Phosphorus Mobility and Speciation Under Dynamic Redox Conditions in Shallow Eutrophic Freshwater Systems

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PHOSPHORUS MOBILITY AND SPECIATION UNDER DYNAMIC REDOX CONDITIONS IN SHALLOW EUTROPHIC FRESHWATER SYSTEMS

A Thesis Presented

by

Austin E. Wilkes

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ABSTRACT

Internal loading of phosphorus (P) from lake sediments can delay the recovery of lakes from eutrophication for years to decades following decreases in external nutrient inputs. While internal P loading is a pervasive problem in freshwater systems, molecular speciation of P in benthic sediments of these systems remains poorly characterized. As different P species will exhibit different responses to changing sediment-water interface (SWI) geochemistry, quantifying P speciation in sediments is a critical step in understanding P dynamics in sediment-water systems. Here, various synchrotron-based techniques were employed to directly probe the bonding environments of P and iron (Fe) in natural and experimentally manipulated lake sediments in order to link chemical speciation to chemical behavior and to identify the geochemical drivers that mediate this linkage. We manipulated SWI redox conditions in mesocosm experiments to investigate the impacts of prolonged anoxia and redox oscillations on P mobility and speciation in sediments. Mesocosm experiments demonstrate that oscillating redox conditions near the SWI may drive accelerated P release from sediments relative to uninterrupted reducing conditions. Sediment P is found to be predominantly associated with Fe oxyhydroxides, calcium carbonate, and apatite minerals in three shallow hyper/eutrophic lakes in northern Vermont. In Missisquoi Bay and Lake Carmi, Fe redox cycling controls P mobility via precipitation and dissolution of Fe oxyhydroxides. In the hypereutrophic Shelburne Pond, the presence of Fe sulfides precludes redox-driven P cycling and P mobility is instead dominated by organic matter mineralization. Our results demonstrate that internal P loading can manifest differently in similar shallow lake systems due to differences in lake configuration, sediment P and Fe speciation, and organic content of sediments. This work demonstrates the potential utility, as well as the limitations, of P K-edge X-ray absorption near edge structure spectroscopy in determining sediment P speciation in freshwater lakes.
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# TABLE OF CONTENTS

**ACKNOWLEDGEMENTS** ......................................................................................... ii

**LIST OF FIGURES** ................................................................................................ v

**LIST OF TABLES** .................................................................................................... vi

**CHAPTER 1: COMPREHENSIVE LITERATURE REVIEW** ................................. 1

1.1 Phosphorus as a Pollutant .................................................................................. 1

1.2 Iron – Phosphorus Interactions ......................................................................... 2

1.3 Internal Phosphorus Loading and Partitioning .................................................. 3

1.4 Phosphorus K-edge XANES .............................................................................. 4

1.4.1 Limitations of P K-edge XANES ................................................................. 5

1.5 Complementary X-ray Absorption Spectroscopies .......................................... 7

1.6 References ......................................................................................................... 9

**CHAPTER 2: ARTICLE FOR SUBMISSION** ....................................................... 15

Abstract .................................................................................................................... 15

2.1 Introduction ........................................................................................................... 15

2.2 Materials and Methods ...................................................................................... 18

2.2.1 Mesocosm Experiments ................................................................................ 18

2.2.2 Phosphorus K-edge XANES Spectroscopy .................................................. 20

2.2.3 Microprobe Analysis ....................................................................................... 21

2.2.4 Iron K-edge EXAFS Spectroscopy ................................................................ 22

2.2.6 Natural Lake Systems .................................................................................... 24

2.2.6-1 Study Sites .................................................................................................. 24

2.2.6-2 Sediment Sampling .................................................................................... 26

