

# Internal Phosphorus Loading and Sediment Phosphorus Fractionation Analysis for Horseshoe Lake, Wisconsin

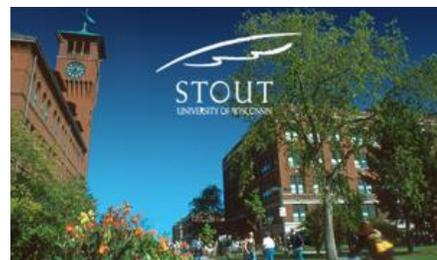
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## **SUMMARY**

Sediment cores were collected from 3 stations in Horseshoe Lake in July, 2016, to examine phosphorus (P) release from sediment under anaerobic conditions and pools of mobile P fractions in the sediment. Overall, rates of P release under anaerobic conditions were moderate, ranging between 2.4 and 3.3 mg/m<sup>2</sup> d. Labile organic P (i.e., sediment P that can be broken down to phosphate by bacteria) dominated the mobile P fraction in the upper 5-cm sediment layer at all stations followed by the iron-bound P fraction. Station 10 and 20 exhibited the highest labile organic and iron-bound P (i.e., subject to release and recycling) concentrations in the upper 5-cm layer. Iron-bound P at these stations fell near the median compared to other lakes. Labile organic P was high at all stations compared to the median. Station 10 and 20 sediment cores exhibited a slight surface concentration maximum in mobile P, suggesting the potential for modest internal P loading. Station 30 mobile P concentrations were relatively constant with increasing sediment depth. This information will need to be combined with information on hypolimnetic anoxia and the Fe:P in order to assess the importance of internal P loading to the P budget and algal blooms in Horseshoe Lake.

## **BACKGROUND**

Phosphorus (P) is a key nutrient that usually limits primary production in freshwater systems. Increased or excess P loading can lead to cultural eutrophication, degradation of water quality, and development of toxic cyanobacterial blooms (Boström et al. 1982, Carpenter et al. 1998, Smith et al. 1998, Cooke et al. 2005, Elser et al. 2007, Havens 2008). Excessive anthropogenic P loading also leads to various problems, such as loss of oxygen, fish kills, and a loss of biodiversity within the lake (Smith and Schindler 2009). Phosphorus sources can originate from the watershed (i.e. external loading) or from P stored as sediment that is later released and recycled into the water column for uptake by algae (i.e. internal loading; Boström 1984, Jeppesen et al. 2005, Mortimer 1941,1942, Nürnberg et al. 1986; Sondergaard et al. 2001). It is important to quantify external and

internal P loading in order to identify important P sources for targeted management strategies.

Tributary soluble P loads are immediately available for algal uptake. Particulate P loads can be deposited as sediment and recycled back into the water column through internal P loading mechanisms (Boström et al. 1982; Carey and Rydin 2011, Mortimer 1942, 1971). Recycling of P from the lake sediment to the water column may play an important role in the P budget of lakes, contributing up to 80% of the total P input (Carey & Rydin 2011, Carpenter 2003, 2005, James et al. 2016, Penn et al. 2000). As a result, internal P loading may mask the effects of external nutrient load reductions and delay lake recovery (Ahlgren 1978, Boström 1984, Cooke et al. 2005, Larsen et al. 1981, Rossi and Premazzi 1991, Ryding 1981, Welch et al. 1986).

Mortimer (1942) described a model for sediment P recycling where  $\text{PO}_4^{3-}$  was initially adsorbed to iron oxyhydroxides or bound as ferric phosphate under aerobic conditions at the thin sediment oxidized microzone (i.e. on the order of mm in thickness) located at the sediment-water interface. Under anaerobic conditions, bacterially mediated reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  resulted in desorption of  $\text{PO}_4^{3-}$  into pore water and diffusion into the overlying water column. Soluble  $\text{Fe}^{2+}$  and  $\text{PO}_4^{3-}$  accumulation in the anoxic hypolimnion over the summer can be accessed directly by vertically migrating algae for growth (James et al. 1992). If there is sufficient Fe relative to P (Fe: P ratio  $> \sim 3.6:1$  mass: mass; Gunnars et al. 2002) in the hypolimnion at turnover, chemical oxidation of Fe during mixing and reaeration is accompanied by adsorption and precipitation of  $\text{PO}_4^{3-}$  and deposition back to the sediment. Thus, iron oxidation-reduction can regulate P release and availability to algae under this scenario.

However, Hasler and Einsele (1948), Caraco et al. (1991, 1993), Kleeberg and Kozerski (1997), Golterman (2001) and others suggested that reduction of sulfate in sediment and reaction with  $\text{Fe}^{2+}$  to insoluble and inert  $\text{FeS}_x$  (solid) disrupted Fe control of P by burying Fe from further interaction with  $\text{PO}_4^{3-}$ . As soluble Fe becomes depleted and the Fe: P ratio declines below  $\sim 3.6:1$  in the hypolimnion, soluble P can become directly entrained

into the epilimnion for uptake rather than removed back to the sediment during mixing and reaeration (James et al. 2016).

Fe~(OOH)~PO<sub>4</sub> or redox-sensitive P can be quantified via extraction with a strong reducing agent (dithionite-bicarbonate; BD; Nürnberg 1988). Additionally, biologically-labile P in the form of bacterial polyphosphates and labile organic P compounds and can be recycled to the overlying water column via mineralization and metabolic breakdown and is extracted with a basic solution (0.1 to 1.0 N NaOH; Psenner and Puckso 1988). Thus, the size of the biologically-labile P pool (i.e., redox-P and labile organic P; subject to recycling and internal P loading) in surface sediment can be quantified for evaluation and be compared to other systems to assess the potential importance as a source of P recycling in lakes.

