734 (±28)[b]	43.5 (±2.9)	331.4 (±15.5)
Wagon	July	109 (±24.5)	22.9 (±0.4)	1.0 (±0.1)	9.2 (±0.0)	973 (±90)	84.5 (±5.1)	1071.5 (±27.2)[a]
Train	Oct	91 (±34.1)	18.7 (±0.3)	9.7 (±0.1)	8.4 (±0.2)	1136 (±156)	285.0 (±7.6)	5148.8 (±132.8)[b]
Yankee	July	299 (±26.3)	23.6 (±0.3)	1.0 (±0.1)	9.6 (±0.1)	1198 (±100)	50.2 (±2.3)[a]	1022.9 (±34.7)[a]
Hill	Oct	643 (±29.8)	18.4 (±0.3)	9.8 (±0.1)	8.4 (±0.0)	1271 (±86)	147.4 (±6.5)[b]	3526.9 (±153.4)[b]

Letters represent significant differences in sediment microbial and chemical properties between seasons determined by Mann–Whitney U test ($p < 0.05$). DIP indicates dissolved inorganic P concentration, whereas G-ase and P-ase represent β-D-glucosidase and phosphatase, respectively.

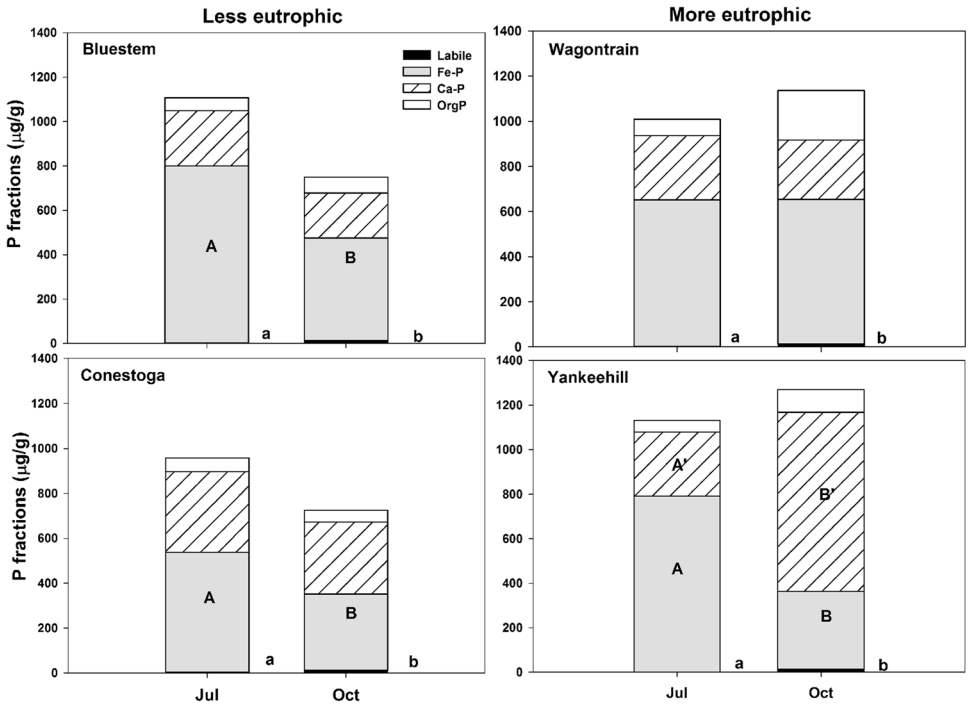

Figure 3. Total sediment P (represented by the height of the bar) and sediment P fractions (for four major sediment P pools) in July and October from each reservoir. Letters represent significant difference ($p < 0.05$) between seasons for each fraction. Lowercase a/b shows the difference of the labile fractions.

findings suggest that as lakes move into the hypereutrophic category (Figure 5D), very high levels of primary production likely alter sediment organic contents and quality, which in turn is decomposed and released to the water column, even under aerobic conditions.