2.3 Results and Discussion ....................................................................................... 26

2.3.1 Phosphorus K-edge XANES ........................................................................ 26

2.3.2 Long-Term Reduction Experiment ............................................................... 30

2.3.3 Redox Oscillation Experiment ...................................................................... 36

2.3.3-1 Phosphorus Speciation in Sediment ......................................................... 37
2.3.3-2 Iron Speciation in Sediment ................................................................. 42
2.3.4 Natural Systems ...................................................................................... 46
  2.3.4-1 Lake Carmi ....................................................................................... 46
  2.3.4-2 Missisquoi Bay .................................................................................. 50
  2.3.4-3 Shelburne Pond ................................................................................ 53
2.4 Implications ................................................................................................. 56
2.5 Supporting Information .............................................................................. 60
2.6 References .................................................................................................. 72
COMPREHENSIVE BIBLIOGRAPHY ................................................................ 80
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Figure 1:</strong> Bulk P K-edge XANES spectra with best fits</td>
<td>29</td>
</tr>
<tr>
<td><strong>Figure 2:</strong> Mesocosm experiment aqueous SRP and Fe concentrations</td>
<td>31</td>
</tr>
<tr>
<td><strong>Figure 3:</strong> Phosphorus μ-XANES spectra from experimental sediment samples</td>
<td>33</td>
</tr>
<tr>
<td><strong>Figure 4:</strong> Iron XANES pre-edge intensity vs. centroid</td>
<td>35</td>
</tr>
<tr>
<td><strong>Figure 5:</strong> Morlet wavelet transform plots of Fe EXAFS data</td>
<td>36</td>
</tr>
<tr>
<td><strong>Figure 6:</strong> Fine scale μ-XRF maps of redox oscillation sediment samples</td>
<td>39</td>
</tr>
<tr>
<td><strong>Supplementary Figure 1:</strong> Bulk P K-edge XANES spectra of P mixtures</td>
<td>60</td>
</tr>
<tr>
<td><strong>Supplementary Figure 2:</strong> Coarse μ-XRF maps of redox oscillation samples</td>
<td>63</td>
</tr>
<tr>
<td><strong>Supplementary Figure 3:</strong> Bulk Fe EXAFS spectra</td>
<td>64</td>
</tr>
<tr>
<td><strong>Supplementary Figure 4:</strong> Scatterplots of μ-XRF data for redox oscillation samples</td>
<td>65</td>
</tr>
<tr>
<td><strong>Supplementary Figure 5:</strong> Lake Carmi dissolved oxygen time series</td>
<td>66</td>
</tr>
<tr>
<td><strong>Supplementary Figure 6:</strong> Scatterplots of μ-XRF data for Lake Carmi samples</td>
<td>67</td>
</tr>
<tr>
<td><strong>Supplementary Figure 7:</strong> Lake Carmi Fe, Mn, and TP time series</td>
<td>68</td>
</tr>
<tr>
<td><strong>Supplementary Figure 8:</strong> Coarse μ-XRF maps of Lake Carmi samples</td>
<td>69</td>
</tr>
<tr>
<td><strong>Supplementary Figure 9:</strong> Scatterplots of μ-XRF data for Shelburne Pond sample</td>
<td>70</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1: Bulk P K-edge XANES Linear combination fitting results</td>
<td>28</td>
</tr>
<tr>
<td>Table 2: Correlation coefficients of P and bonding elements in ( \mu )-XRF maps</td>
<td>40</td>
</tr>
<tr>
<td>Supplementary Table 1: P mixtures XANES LCF results</td>
<td>61</td>
</tr>
<tr>
<td>Supplementary Table 1: Iron EXAFS LCF results</td>
<td>62</td>
</tr>
<tr>
<td>Supplementary Table 2: Summary geochemical data for Missisquoi Bay 2015</td>
<td>71</td>
</tr>
</tbody>
</table>
CHAPTER 1: COMPREHENSIVE LITERATURE REVIEW

1.1 Phosphorus as a Pollutant

Phosphorus is an essential macronutrient for all forms of life and is often the limiting nutrient for biological productivity in freshwater systems. In excess, P can be a pollutant and driver of eutrophication and cyanobacterial harmful algal blooms (HABs) in lakes. Cyanobacterial HABs result in a variety of undesirable water quality issues including impairment to recreational activities, reduced aesthetics, and the production of toxins that can be harmful to aquatic and terrestrial wildlife as well as human health (Brooks et al. 2015). As cyanobacterial HABs have become an increasingly frequent and severe water quality issue globally (Brooks et al. 2015; O’Neil et al. 2012), understanding the pathways by which P becomes bioavailable in sediment-water systems has become increasingly important.

Traditionally, surface water eutrophication has been attributed to increased external inputs of P originating from agricultural runoff (Daniel et al. 1998) and strategies to mitigate eutrophication have focused on agricultural best-management practices (Sharpley et al. 1994). However, internal cycling of P between sediments and the water column may drive eutrophication-related issues in receiving water bodies for years to decades following reductions in external nutrient inputs (Schindler, 2006; Søndergaard et al. 2003). This is particularly a concern in relatively shallow water bodies where sediment P can become bioavailable due to transient mixing and stratification (Giles et al. 2016). As policy and infrastructure improvements that curtail external P loading become
more widespread, the relative importance of internal P loading to total P budgets in
eutrophic water bodies will likely increase (Reddy et al. 2011).

1.2 Iron – Phosphorus Interactions

The reactivity of P as an anion (primarily as HPO$_4^{2-}$) and its high affinity for cationic
mineral surfaces gives rise to a propensity for P to remain in solid phase (Sharpley et al.
2013). As a result, excess P from historical and current agricultural fertilizer applications
can accumulate at various locations along the land-freshwater continuum, including in
sediments of receiving water bodies (Sharpley et al. 2013). This “legacy P” (Kleinman et
al. 2011) may then cycle between sediments and the water column in response to various
chemical, physical, and biological drivers (Giles et al. 2016; Søndergaard et al. 2003).