## **OBJECTIVES**

The objectives of this investigation were to determine rates of phosphorus (P) release from sediments under laboratory-controlled anaerobic conditions and to quantify biologically-labile (i.e., subject to recycling) P fractions for sediment collected in Horseshoe Lake, WI. Findings from this research will be important in evaluating the importance of internal P loading to the overall P economy of the lake.

## **APPROACH**

*Laboratory-derived rates of P release from sediment under anaerobic conditions:*

Sediment cores were collected from 3 stations (Figure 1 and Table 1) in Horseshoe Lake in July, 2016, for determination of rates of P release from sediment under anaerobic conditions (Figure 2). Cores were drained of overlying water and the upper 10 cm of sediment was transferred intact to a smaller acrylic core liner (6.5-cm dia and 20-cm ht) using a core remover tool. Surface water collected from the lake was filtered through a glass fiber filter (Gelman A-E), with 300 mL then siphoned onto the sediment contained in the small acrylic core liner without causing sediment resuspension. Sediment

incubation systems consisted of the upper 10-cm of sediment and filtered overlying water contained in acrylic core liners that were sealed with rubber stoppers. They were placed in a darkened environmental chamber and incubated at a constant temperature (20 °C). The oxidation-reduction environment in the overlying water was controlled by gently bubbling nitrogen (anaerobic conditions, 2 replicates per station) through an air stone placed just above the sediment surface in each system (Figure 3). Bubbling action insured complete mixing of the water column but did not disrupt the sediment.

Water samples for soluble reactive P were collected from the center of each system using an acid-washed syringe and filtered through a 0.45 µm membrane syringe filter (Nalge). The water volume removed from each system during sampling was replaced by addition of filtered lake water preadjusted to the proper oxidation-reduction condition. These volumes were accurately measured for determination of dilution effects. Soluble reactive P was measured colorimetrically using the ascorbic acid method (APHA 2005). Rates of P release from the sediment ( $\text{mg/m}^2 \text{ d}$ ) were calculated as the linear change in mass in the overlying water divided by time (days) and the area ( $\text{m}^2$ ) of the incubation core liner. Regression analysis was used to estimate rates over the linear portion of the data.

*Sediment chemistry:* Additional sediment cores collected from station 10, 20, and 30 were sectioned vertically over the upper 20-cm layer to evaluate variations in sediment physical-textural and chemical characteristics. These cores were sectioned at 1-cm intervals over the first 6 cm, at 2-cm intervals between 6 and 10 cm, and at 2.5-cm intervals below 10 cm. Sections were analyzed for moisture content (%), sediment density ( $\text{g/cm}^3$ ), loss on ignition (i.e., organic matter content, %), loosely-bound P, iron-bound P, labile organic P, and aluminum-bound P (all expressed at mg/g). A known volume of sediment was dried at 105 °C for determination of moisture content and sediment density and burned at 550 °C for determination of loss-on-ignition organic matter content (Håkanson and Jansson 2002).

Phosphorus fractionation was conducted according to Hieltjes and Lijklema (1980), Psenner and Puckso (1988), and Nürnberg (1988) for the determination of ammonium-

chloride-extractable P (loosely-bound P), bicarbonate-dithionite-extractable P (i.e., iron-bound P), and sodium hydroxide-extractable P (i.e., aluminum-bound P; Table 2). A subsample of the sodium hydroxide extract was digested with potassium persulfate to determine nonreactive sodium hydroxide-extractable P (Psenner and Puckso 1988). Labile organic P was calculated as the difference between reactive and nonreactive sodium hydroxide-extractable P.

The loosely-bound and iron-bound P fractions are readily mobilized at the sediment-water interface as a result of anaerobic conditions that result in desorption of P from sediment and diffusion into the overlying water column (Mortimer 1971, Boström 1984, Nürnberg 1988). The sum of the loosely-bound and iron-bound P fractions represent redox-sensitive P (i.e., the P fraction that is active in P release under anaerobic and reducing conditions). In addition, labile organic P can be converted to soluble P via bacterial mineralization (Jensen and Andersen 1992) or hydrolysis of bacterial polyphosphates to soluble phosphate under anaerobic conditions (Gächter et al. 1988; Gächter and Meyer 1993; Hupfer et al. 1995). The sum of redox-sensitive P and labile organic P collectively represent biologically-labile P. This fraction is generally active in recycling pathways that result in exchanges of phosphate from the sediment to the overlying water column and potential assimilation by algae. In contrast, aluminum-bound P is more chemically inert and subject to burial rather than recycling. But it provides important background information if an alum treatment is being considered to reduce internal P loading.

## **RESULTS AND INTERPRETATION**

P mass and concentration increased approximately linearly in the overlying water column of sediment systems maintained under anaerobic conditions (Figure 4). Linear increases in P concentration between day 0 and 7 were used in rate calculation. The mean P concentration maximum in the overlying water end of the incubation period was moderate for all stations. It was greatest in station 10 (0.225 mg/L) and 20 (0.231 mg/L)

and lower for station 30 (0.115 mg/L) sediment incubation systems (Table 3). The mean rate of P release under anaerobic conditions was also moderate to moderately low, ranging between 2.4 and 3.3 mg/m<sup>2</sup> d (Table 3) but indicative of mesotrophic to eutrophic conditions (Nürnberg 1988). Compared to other lakes in the region, the anaerobic P release rates for Horseshoe Lake sediments fell near but below the median for station 10 and 20 sediment cores (Figure 5). The anaerobic P release rate from station 30 sediments fell below the lower 25% quartile compared to other lakes in the region (Figure 5).

Averaged over the upper 5 cm, sediment moisture content was very high, while wet and dry bulk densities were low (i.e., low mass of sediment per volume), for all stations in Horseshoe Lake, suggesting very flocculent sediments with high porosity (i.e., high volume of interstitial spaces for porewater; Table 4). Organic matter content in the 5-cm surface layer was moderate high, ranging between 33% and 39% (Table 4). Organic matter content reflects labile (i.e., can be used in bacterial metabolism) and refractory (i.e., resistant to bacterial breakdown) organic carbon.