In our study, the ''less eutrophic'' Bluestem and Conestoga released P under anaerobic conditions, but retained P during aerobic conditions (Figure 2). This pattern fits with the expected mechanism of internal P loading in a eutrophic lake (Figure 5C). Increased P retention (that is, lower internal loading) under aerobic conditions confirms the importance of redox-sensitive Fe–P interactions as a control on internal P loading (Søndergaard and others 1996; Penn and others 2000). The Fe–P pool dominated the sediment P under both summer (anaerobic) and fall (aerobic conditions; Figure 3), which also supports the importance of Fe–P interaction. High abundance of Fe–P pool in this study is interesting as other studies found relatively smaller fraction of Fe–P in the sediments (Lake and others 2007; Wilson and others 2010). Previous studies demonstrated that sediment Fe–P fractions increase with eutrophication (Søndergaard and others 1996; Gonsiorczyk and others 1998), and the shift can become more pronounced under hypereutrophic conditions (Kisand 2005; Wilson and others 2010).

In contrast to the ''less eutrophic'' lakes, P retention in ''more'' eutrophic lakes decreased under aerobic conditions, indicating enhanced internal P loading under aerobic conditions (Figure 2). Excessive available P stimulates algal growth. Up to 90% of primary production (for example, algae) sinks in lake sediments (Baines and Pace 1994), thereby increasing sediment organic P fractions in summer and fall. Fresh organic matter coupled to post-turnover hypolimnetic aeration promotes mineralization (Fontaine and others 2007; Song and others 2007), leading to additional internal P loading under aerobic conditions. Compared to previous studies (Olila and others 1995; Søndergaard and others 1996), the sediment organic P fraction (Figure 3) was much smaller, suggesting fast mineralization rates in hypereutrophic lake sediments. The discernible increase in organic P fractions in the fall compared to summer indicates higher productivity in our more eutrophic lakes compared to our less eutrophic lakes (Figure 3). This relatively larger pool of fresh organic matter, combined with newly replenished (post-turnover) hypolimnetic oxygen, stimulates organic decomposition as indicated by increased enzyme activity (Figure 4). Thus, we demonstrate that biological processes, including organic matter mineralization via enzymatic breakdown, can increase internal P loading above and beyond the commonly cited controls of oxygen-driven Fe–P sorption chemistry.

We are not suggesting that the chemical and biological controls are exclusive of each other; in fact, they likely support one another in hypereu-

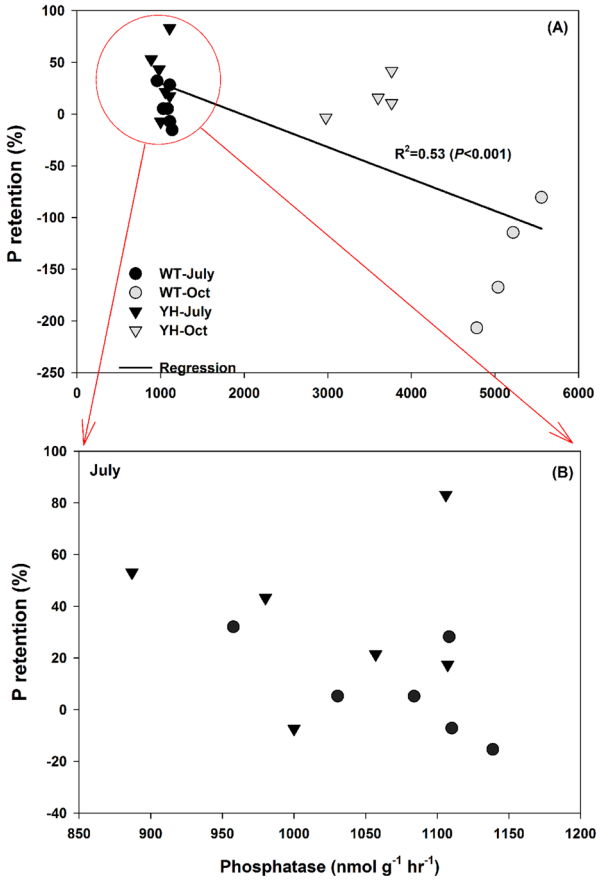

Figure 4. Relationships of phosphatase activities and P retention in WagonTrain and YankeeHill reservoirs for both sampling seasons (**A**) and July only (**B**).

trophic lakes. Future studies aimed at quantitative comparisons between chemical and biological controls on internal P loadings and how that balance changes under a wider range of lake trophic statuses would support our findings and provide additional context for our work. In this study, we suggest that P release is controlled by classic redox chemistry dynamics during the summer period, but the post-turnover period is controlled by stimulation of biological processes (for example, note the significant decrease in Fe–P fraction in YankeeHill, Figure 3). This functionally creates a perpetual P cycle in lake sediments, wherein oxygen is a controlling factor in both scenarios, but uses very different mechanisms (Figure 5).