The solubility (and thus mobility) of P in aquatic systems is often tightly linked to
that of iron (Fe), which is generally insoluble as Fe$^{3+}$ but soluble as Fe$^{2+}$ (Schwertmann
1991). Thus, P mobility in these systems can be redox-sensitive and may be influenced
by changing redox conditions near the sediment-water interface (SWI). Numerous studies
have described the significant role that reactive Fe phases can play in governing P
behavior in sediment-water systems (Giles et al. 2016; Kraal et al. 2015a; Li et al. 2015;
Parsons et al. 2017; Schroth et al. 2015; Smith et al. 2011). Nanoparticulate Fe
oxyhydroxides specifically, are known to be highly effective scavengers of dissolved P
under oxic conditions due to their high surface area and positive charge (Smith et al.
2011). These nanoparticle phases are metastable with respect to time (Schwertmann et al.
1999) and are highly susceptible to reductive dissolution under hypoxic and anoxic
conditions, which results in the release of sorbed P (Rozan et al. 2002; Sundby et al. 1992). This mobilized P may then be sequestered in sediment pore water, released to the overlying water column, or reincorporated into various mineral phases depending on the position of the redox front (Smith et al. 2011) and bottom and pore water concentrations of sulfur (Kraal et al. 2015b; Li et al. 2015) and nitrate (Parsons et al. 2017).

1.3 Internal Phosphorus Loading and Partitioning

Internal P loading disproportionately impacts shallow eutrophic lakes, where legacy P is often abundant and sediment surface area is relatively large compared to the volume of overlying water (Søndergaard et al. 2003). Under calm lake conditions (low wind and riverine inputs), transient thermal stratification can rapidly set up, precluding lake mixing and preventing reaeration of the SWI (Friedrich et al. 2014). These conditions initiate a microbially mediated positive feedback loop, where organic matter remineralization releases P into the water column and consumes oxygen near the SWI. In turn, this amplifies reducing conditions, promoting the dissolution of Fe oxyhydroxides and leading to further P release from sediment (Jilbert et al. 2011). While the increased mobility of P under persistent anoxia is well documented (Krom and Berner, 1981; Penn et al. 2000; Rozan et al. 2002), redox conditions in shallow systems are rarely static and are influenced by light, wind, ice cover, temperature, and microbial populations (Golosov et al. 2007). Wind-driven mixing in particular can disrupt thermal stratification and reoxygenate the SWI on very short timescales (Isles et al. 2015; Smith et al. 2011), which
may result in a rapid switching in the P sink/source function of the sediment (Kraal et al. 2015b).

While dynamic redox conditions can exert substantial control on P mobility by governing the solubility of Fe oxyhydroxides, P can also be sequestered in sediments through sorption to calcite (Sø et al. 2011), or through interaction with Ca$^{2+}$, Al$^{3+}$ or Fe$^{2+/3+}$ to form various phosphate minerals. Additionally, lake sediment P has been shown to occur as polyphosphate (Hupfer et al. 2004) and various organic species including inositol hexakisphosphate, phosphate monoesters, and diesters (Giles et al. 2015). As different sediment P species will exhibit varying degrees of stability in response to SWI geochemistry, the chemical P species present in any sediment-water system will determine the processes that control the initiation and magnitude of internal loading in that system. For example, Fe-bound P may be released to the water column under anoxic conditions, while Ca- or Al-bound P may be stable under the same conditions (Rothe et al. 2016). Similarly, low pH may drive the dissolution of CaCO$_3$ and release of associated P, but would also increase the sorptive capacity of Fe oxyhydroxides, imparting an unknown net effect on P flux (Penn et al. 2000). Speciation of P in sediment is thus a critical determinant of potential P mobility in aquatic systems.

1.4 Phosphorus K-edge XANES

Traditionally, sequential extraction methods have been used to characterize P-pools in benthic sediment (Li et al. 2015; Parsons et al. 2017; Ruttenberg et al. 2009). Although this method is a valuable tool to operationally quantify P pools of different reactivity in
sediment, it is indirect and provides limited information related to the speciation of P in these pools. Recently, P K-edge X-ray absorption near edge structure (XANES) spectroscopy has been utilized to directly characterize P species in marine sediments (Dijkstra et al. 2016; Egger et al. 2015; Kraal et al. 2015a; 2017; Li et al. 2015) and soils (Abdala et al. 2015; Ajiboye et al. 2008; Alotaibi et al. 2018; Beauchemin et al. 2003; Brandes et al. 2007; Hesterberg et al. 2017; Liu et al. 2015; 2017; Luo et al. 2017; Prietzel et al. 2013; Rivard et al. 2016; Schmieder et al. 2018). However, with the exception of one study examining lake sediment samples dating 2000 – 10,000 years old (Giguet-Covex et al. 2013), the application of this technique to freshwater sediments is notably absent from the literature. Phosphorus K-edge XANES is a highly selective synchrotron-based spectroscopic technique that can be used to identify and estimate the quantities of specific inorganic P species (e.g. Al-, Ca-, Fe-bound P) in mixed-mineral samples by probing the molecular bonding environment around P atoms (Liu et al. 2017). This technique is advantageous over selective extraction schemes as it is non-destructive, requires minimal sample preparation, and provides direct measurement of the local structural and electronic environment of P (Ajiboye et al. 2007).