Vertically in the sediment column, sediment moisture content was very high in the surface 1-cm section at all stations, exceeding 95% (Figure 6). Moisture content declined and was relatively constant below 5 cm; however, it exceeded 90% again suggesting very flocculent sediment consisting primarily of porewater. Sediment densities were very low in the upper 5 cm, approaching that of water (i.e., 1 g/cm<sup>3</sup>), and increased with increasing sediment depth. This pattern is typical and reflects sediment compaction over time (i.e., compression and consolidation of deeper sediment). Organic matter content tended to be higher in the surface sediments and declined with increasing sediment depth, exhibiting a minimum at ~ 5 to 7 cm, particularly at station 10 and 20. Organic matter content then increased slightly below ~ 7 cm at all stations. This pattern may reflect historical watershed loading patterns to the lake.

The biologically-labile P (i.e., subject to recycling back to the overlying water column; loosely-bound P, iron-bound P, and labile organic P) in the upper 5-cm sediment layer

was moderately high, ranging between 1.12 mg/g and 1.24 mg/g at station 10 and 20 and 0.82 at station 30 (Figure 7 and Table 5). Labile organic P accounted for over 50% of the biologically-labile P fraction in the upper 5 cm at all stations, ranging between 0.65 mg/g and 0.74 mg/g (Figure 7). Concentrations of this fraction were also high relative to other lakes in the region (Figure 8). Iron-bound P was the next dominant biologically-labile P fraction (Figure 7) and concentrations fell above the median compared to other lakes in the region, particularly for the upper 5-cm sections from station 10 and 20 (Figure 8). Iron-bound P was much lower in the upper 5 cm of the station 30 sediment core, falling within the lower 25% quartile compared to regional lakes (Figure 7 and 8). Loosely-bound P represented < 1% of the biologically-labile P at all stations (Figure 7) and were relatively low compared to other lakes in the region (Figure 8). Aluminum-bound P concentrations in the upper 5-cm sediment layer were also relatively high (Figure 8). Overall, redox-P concentrations fell near the median while biologically-labile P concentrations were well above the median compared to other lakes in the region (Figure 9). The latter pattern was attributed to high labile organic P.

Vertically in the sediment column, labile organic P was the dominant mobile P fraction at all stations (Figure 10). Concentrations of labile organic P and iron-bound P exhibited a surface peak in the station 10 core. Peak labile organic P concentrations were also observed between 0 and 4 cm at station 20 and at the sediment surface (0 to 1 cm) at station 30. However, iron-bound P concentrations were relatively constant below the sediment surface section at all stations. Overall, vertical profiles in redox-P and biologically-labile P at station 10 suggested only a very modest to no buildup of potentially-mobile P in the upper sediment layer. In contrast, surface P concentration maxima in eutrophic lake sediments reflect high deposition and recycling in excess of diagenesis and burial (Carey and Rydin 2011, Malmaeus et al. 2012). Concentrations of these constituents were nearly constant versus increasing sediment depth at station 20 and 30, suggesting negligible accumulation of potentially mobile P in the surface sediments.

## RECOMMENDATIONS

Sediment internal P loading from anaerobic sediment in Horseshoe lake is currently modest but more information is needed to assess the role of this P source in potentially driving summer and fall algal blooms (particularly cyanobacteria). Rates derived from this study can be combined with dissolved oxygen profiles to estimate lake wide internal P loading for comparison with watershed tributary P sources. This analysis will be important in evaluating P sources in the lake that need to be targeted for management.

An unknown is the role of iron in hypolimnetic P availability for algal uptake. Iron oxyhydroxides can adsorb soluble P and keep it trapped in the hypolimnion during turnover and reoxygenation if the Fe:P ratio is high. Under this scenario, availability of internal P loads is usually minor unless algae can vertically migrate into the anoxic hypolimnion for direct P uptake. If the Fe:P ratio is relatively low, there will not be enough iron oxyhydroxide to bind all the soluble P and it will become entrained into the surface waters during mixing periods and assimilated by algae for growth. If watershed tributary P loads are low in the fall but a massive algal bloom occurs during turnover, it is likely that the Fe:P ratio is low. More information is needed on summer iron and dissolved oxygen patterns in Horseshoe Lake to evaluate the availability of internal P loads for algal uptake.

I recommend that a vertical water sampling profile (i.e., 1-m intervals from the surface to the bottom at station 10 and 20) be collected in late August for analysis of soluble iron and phosphorus in the anoxic hypolimnion to determine the Fe:P ratio and potential for entrainment of soluble P during mixing periods. I also recommend collecting samples for chlorophyll analysis in August to evaluate the potential for vertical migration into the hypolimnion.

Additionally, it would be valuable to attempt to construct a hydrological and P budget for the lake to evaluate important P sources contributing to algal blooms. Dominant P sources can then be targeted for management. Simple empirical modeling can be

conducted to assess the impact of P loading reduction on mean summer water quality. Water quality goals can be established and management scenarios developed to improve Horseshoe Lake.

## **ACKNOWLEDGMENTS**

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Table 1. Sediment sampling locations in Horseshoe Lake.				
Station	Water depth		East	North
	(ft)	(m)		
10	40	12	45.43650	-92.17460
20	51	16	45.43604	-92.16560
30	35	11	45.44157	-92.15822

Table 2. Sediment sequential phosphorus (P) fractionation scheme, extractants used, and definitions of recycling potential.