In addition to the biological mechanism of increased productivity–decomposition under aerobic conditions described herein, potential alternative controls on P cycling include: (1) bacterial polyphosphate retention–release, (2) P release coupled to sulfate reduction, and (3) pH-dependent chemical reaction of P with sediment minerals (Wetzel 2001; Hupfer and Lewandowski 2008). Bacterial polyphosphate P release occurs when certain bacteria accumulate polyphosphate in their cells and release it on cell lysis (Streichan and others 1990; Vasiliadis and others 1990). Given that our more eutrophic reservoirs released P under aerobic conditions, polyphosphate P cycling likely does not explain aerobic P loadings in hypereutrophic reservoirs. Alternatively, high sulfate concentrations under anaerobic conditions can mobilize P by liberating it from Fe, which binds strongly with sulfide (the product of sulfate reduction, Caraco and others 1993). The interaction between S and Fe is a common P release mechanism in sulfate rich environments (for example, coastal regions, Blomqvist and others 2004; Caraco and others 1989). Sulfate reduction requires anoxic conditions; again, our more eutrophic reservoirs exhibit greater P release with aerobic conditions. Thus, coupled Fe–S cycling is not a likely mechanism explaining greater P release in these lakes. Finally, increases in pH alter carbonate chemistry and induce co-precipitation of P with $CaCO_3$ (Boström and others 1988; Boers and others 1998). YankeeHill, the most productive of the four reservoirs, had the highest pH (Table 2), which likely induced a large Ca–P sediment fraction (Figure 3). Ca–P co-precipitation may be responsible for additional P retention of YankeeHill, resulting in positive P retention in both aerobic and anaerobic conditions. However, because YankeeHill retained less P under aerobic condition when Ca–P fraction significantly increased, it is likely not the main mechanism driving reduced P retention under aerobic conditions. Given the strong evidence for the production–decomposition biological control we described above, we believe that these alternative mechanisms are less likely to explain internal P dynamics in our hypereutrophic reservoirs.

The sediment core flow-through approach does not account for bioturbation, littoral P release, wind resuspension; thus, this method could underestimate internal P loadings in lakes (Welch and Cooke 2005; Gibbs and others 2011; Das and others 2012). However, comparisons of internal P loading estimated by mass-balance budgets (for example, surface water monitoring) and experimental measurements (that is, flow-through core incubation) show no significant difference (Nürnberg 1987). This finding suggests that unaccounted P sources in flow-through core experiments are usually minimal (Nürnberg 2009a). Moreover, the flow-through approach is effective for manipulation while maintaining the in situ structural, geochemical, and microbiological features of the