1.4.1 Limitations of P K-edge XANES

Although P K-edge XANES can provide valuable insight into bulk P speciation in sediment, the limitations of this technique must be considered in order to properly constrain accurate interpretations of results. First, and perhaps most important, is that P species in unknown samples are more difficult to distinguish and quantify with K-edge
XANES than other elements such as S or Mn (Beauchemin et al. 2003). As P in natural systems is overwhelmingly present as \(\text{HPO}_4^{2-}\) in the +5 oxidation state, the energy position of the white line—the discrete energy level with the maximum probability of photon absorption—does not shift significantly between different species, leaving discrimination amongst species up to subtle differences in pre- and post-edge spectral features (Prietzel et al. 2013). Second, and partially owing to the spectral similarities aforementioned, is that P K-edge XANES is not sensitive enough to accurately identify species representing \(< 5\%\) of total sample P content (Werner and Prietzel, 2015). It is therefore typical to be able to differentiate only 3-5 different chemical species in a given sample while species present at lower concentrations may not be detected (Kizewski et al. 2011; Liu et al. 2017). Lastly, and again owing to spectral similarities, is the difficulty of identifying organic P species in natural samples. While not an inherent limitation of XANES, in practice, the relatively broad and featureless spectra of common organic P compounds like phytic acid make identification and quantification of these species in uncharacterized samples challenging (Ajiboye et al. 2008; Beauchemin et al. 2003; Kraal et al. 2015a).

Despite these limitations, the recent application of synchrotron-based X-ray absorption spectroscopy (XAS) to probe benthic P species in sediment-water systems has proven to be extremely fruitful. Li et al. (2015) used P XANES and other synchrotron-based techniques to demonstrate that ferric Fe was a major P sink even in anoxic sediments of the Chesapeake Bay. Using P and Fe XANES, Dijkstra et al. (2016) and Egger et al. (2015) both showed that vivianite was a significant P sink in anoxic
sediments of the Baltic Sea. And, with P and Fe XANES and synchrotron-based micro-X-ray fluorescence (µ-XRF), Kraal et al. (2017) demonstrated the significant role that CaCO₃ plays in P retention in euxinic sediments of the Black Sea. While these studies highlight the utility of this technique for characterizing P speciation (and thus P behavior) in benthic environments, they also emphasize the need for complementary techniques to validate the results from and overcome the limitations of, P K-edge XANES.

1.5 Complementary X-ray Absorption Spectroscopies

As P is often associated with Fe in aquatic systems, Fe K-edge XANES and extended X-ray absorption fine structure (EXAFS) spectroscopy have been used in concert with P XANES to corroborate estimations of P speciation in sediments (Egger et al. 2015; Dijkstra et al. 2016; Kraal et al. 2017; Li et al. 2015). Iron EXAFS is a synchrotron-based technique in which detailed structural information such as interatomic distances and coordination numbers of Fe phases present in samples can be determined based on oscillations in X-ray absorption arising from the backscatter of photoelectrons off of neighboring atoms (Rehr et al. 2000). Additionally, the position and integrated intensity of the pre-edge feature in Fe XANES spectra can be used to quantitatively determine the average Fe oxidation state in mixed-valence samples (Wilke et al. 2001). This information can be used to determine the Fe speciation in bulk samples, i.e. fraction of Fe-oxides, sulfides, silicates, etc. Unlike P K-edge XANES, Fe EXAFS has occasionally been utilized to investigate Fe speciation in sediments from freshwater lakes (Cosmidis et al. 2014; Spadini et al. 2003). This method is inherently complementary to P K-edge
XANES, as it can validate the presence of Fe oxyhydroxides or Fe phosphates in samples where spectral ambiguity precludes their identification from P XANES spectra alone. Furthermore, Fe EXAFS may be useful in cases where noisy P XANES data (typically due to low P concentrations in natural samples) make the distinction between P sorbed on Fe- or Al-oxides or clays, difficult (Beauchemin et al. 2003).

Spatially resolved synchrotron-based spectroscopies are another way to enhance the sensitivity of XAS analysis to P speciation in sediments (Hesterberg et al. 2017). So-called microprobe measurements include μ-XRF, which provides in situ mapping of P distribution and co-localization with other elements, and microscopically focused XANES (μ-XANES), which can be used to probe P in micron-scale spots (Liu et al. 2017). These techniques are particularly useful in overcoming the limitations of P K-edge XANES, as they can identify minor P species that may be obscured in bulk analysis despite being biogeochemically relevant (Hesterberg et al. 2017). Elemental mapping via μ-XRF can also corroborate results from bulk P XANES analysis by confirming the spatial colocation of P with presumed P bonding elements (Rivard et al. 2016). Each of these spectroscopic techniques possesses its own strengths and shortcomings. In order to accurately constrain P speciation in lake sediments and ultimately relate chemical speciation to chemical behavior, a combination of various techniques that can validate and complement one another is certainly the best approach.
1.6 References


CHAPTER 2: ARTICLE FOR SUBMISSION

Phosphorus Mobility And Speciation Under Dynamic Redox Conditions In Shallow Eutrophic Freshwater Systems