Variable	Extractant	Recycling Potential
Loosely-bound P	1 M Ammonium Chloride	Biologically labile; Soluble P in interstitial water and adsorbed to $\text{CaCO}_3$ ; Recycled via direct diffusion, eH and pH reactions, and equilibrium processes
Iron-bound P	0.11 M Sodium Bicarbonate-dithionate	Biologically labile; P adsorbed to iron oxyhydroxides ( $\text{Fe}(\text{OOH})$ ); Recycled via eH and pH reactions and equilibrium processes
Labile organic P	Persulfate digestion of the NaOH extraction	Biologically labile; Recycled via bacterial mineralization of organic P and mobilization of polyphosphates stored in cells
Aluminum-bound P	0.1 N Sodium Hydroxide	Biologically refractory; Al-P minerals with a low solubility product

**Table 3. Mean (1 standard error in parentheses; n = 3) rates of phosphorus (P) release under aerobic and anaerobic conditions for sediments collected in Horseshoe Lake.**

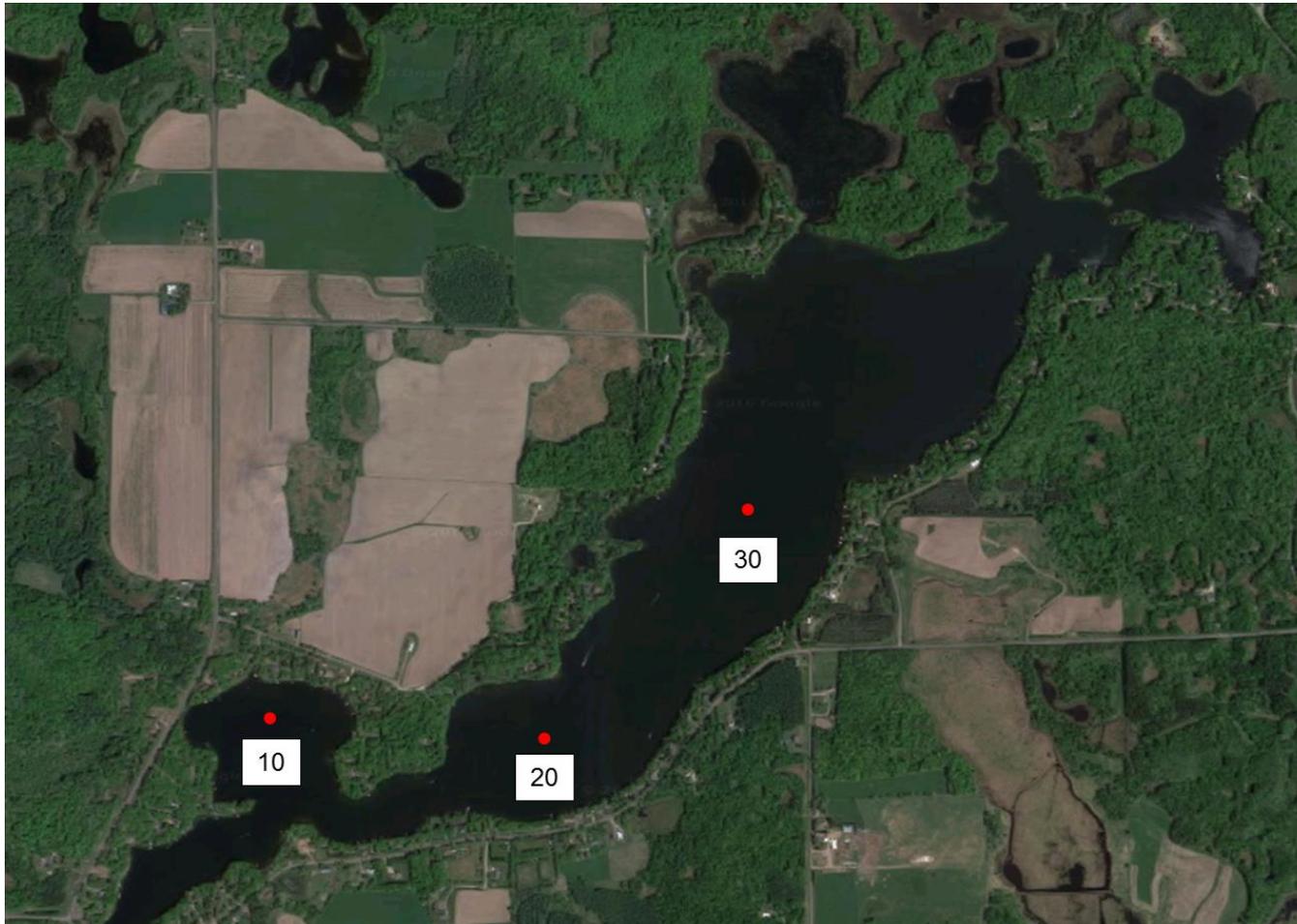
Station	Diffusive P Flux	
	Anaerobic (mg m <sup>-2</sup> d <sup>-1</sup> )	(mg/L)
10	2.52	0.225
20	3.32	0.231
30	2.37	0.115

**Table 4. Textural characteristics in the upper 5-cm sediment layer.**

Station	Moisture Content (%)	Wet Bulk Density (g/cm <sup>3</sup> )	Dry Bulk Density (g/cm <sup>3</sup> )	Loss-on-ignition (%)
10	94.8	1.023	0.054	32.8
20	95.4	1.019	0.050	39.4
30	93.2	1.029	0.071	33.5

**Table 5. Phosphorus fractions in the upper 5-cm layer of Horseshoe Lake sediments.**

Station	Loosely-bound P (mg/g DW)	Iron-bound P (mg/g DW)	Labile organic P (mg/g DW)	Aluminum-bound P (mg/g DW)	Redox-P (mg/g DW)	Bio-labile P (mg/g DW)
10	0.023	0.479	0.736	0.462	0.502	1.238
20	0.016	0.395	0.708	0.435	0.411	1.119
30	0.018	0.159	0.649	0.362	0.177	0.826



*Figure 1. Sediment sampling stations in Horseshoe Lake, WI.*



*Figure 2. Sediment core apparatus and sediment coring on Horseshoe Lake.*



*Figure 3. Sediment core incubation systems for determination of laboratory-derived rates of phosphorus release from sediments under anaerobic conditions.*