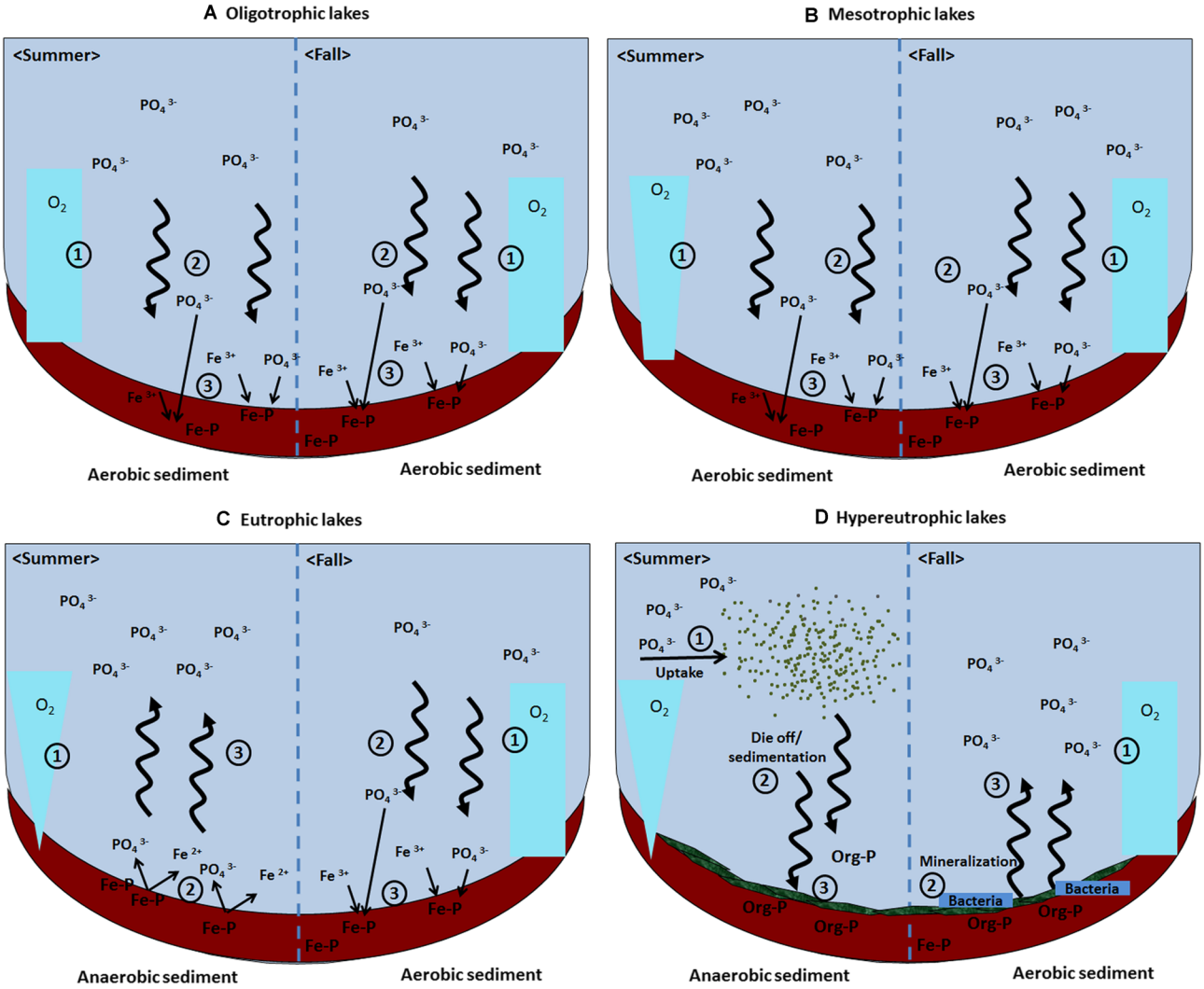

Figure 5. A conceptual diagram comparing internal P dynamics in lakes along the eutrophication gradient of oligotrophic to hypereutrophic lakes. Numbers in circles guide the sequence of chemical and biological interactions with P during summer and fall at each trophic state. In oligotrophic (**A**) and mesotrophic (**B**) lakes, the hypolimnion stays relatively well oxygenated (1), which results in phosphorus that enters sediments (2) to remain bound through coupled iron (Fe) precipitation (3). In eutrophic lakes (**C**), the hypolimnion goes anoxic during summer (1), which causes the Fe-bound P to be released from sediments (2) into the hypolimnion (3); fall turnover oxygenates the hypolimnion and results in the P binding with Fe. In hypereutrophic lakes (**D**), excessive algal growth consumes P (1) and sediments (2) to the lake bottom (3) where it is decomposed; fall turnover oxygenates the hypolimnion, stimulating bacterial decomposition of this organic matter and fueling additional internal P loading.

sediment (Roychoudhury and others 1998; Gibbs and others 2011). The flow-through sediment incubation in this study shows contrasting internal P loading patterns among reservoirs, which allowed us to identify internal P loading mechanisms in hypereutrophic lakes.