Austin Wilkes

Abstract

While internal loading of phosphorus (P) is recognized as a driver of eutrophication-related issues in many freshwater systems, molecular speciation of P in benthic sediments of these systems remains poorly characterized. As different P species will exhibit different responses to changing sediment-water interface (SWI) geochemistry, quantifying P speciation in sediments is a critical step in understanding P dynamics in sediment-water systems. Here, various synchrotron-based techniques were employed to directly probe the bonding environments of P and iron (Fe) in natural and experimentally manipulated lake sediments in order to link chemical speciation to chemical behavior and to identify the geochemical drivers that mediate this linkage. We manipulated SWI redox conditions in mesocosm experiments to investigate the impacts of prolonged anoxia and redox oscillations on P mobility and speciation in sediments. Mesocosm experiments demonstrate that oscillating redox conditions near the SWI may drive accelerated P release from sediments relative to uninterrupted reducing conditions. Sediment P was found to be predominantly associated with Fe oxyhydroxides, calcium carbonate, and apatite minerals in three shallow hyper/eutrophic lakes in northern Vermont. In Missisquoi Bay and Lake Carmi, Fe redox cycling controls P mobility via precipitation and dissolution of Fe oxyhydroxides. In the hypereutrophic Shelburne Pond, the presence of Fe sulfides precludes redox-driven P cycling and P mobility is instead dominated by organic matter mineralization. Our results demonstrate that internal P loading can manifest differently in similar shallow lake systems due to differences in lake configuration, sediment P and Fe speciation, and organic content of sediments.

2.1 Introduction

Phosphorus is the dominant limiting nutrient and driver of eutrophication and harmful cyanobacteria blooms in many freshwater systems. As cyanobacterial blooms have become increasingly frequent and severe at a global scale (Brooks et al. 2015; O’Neil et al. 2012), understanding the processes that promote P bioavailability in the water column have become increasingly important. Traditionally, surface water eutrophication has been
attributed to increased external inputs of P originating from agricultural runoff (Daniel et al. 1998) and strategies to mitigate eutrophication have focused on agricultural best-management practices (Sharpley et al. 1994). However, internal loading of “legacy P” from lake sediments may drive eutrophication-related issues for years to decades following reductions in external nutrient inputs (Schindler, 2006; Søndergaard et al. 2003). This is particularly important in shallow systems, where benthic-derived P can become bioavailable due to transient mixing and stratification (Giles et al. 2016).

Internal loading of P in shallow lakes can occur under reducing conditions near the sediment-water interface (SWI) due to the reductive dissolution of surface-sediment Fe oxides and simultaneous release of associated P (Giles et al. 2016; Joung et al. 2017; Søndergaard et al. 2003). This released P can be sequestered in sediment pore water where it may be reincorporated into various mineral phases (Kraal et al. 2015b; Parsons et al. 2017) or escape into the overlying water column where it may be available for biological uptake. In lakes, periods of low wind and riverine inputs coupled with strong sunlight can promote the development of a thermally stratified water column. This can result in P release from sediment as ongoing microbial respiration consumes oxygen near the SWI and lack of mixing prevents re-aeration of sediments. Although the increased mobility of P under persistent anoxia is well documented (Krom and Berner, 1981; Penn et al. 2000; Rozan et al. 2002), the extent to which oxic-anoxic cycling impacts P mobility and speciation in sediments is still poorly understood (Parsons et al. 2017). As redox conditions in shallow systems are highly dynamic and sensitive to light, wind, ice
cover, temperature, and microbial populations (Golosov et al. 2007), the timing and duration of oxic-anoxic cycling may affect P release and redistribution within sediment on very short timescales.

In contrast to stratified conditions, a well-mixed water column will maintain oxidizing conditions at the SWI and may result in the retention of P through sorption to Fe oxides (Søndergaard et al. 2003). Surface sediments can therefore act as either a source or sink for dissolved P depending on the position of the redoxcline in relation to the SWI (Giles et al. 2016; Joung et al. 2017; Parsons et al. 2017). Additionally, the chemical P species present in sediments of any lake system may govern the extent to which internal loading occurs in that system. Sedimentary phosphorus can exist in many different inorganic species through sorption to minerals like calcite or Fe oxyhydroxides (Sø et al. 2011; Peretyazhko and Sposito, 2005) or through interaction with Ca^{2+}, Al^{3+}, or Fe^{2+/3+} to form various phosphate minerals. Lake sediment P has also been shown to exist as polyphosphate (Hupfer et al. 2004) and various organic species including inositol hexakisphosphate, phosphate monoesters, and diesters (Giles et al. 2015). Intuitively, these different chemical P species will exhibit different degrees of stability or reactivity in response to dynamic SWI geochemistry. Speciation of P in sediment is thus a critical determinant of potential P mobility, and bioavailability, in aquatic systems.

In this study, we examine the speciation of P and Fe in sediments and the redox-driven cycling of P and Fe across the SWI in response to natural and experimental redox
fluctuations. For the first time, P K-edge X-ray absorption near edge structure (XANES) spectroscopy is used to directly measure legacy P speciation in freshwater lake sediments. Multiple other synchrotron-based techniques (Fe K-edge XANES/EXAFS, µ-XRF) are utilized to further constrain the bonding environment of P in sediments subjected to different redox treatments while P release (or sequestration) is measured via concurrent water sampling. Experimental results are then used as a framework for interpreting X-ray absorption spectroscopy (XAS) data collected on sediments from various nearby lakes under naturally changing redox conditions. Our objective is to determine the impact of redox fluctuations on P release, sequestration and partitioning in shallow eutrophic freshwater lakes. Our results provide insight into the drivers of internal loading and sequestration of P in these systems and the spatial and temporal variability of these drivers, as well as the molecular P and Fe species most active in P cycling across the SWI.