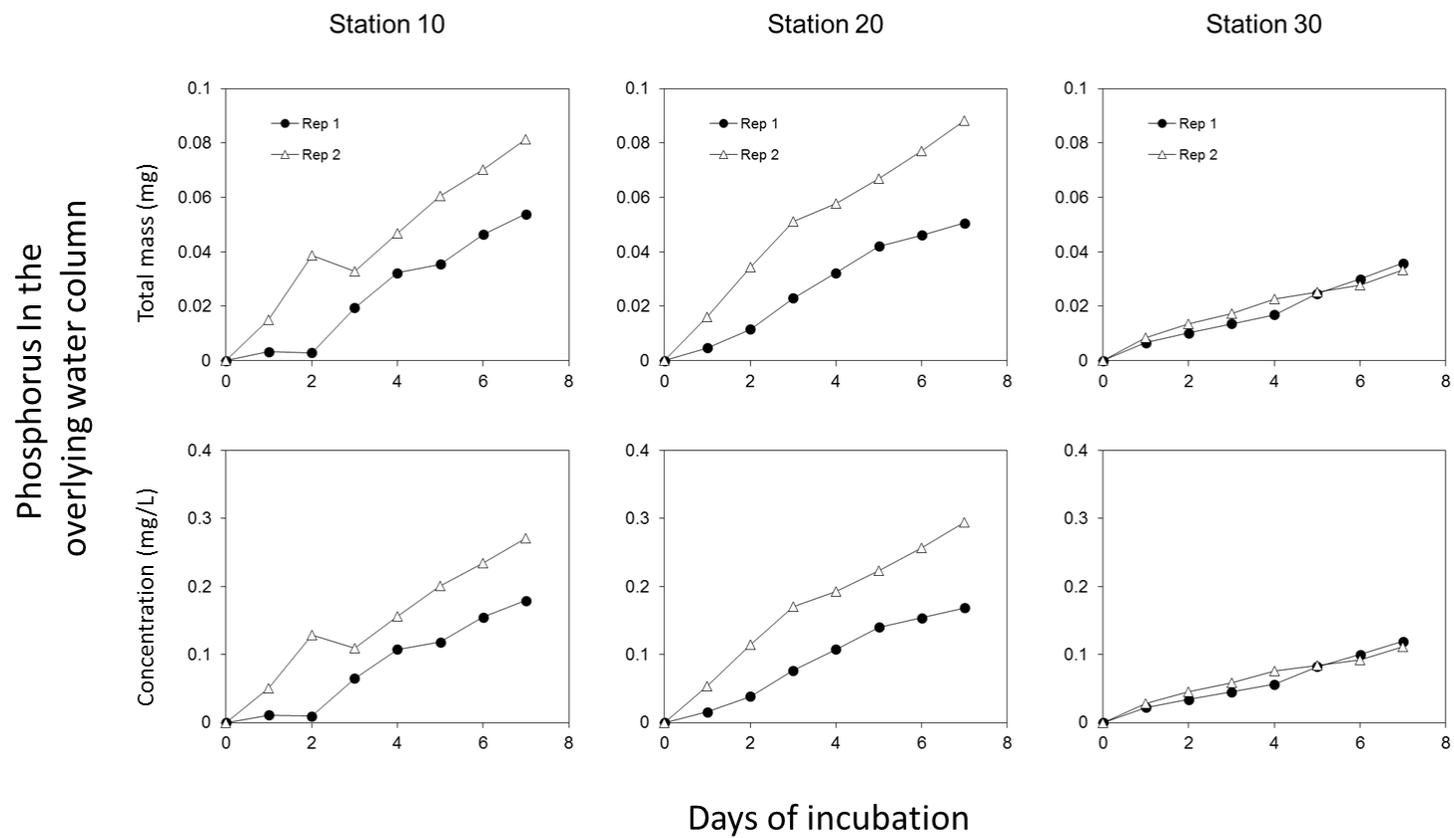
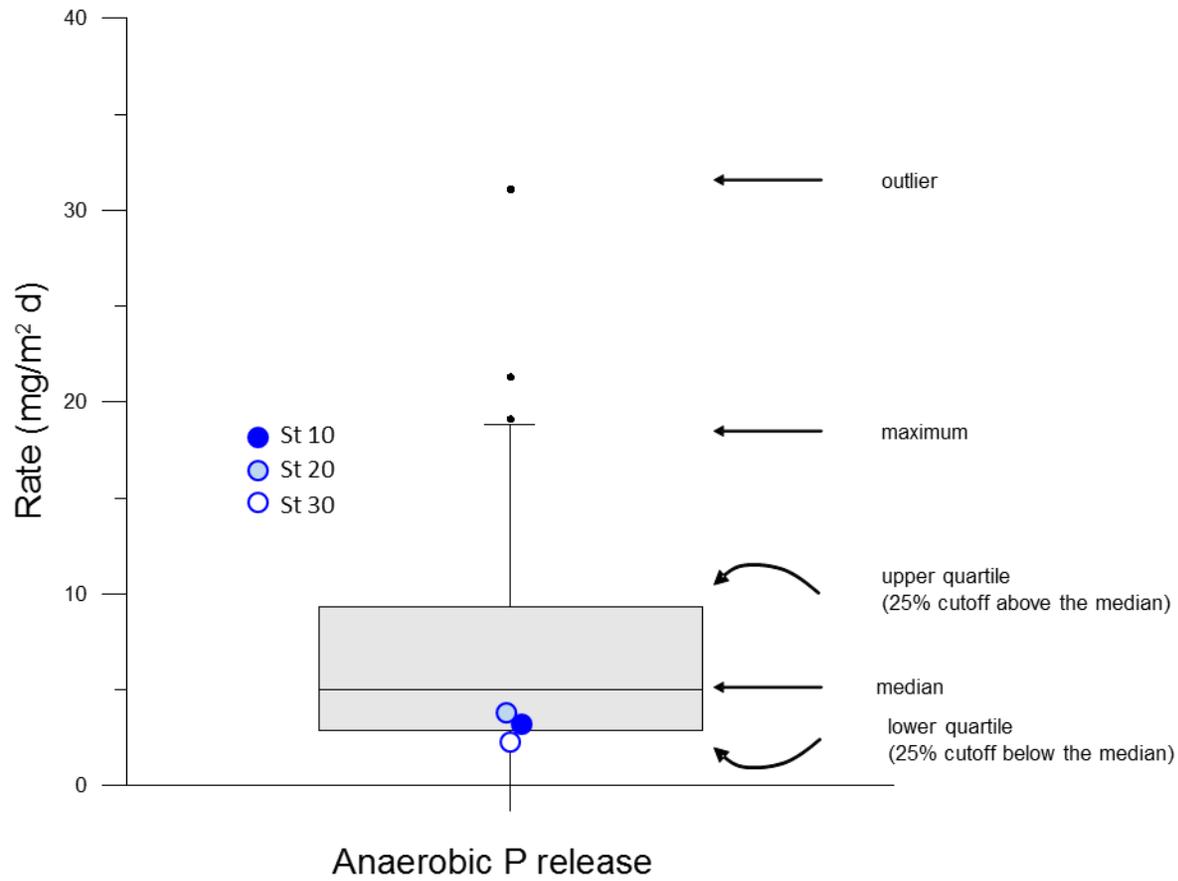