We theorize that as lakes become more eutrophic (and hypereutrophic), control on internal P loading may shift toward a combination of biological controls (for example, productivity, decomposition), in addition to recognized chemical controls of oxygen availability and Fe redox chemistry (Figure 5). That is, as lakes become more eutrophic, biological mechanisms begin to amplify internal P loading, creating a perpetual cycle of internal P loading. Amplified internal loadings in hypereutrophic lakes likely delay lake recovery and the successes of restoration efforts (Jeppesen and others 2005; Hamilton 2012). In addition, these biological controls on internal P loadings may partly explain the limited effectiveness of lake restoration and water quality management techniques (Özkundakci and

others 2011). For example, reaeration of the hypolimnion in a hypereutrophic lake may stimulate biological breakdown of P, resulting in increased internal P loading. Thus, a comprehensive understanding of the controls on internal P dynamics, including alternative and additional mechanisms such as biological processes described herein, should be considered to enhance understanding of lake restoration and water quality management techniques.

Take Home Messages

1. We show that organic matter decomposition and extracellular enzymes stimulated by aerobic conditions control internal P loading in hypereutrophic lakes; this control is in addition to the well-documented mechanism of anaerobic iron-bound phosphorus (P) release.
2. We demonstrate that lake trophic status and sediment chemistry determine whether biological or chemical controls dominate internal P loading.
3. We theorize that as lakes become more eutrophic, biological mechanisms begin to amplify internal P loading by acting under both anaerobic and aerobic conditions, thus creating a perpetual cycle of internal P loading.

ACKNOWLEDGEMENTS

This study was funded by Nebraska Department of Environmental Quality. We also thank to Craig Adams, David Moscicki, Cain Silvey, and Carrie Adkisson for their field and laboratory support. We thank the Kansas Biological Survey–Friday Ecology Seminar group for stimulating conversations on these ideas. We also thank the anonymous reviewers for providing valuable comments to improve this manuscript.

REFERENCES

Azzoni R, Giordani G, Viaroli P. 2005. Iron-sulphur-phosphorus interactions: Implications for sediment buffering capacity in a mediterranean eutrophic lagoon (Sacca di Goro, Italy). Hydrobiologia 550:131–48.

Baines SB, Pace ML. 1994. Relationships between suspended particulate matter and sinking flux along a trophic gradient and implications for the fate of planktonic primary production. Can J Fish Aquat Sci 51:25–36.

Blomqvist S, Gunnars A, Elmgren R. 2004. Why the limiting nutrient differs between temperate coastal seas and freshwater lakes: A matter of salt. Limnol Oceanogr 49:2236–41.

Boers PCM, Van Raaphorst W, Van Der Molen DT. 1998. Phosphorus retention in sediments. Water Sci Technol 37:31–9.

Boström B, Andersen JM, Fleischer S, Jansson M. 1988. Exchange of phosphorus across the sediment-water interface. Hydrobiologia 170:229–44.

Caraco NF, Cole JJ, Likens GE (1989) Evidence for sulphate-controlled phosphorus release from sediments of aquatic systems. Nature 341:316–8. http://www.nature.com/doifinder/10.1038/341316a0.

Caraco NF, Cole JJ, Likens GE. 1993. Sulfate control of phosphorus availability in lakes - A test and re-evaluation of Hasler and Einsele's model. Hydrobiologia 253:275–80.

Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol Appl 8:559–68.

Carpenter SR, Booth EG, Kucharik CJ, Lathrop RC. 2015. Extreme daily loads: role in annual phosphorus input to a north temperate lake. Aquat Sci 77:71–9.

Carpenter SR, Ludwig D, Brock WA. 1999. Management of eutrophication for lakes subject to potentially irreversible change. Ecol Appl 9:751–71.

Das J, Daroub SH, Bhadha JH, Lang TA, Josan M. 2012. Phosphorus release and equilibrium dynamics of canal sediments within the everglades agricultural area, Florida. Water Air Soil Pollut 223:2865–79.

Diaz OA, Daroub SH, Stuck JD, Clark MW, Lang TA, Reddy KR. 2006. Sediment inventory and phosphorus fractions for Water Conservation Area canals in the Everglades. Soil Sci Soc Am J 70:863–71.

Ding S, Han C, Wang Y, Yao L, Wang Y, Xu D, Sun Q, Williams PN, Zhang C. 2015. In situ, high-resolution imaging of labile phosphorus in sediments of a large eutrophic lake. Water Res 74:100–9.

Ding S, Wang Y, Wang D, Li YY, Gong M, Zhang C. 2016. In situ, high-resolution evidence for iron-coupled mobilization of phosphorus in sediments. Sci Rep 6:24341.