2.2 Materials and Methods

2.2.1 Mesocosm Experiments

Redox manipulation experiments were performed with sediment cores collected from our long-term monitoring site in Missisquoi Bay using SCUBA in August 2018. The intact sediment cores and overlying lake water were sealed in the glass coring tubes used for collection and allowed to settle out in the presence of oxygen for 21 days. Gas lines were fed into each sealed coring tube, just below the water’s surface, to supply air or nitrogen in order to induce oxidizing or reducing conditions, respectively, at the SWI. Following
this pre-treatment period, seven replicate sediment cores were subjected to redox oscillations by alternating between 7-day periods of intermittent nitrogen bubbling and 90-minute periods of air bubbling. Two cores were subjected to extended reducing conditions by periodically bubbling with nitrogen for 47 days. Cores were stored at room temperature in the dark for the duration of the experiments. Water samples from each core were collected from 2 cm above the SWI using a syringe at routine intervals throughout the experiments. These samples were filtered using 0.22 µm sterile syringe filters and preserved for analysis of trace metals and P. Soluble reactive phosphorus (SRP) was measured using direct colorimetric analysis while dissolved trace metal concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS).

Redox conditions at the SWI and across vertical profiles of the sediment pore water and the overlying water column were monitored in situ using a Au-Hg amalgam voltammetric electrode. Sediment sampling was performed at discrete points throughout the experiment by sacrificing one of seven replicate cores, siphoning off the water without disturbing the SWI, and collecting a vertically resolved ‘freeze-core’. This was performed by driving a hollow stainless steel spike into the sediment core and continuously refilling it with liquid nitrogen in order to freeze the surrounding sediment to the spike. These sediment freeze cores were then stored in the freezer (-20º C) before being sectioned vertically into 1-cm increments using a bandsaw. Splits of sectioned sediments were lyophilized and preserved for XAS analysis. For the purpose of this study, only the top centimeter of sediments were examined.
2.2.2 Phosphorus K-edge XANES Spectroscopy

Bulk phosphorus XANES spectra were collected in fluorescence mode at the Soft X-ray Microcharacterization Beamline (SXRMB) at the Canadian Light Source (CLS) in Saskatoon, Canada. The storage ring had an energy of 2.9 GeV and a maximum current of 250 mA. The beamline was equipped with a Si (111) double crystal monochromator and a 7-element silicon drift detector. Freeze-dried powdered samples were spread as thin homogeneous layers onto double-sided carbon tape and placed in the vacuum chamber. Phosphate sorbed on ferrihydrite was used to calibrate the edge energy ($E_0$) of all sample spectra to 2152.0 eV. Three scans were collected for each sample using a step size of 2 eV from -20 to -6 eV, 0.15 eV from -6 to 25 eV, and 0.75 eV from 25 to 60 eV, all with respect to $E_0$. All P reference compounds were either sourced from local mineral collections or synthesized using reagent grade chemicals. Goethite and 2-line ferrihydrite were prepared following the methods outlined in Schwertmann and Cornell (1991). Calcite was prepared according to the procedure of Boyjoo et al. (2014). All phosphate adsorption was performed using batch experiments following the procedure described by Prietzel et al. (2016). Aluminum phosphate was purchased in reagent grade from Sigma Aldrich. All other P mineral standards were sourced from local mineral collections.

Spectrum averaging, baseline correction, post-edge normalization, and linear combination fitting were performed using the software SIXPack (Webb, 2005). Triplicate spectra were first examined visually for drifts, glitches and general quality before being averaged. All averaged spectra were baseline-corrected from -23 to -10 eV relative to $E_0$.  

20
Post-edge normalization to an edge jump of 1.0 was performed from +35 to +55 eV, relative to $E_0$. Linear combination fitting (LCF) of unknown spectra was performed from 2140 to 2180 eV while allowing $E_0$ to float and forcing weighting factors to be positive. Weighting factors were not forced to sum to one, but were instead renormalized to sum to one following the final fit. As previous work has shown that P proportions smaller than 5% are unreliable and often erroneous (Werner and Prietzel, 2015), only weighting factors $>5\%$, were included in final fits. Quality of fit was assessed based on R-factors, how close the weighted factor totals came to 1.0 and visual inspection of fitting mismatch at discrete spectral features characteristic of specific P-bonding environments.