Figure 4. Changes in soluble reactive phosphorus mass (upper panel) and concentration (lower panel) in the overlying water column under anaerobic conditions versus time for sediment cores collected in Horseshoe Lake.



*Figure 5. Box and whisker plot comparing the anaerobic phosphorus (P) release rate measured in Horseshoe Lake with statistical ranges for lakes in Minnesota.*

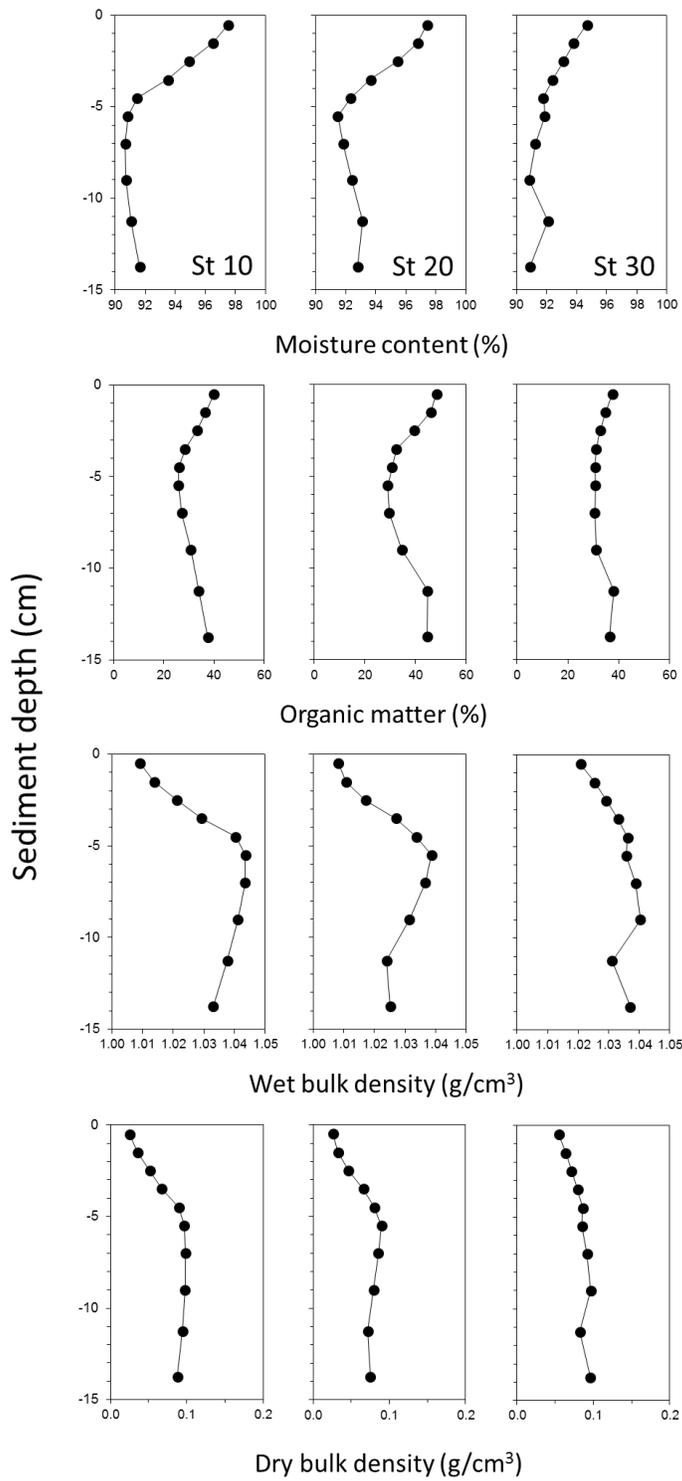


Figure 6. Vertical variations in moisture content, organic matter content, and wet and dry bulk.