Downing JA, Prairie YT, Cole JJ, Duarte CM, Tranvik LJ, Striegl RG, McDowell WH, Kortelainen P, Caraco NF, Melack JM. 2006. The global abundance and size distribution of lakes, ponds, and impoundments. Limnol Oceanogr 51:2388–97.

Fontaine S, Barot S, Barré P, Bdioui N, Mary B, Rumpel C. 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450:277–80.

Freeman C, Liska G, Ostle NJ, Jones SE, Lock MA. 1995. The use of fluorogenic substrates for measuring enzyme activity in peatlands. Plant Soil 175:147–52.

Gächter R, Wehrli B. 1998. Ten Years of Artifical Mixing and Oxygenation: No Effect on the Internal Phosphorous Loading of Two Eutrophic Lakes. Environ Sci Technol 32:3659–65.

Galvez-Cloutier R, Sanchez M. 2007. Trophic status evaluation for 154 lakes in Quebec, Canada: Monitoring and recommendations. Water Qual Res J Canada 42:252–68.

Genkai-Kato M, Carpenter SR. 2005. Eutrophication due to phosphorus recycling in relation to lake morphometry, temperature, and macrophytes. Ecology 86:210–19.

Gibbs MM, Hickey CW, Özkundakci D. 2011. Sustainability assessment and comparison of efficacy of four P-inactivation agents for managing internal phosphorus loads in lakes: Sediment incubations. Hydrobiologia 658:253–75.

Gonsiorczyk T, Casper P, Koschel R. 1998. Phosphorus-binding forms in the sediment of an oligotrophic and an eutrophic hardwater lake of the Baltic Lake District (Germany). Water Sci Technol 37:51–8.

Hamilton SK. 2012. Biogeochemical time lags may delay responses of streams to ecological restoration. Freshw Biol 57:43–57.

Hupfer M, Lewandowski J. 2008. Oxygen controls the phosphorus release from lake sediments—A long-lasting paradigm in limnology. Int Rev Hydrobiol 93:415–32.

Jeppesen E, Søndergaard M, Jensen JP, Havens KE, Anneville O, Carvalho L, Coveney MF, Deneke R, Dokulil MT, Foy B, Gerdeaux D, Hampton SE, Hilt S, Kangur K, Köhler J, Lammens EHHR, Lauridsen TL, Manca M, Miracle MR, Moss B, Nõges P, Persson G, Phillips G, Portielje R, Romo S, Schelske CL, Straile D, Tatrai I, Willén E, Winder M. 2005. Lake responses to reduced nutrient loading - An analysis of contemporary long-term data from 35 case studies. Freshw Biol 50:1747–71.

Kasprzak P, Padisák J, Koschel R, Krienitz L, Gervais F. 2008. Chlorophyll a concentration across a trophic gradient of lakes: An estimator of phytoplankton biomass? Limnologica 38:327–38.

Kisand A. 2005. Distribution of sediment phosphorus fractions in hypertrophic strongly stratified Lake Verevi. Hydrobiologia 547:33–9.

Lake BA, Coolidge KM, Norton SA, Amirbahman A. 2007. Factors contributing to the internal loading of phosphorus from anoxic sediments in six Maine, USA, lakes. Sci Total Environ 373:534–41.

Mortimer CH. 1942. The exchange of dissolved substances between mud and water in lakes. J Ecol 30:147–201.

Mortimer CH. 1971. Chemical exchanges between sediments and water in the Great Lakes-speculations on probable regulatory mechanisms. Limnol Oceanogr 16:387–404.

Nebraska Department of Environmental Quality. 2016. 2016 Surface Water Quality Integrated Report.

Nürnberg GK. 1987. A comparison of internal phosphorus loads in lakes with anoxic hypolimnia: Laboratory incubation versus in situ hypolimnetic phosphorus accumulation. Limnol Oceanogr 32:1160–4.

Nürnberg GK. 1996. Trophic state of clear and colored, soft- and hardwater lakes with special consideration of nutrients, anoxia, phytoplankton and fish. Lake Reserv Manag 12:432–47.

Nürnberg GK. 2009a. Assessing internal phosphorus load – Problems to be solved. Lake Reserv Manag 25:419–32.