### 2.2.3 Microprobe Analysis

Microprobe measurements were also conducted on the SXRMB at the CLS. Micro-X-ray fluorescence (µ-XRF) and micro-X-ray absorption near edge structure (µ-XANES) measurements were collected on natural and experimental sediment samples to map the distribution of P at the microscale and further constrain the bonding environment of P in these sediments. Microprobe measurements were conducted on freeze-dried and homogenized sediment samples, spread in thin even layers onto doubled-sided carbon tape. Elemental mapping with µ-XRF was performed at 7130 eV using a Bruker detector. In order to allow for meaningful statistical comparisons between samples, coarse scans were first collected over large (2x2 mm) randomly selected areas of each sediment sample using a step size of 50 µm x 50 µm and a 1-second dwell time. These coarse maps allowed for the identification of regions of interest (i.e. regions with high P counts) within samples where smaller maps could then be collected at higher resolution, as
reported in Liu et al (2017). High-resolution maps were collected using a 10 µm x 10 µm step size and a dwell time of 1 second. These maps were acquired in regions where P counts were at or near their maximum values in the 2x2 mm maps and were used to collect spatially resolved P K-edge µ-XANES spectra at discrete P hot spots. All µ-XRF data were processed in SMAK (Webb, 2005) and R (R Core Team, 2018) while all µ-XANES spectra were processed using SIXPack (Webb, 2005).

2.2.4 Iron K-edge EXAFS Spectroscopy

Iron speciation in the experimental sediments was investigated using Fe K-edge extended X-ray fine structure (EXAFS) spectroscopy on beamline 4-1 (Stanford Synchrotron Radiation Lightsource). Freeze-dried sediments were spread in thin layers onto Kapton tape, mounted on a stainless steel sample holder, and placed at a 45º angle to the incident X-ray beam. Measurements were collected in fluorescence mode using a passivated implanted planar silicon (PIPS) detector positioned behind a Mn-foil filter at 90º to the beam. A Si (220) crystal was used as the monochromator. Energy calibration was performed using Fe foil and $E_0$ was set to 7111.08 eV (Wilke et al. 2001). All XANES spectra were calibrated, background-subtracted, and normalized using the software SIXPack (Webb, 2005). The EXAFS portion of each normalized spectrum was isolated by subtracting a background spline function from the post-edge region. These spectra were then $k^2$-weighted before being Fourier transformed (FT) using a Kaiser-Bessel window. Iron speciation was quantified using linear combination fitting in the EXAFS region from 2-10 Å$^{-1}$ and in the XANES region from 7100-7160 eV using a wide range of naturally occurring Fe-bearing minerals as references. Final fits were forced to include
only non-negative loadings > 5%, while allowing energy to float. This value was chosen based on findings from O’Day et al. (2004), which demonstrated that a practical detection limit for most Fe minerals in mixtures is ~ 5%. Linear combination fitting indicated that all sample spectra could be fit using a combination of ferric hydroxide (Fe(OH)$_3$), hornblende, magnetite (Fe$_3$O$_4$), and pyrite (FeS$_2$). Ferric hydroxide, hornblende, and pyrite were used as analogs for Fe oxyhydroxides, Fe in silicates, and Fe sulfides, respectively. Due to noise in the high k-range of most Fe EXAFS spectra, LCF results from the XANES region were relied upon to estimate the proportions of Fe mineral classes present in sediment samples. According to O’Day et al. (2004), XANES spectra for Fe sulfides, oxides/hydroxides, phyllosilicates, and spinels (such as magnetite) contain features that allow for identifying and distinguishing amongst these mineral classes in complex mixtures. As such, the Fe speciation results presented here are meant to represent Fe mineral classes rather than individual mineral species. Results from Fe EXAFS LCF are presented alongside XANES LCF results, but should be interpreted with caution due to the higher degree of noise and fitting error associated with these spectra.

For further analysis of backscattering atoms in higher coordination shells, the wavelet transform (WT) method was used with the FORTRAN program HAMA (Funke et al. 2005). The Morlet WT was performed on $k^3$-weighted EXAFS spectra in the k-range of 2-12 Å$^{-1}$ and R-range of 2-4 Å (not phase-corrected) in order to connect FT peaks to energies where backscattering takes place (Karlsson et al. 2008). For high resolution WT plots of the second and third shell, the frequency ($\eta$) was set to 8 while the half-width of
the Gaussian window (\(\sigma\)) was set to 1, following the parameters used by Karlsson and Persson (2010). Average Fe coordination and oxidation state were examined based on the methods outlined in Wilke et al. (2001). Pre-edge peaks were isolated by subtracting a cubic spline function fit to the background absorption in the energy range a few eV above and below the pre-edge peak. These background-subtracted peaks were then fit with two pseudo-Voigt functions (50% Gaussian, 50% Lorentzian) using Fityk (Wojdyr 2010). Pre-edge centroid position and integrated intensity were calculated from these fits.