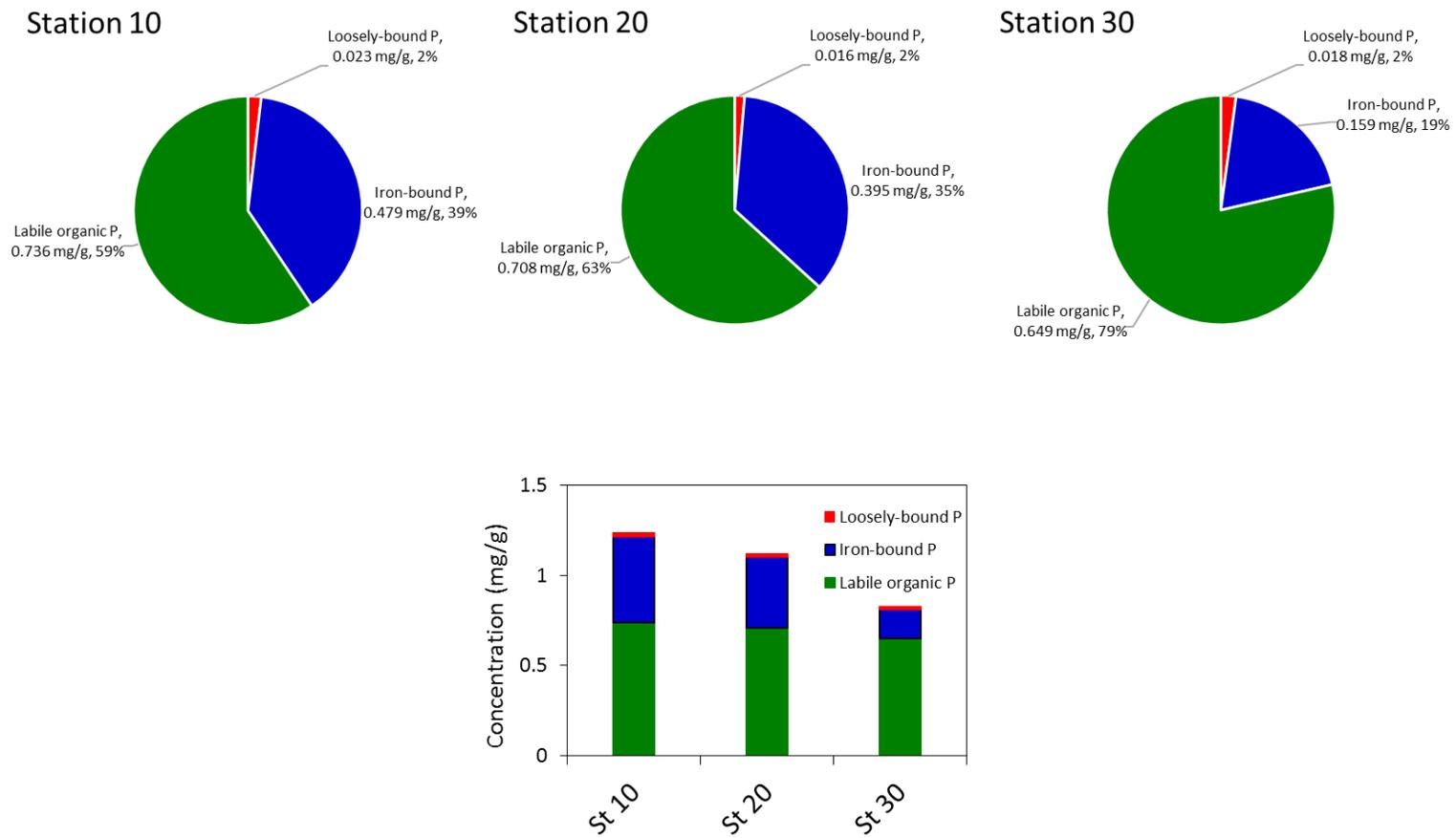


Figure 7. Composition of biologically-labile phosphorus (P) in the upper 5-cm surface sediment layer of Horseshoe Lake. Loosely-bound, iron-bound, and labile organic P are biologically reactive (i.e., subject to recycling). Values next to each label represent concentration ( $\text{mg/g}$ ) and percent of the biologically-labile P concentration, respectively.

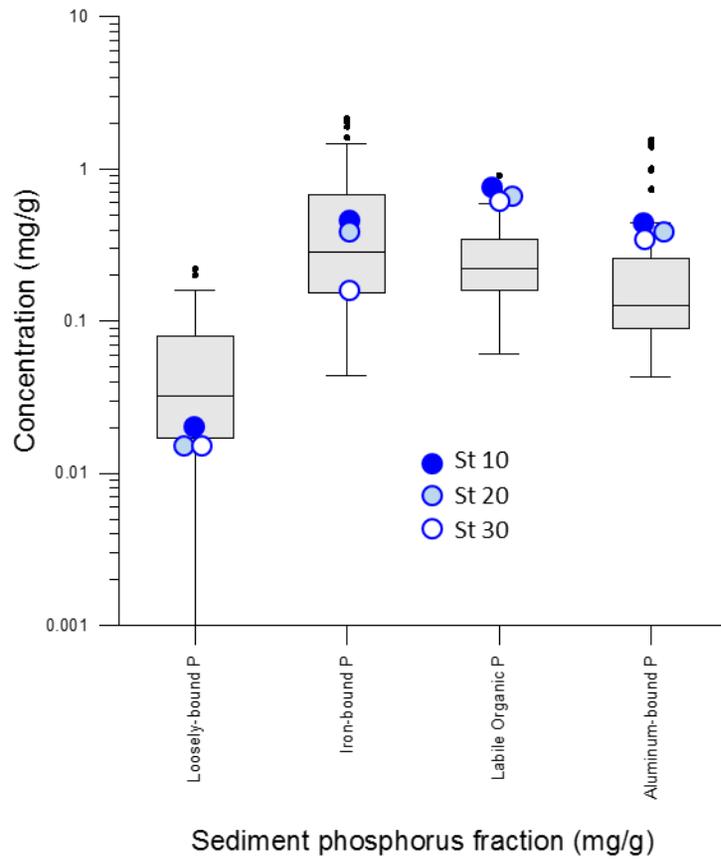


Figure 8. Box and whisker plots comparing various sediment phosphorus (P) fractions measured for sediment collected in Horseshoe Lake, WI, with statistical ranges for lakes in nearby Minnesota. Loosely-bound, iron-bound, and labile organic P are biologically-labile (i.e., subject to recycling) and aluminum-bound, calcium-bound, and refractory organic P are more inert to transformation (i.e., subject to burial). Please note the logarithmic scale.