Nürnberg GK. 2009b. Lake and Reservoir Management Assessing internal phosphorus load -Problems to be solved. Lake Reserv Manag 25:419–32.

Nusch EA. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. Arch für Hydrobiol der Limnol 14:14–36.

Olila OG, Reddy KR, Harris WG. 1995. Forms of distribution of inorganic phosphorus in sediments of two shallow eutrophic lakes in Florida, pp 147–61.

Özkundakci D, Duggan IC, Hamilton DP. 2011. Does sediment capping have post-application effects on zooplankton and phytoplankton? Hydrobiologia 661:55–64.

Penn MR, Auer MT, Doerr SM, Driscoll CT, Brooks CM, Effler SW. 2000. Seasonality in phosphorus release rates from the sediments of a hypereutrophic lake under a matrix of pH and redox conditions. Can J Fish Aquat Sci 57:1033–41.

Pettersson K. 1998. Mechanisms for internal loading of phosphorus in lakes. Hydrobiologia 373–374:21–5.

Petticrew EL, Arocena JM. 2001. Evaluation of iron-phosphate as a source of internal lake phosphorus loadings. Sci Total Environ 266:87–93.

Reddy KR, Kadlec RH, Flaig E, Gale PM. 1999. Phosphorus Retention in Streams and Wetlands: A Review. Crit Rev Environ Sci Technol 29:83–146.

Roychoudhury AN, Viollier E, Van Cappellen P. 1998. A plug flow-through reactor for studying biogeochemical reactions in undisturbed aquatic sediments. Appl Geochemistry 13:269–80.

Sinke AJC, Cornelese A, Keizer P, Tongeren OFR, Cappenberg TE. 1990. Mineralization, pore water chemistry and phosphorus release from peaty sediments in the eutrophic Loosdrecht lakes, The Netherlands. Freshw Biol 23:587–99.

Smith VH, Schindler DW. 2009. Eutrophication science: where do we go from here? Trends Ecol Evol 24:201–7.

Søndergaard M, Windolf J, Jeppesen E. 1996. Phosphorus fractions and profiles in the sediment of shallow Danish lakes as related to phosphorus load, sediment composition and lake chemistry. Water Res 30:992–1002.

Song K, Xenopoulos MA, Buttle JM, Marsalek J, Wagner ND, Pick FR, Frost PC. 2013. Thermal stratification patterns in urban ponds and their relationships with vertical nutrient gradients. J Environ Manage 127:317–23.

Song KY, Zoh KD, Kang H. 2007. Release of phosphate in a wetland by changes in hydrological regime. Sci Total Environ 380:13–18.

Stoddard JL, Van Sickle J, Herlihy AT, Brahney J, Paulsen S, Peck DV, Mitchell R, Pollard AI. 2016. Continental-Scale Increase in Lake and Stream Phosphorus: Are Oligotrophic Systems Disappearing in the United States? Environ Sci Technol 50:3409–15.

Streichan M, Golecki JR, Schön G. 1990. Polyphosphate-accumulating bacteria from sewage plants with different proceses for biological phosphorus removal. FEMS Microbiol Lett 73:113–24.

Thomas, S, Chandrakiran, S., Hoagland K. 2013. Examining toxic cyanobacterial blooms in Nebraska reservoirs: Landscape controls and the within-reservoir mechanisms.

Tilman D, Fargione J, Wolff B, D'Antonio C, Dobson A, Howarth R, Schindler D, Schlesinger WH, Simberloff D, Swackhamer D. 2001. Forecasting agriculturally driven global environmental change. Science 292:281–4.

Vasiliadis G, Duncan A, Bayly RC, May JW. 1990. Polyphosphate production by strains of Acinetobacter. FEMS Microbiol Lett 70:37–40.

Welch EB, Cooke GD. 2005. Internal Phosphorus Loading in Shallow Lakes: Importance and Control. Lake Reserv Manag 21:209–17.

Wetzel RG. 2001. Limnology: Lake and River Ecosystems.

Wilson TA, Amirbahman A, Norton SA, Voytek MA. 2010. A record of phosphorus dynamics in oligotrophic lake sediment. J Paleolimnol 44:279–94.

Withers PJA, Jarvie HP. 2008. Delivery and cycling of phosphorus in rivers: A review. Sci Total Environ 400:379–95.