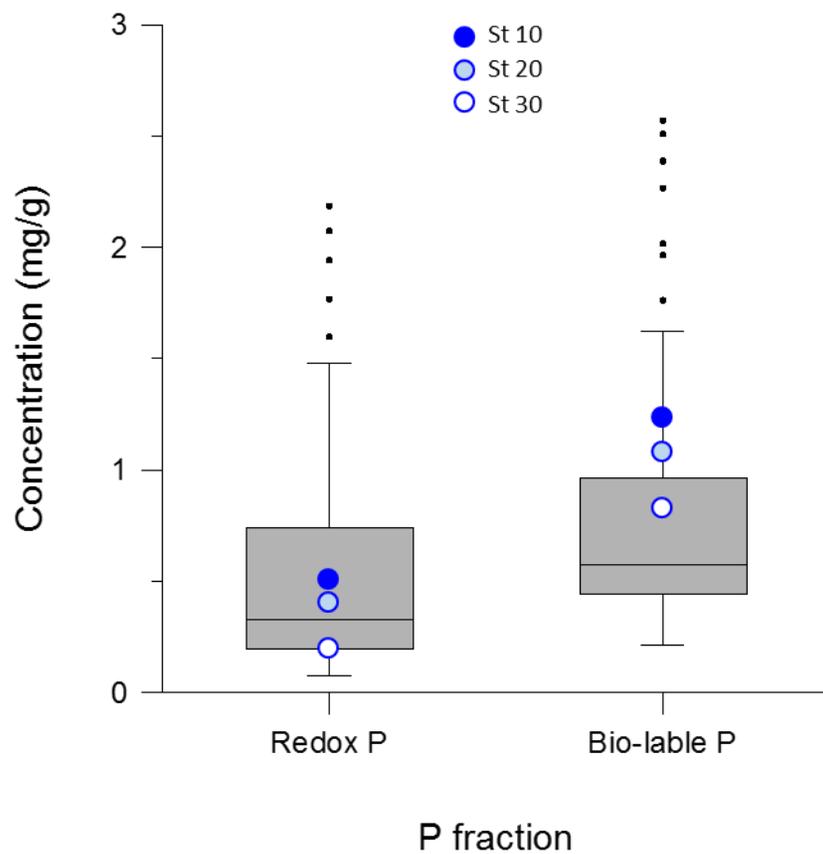


Figure 9. Box and whisker plots comparing redox-P (i.e., sum of the loosely-bound and iron-bound P fractions) and biologically-labile P (i.e., the sum of redox-P and labile organic P fractions) for sediment collected in Horseshoe Lake, WI, with statistical ranges for lakes in nearby Minnesota.

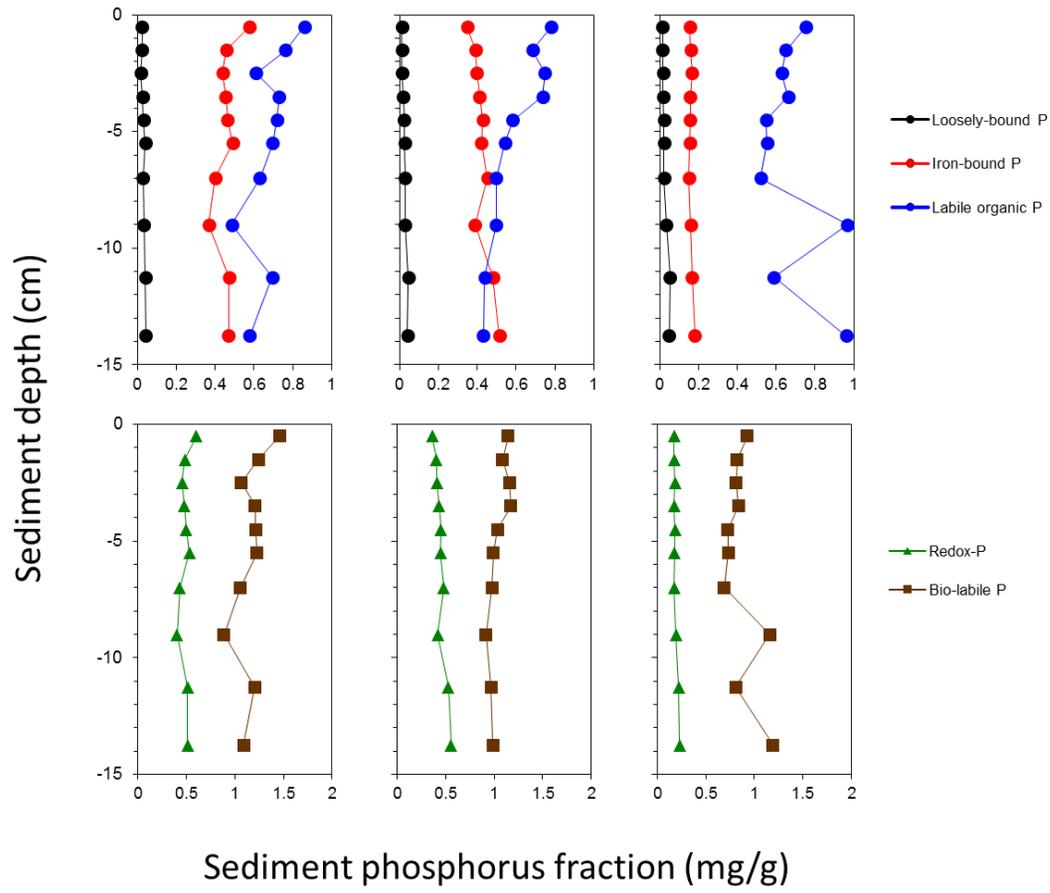


Figure 10. Vertical variations in loosely-bound phosphorus (P), iron-bound P, labile organic P, redox-P, and biologically-labile P