2.2.5 Natural Lake Systems

2.2.5-1 Study Sites

Missisquoi Bay is a shallow (mean depth 2.8 m; max depth 4 m) polymictic eutrophic bay in the northeastern arm of Lake Champlain. The bay has a surface area of 77.5 km\(^2\) and drains a catchment of 3105 km\(^2\) (Levine et al. 2012). Missisquoi Bay is largely isolated from the main lake, connected only by a small channel at its southern end. The bay experiences regular cyanobacteria blooms in the summer months as a result of current and historical anthropogenic nutrient loading (Levine et al. 2012) and large watershed to lake surface area ratio of \(~ 40\) (Joung et al. 2017). Additionally, the large sediment surface area to water volume ratio and hydrodynamic isolation of Missisquoi Bay make it particularly susceptible to internal loading of legacy P in the summer, as transient thermal stratification can set up rapidly under calm conditions. The surface sediments are relatively organic-poor with organic carbon content ranging from 4 to 5% (Joung et al. 2017).
Lake Carmi is a dimictic eutrophic lake located 20 km east of Missisquoi Bay in Franklin, VT. The lake has a surface area of 5.7 km$^2$, mean and maximum depths of 4 m and 10 m, respectively, and a catchment area of 31.2 km$^2$ (VTDEC, 2018). The watershed to lake surface area ratio is ~ 5, which is much lower than that of Missisquoi Bay. The mean organic carbon content of the surface sediments is 11.5%. Lake Carmi is also deeper and less prone to transient wind-driven mixing than Missisquoi Bay. Thus, it affords the opportunity to study the effects of prolonged seasonal stratification on benthic P speciation. Like Missisquoi Bay, Lake Carmi has experienced persistent and severe cyanobacteria blooms in the summer months. To study the timing and magnitude of stratification in the lake, two surface buoys were deployed in the deepest section in June 2018, which collected high frequency temperature and dissolved oxygen measurements throughout the water column.

Shelburne Pond is a polymictic hypereutrophic lake located 70 km south of Missisquoi Bay. The lake has a surface area of 1.8 km$^2$, mean and max depths of 3.4 m and 7.6 m (Ferber et al. 2004), and a catchment area of 19 km$^2$ (Joung et al. 2017). The watershed to lake surface area ratio of ~ 11 is greater than that of Lake Carmi but still far lower than that of Missisquoi Bay (Joung et al. 2017). Surface sediments of Shelburne Pond are relatively organic-rich compared to Missisquoi Bay and Lake Carmi, with organic carbon content ranging from 20 to 25% (Joung et al. 2017). Cyanobacteria blooms also occur frequently throughout the summer months (Ferber et al. 2004).
2.2.5-2 Sediment Sampling

In order to compare experimental P dynamics to those observed in natural systems, sediment sampling was conducted at Missisquoi Bay, Lake Carmi, and Shelburne Pond using a gravity-coring device. Numerous cores were collected throughout the year at Missisquoi Bay in 2015. Two cores were collected from Lake Carmi in 2018; one near peak stratification on August 8 and another following lake turnover on October 3. One core was collected from Shelburne Pond on August 7, 2018. Cores were sealed with aluminum tape, wrapped in aluminum foil, and stored upright in a cooler during transportation back to the lab to prevent reactions with light and air. The overlying lake water was siphoned off from each coring tube before sectioning sediments into 1 cm increments. These subsamples were then lyophilized for XAS and P extractions. Phosphorus extractions were performed in triplicate and concentrations were determined using inductively coupled plasma optical emission spectrometry (ICP-OES).

2.3 Results and Discussion

2.3.1 Phosphorus K-edge XANES

Linear combination fitting of bulk P K-edge XANES spectra demonstrated systematic changes in P speciation, with the fraction of P sorbed to Fe oxyhydroxides varying by up to 26% across experimental samples spanning highly contrasting redox states (Table 1). From a visual standpoint, all XANES spectra appeared very similar (Fig. 1), with subtle differences present in the intensity of the pre-edge feature arising from Fe(III)-O-P bonding (Franke and Hormes, 1995; Khare et al. 2007). All sample spectra closely
resembled the standard spectrum of phosphate adsorbed to ferrihydrite (Fig. 1), which suggested that a large fraction of P in these sediments was associated with Fe oxyhydroxides under all investigated redox conditions. Linear combination fitting results reflected this, as the fraction of P sorbed to Fe oxyhydroxides was the largest component in the best fits for most samples (Table 1). These results imply that the average P bonding environment may be similar across all samples, despite the various redox conditions different samples were subjected to. Comparable findings were reported by Rivard et al. (2016), who demonstrated that bulk P XANES spectra from agricultural soil samples representing various different cropping and fertilization practices were all alike. In this study, P speciation as determined by K-edge XANES is assumed to represent only inorganic P species, as numerous previous studies have shown the relative insensitivity of this technique towards distinguishing and quantifying organic P species in uncharacterized samples (Ajiboye et al. 2008; Beauchemin et al. 2003; Kraal et al. 2015a).
Table 1: Linear combination fitting results of all bulk P K-edge XANES spectra from 2140 to 2180 eV. Fh-P is phosphate adsorbed on ferrihydrite. Gt-P is phosphate adsorbed on goethite. Fe$^{3+}$-bound is the sum of contributions of phosphate sorbed on ferrihydrite and phosphate sorbed on goethite.

<table>
<thead>
<tr>
<th></th>
<th>Fh-P</th>
<th>Gt-P</th>
<th>Vivianite</th>
<th>CaCO$_3$-P</th>
<th>Biogenic apatite</th>
<th>Fe$^{3+}$-bound</th>
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<td>Initial Ox.</td>
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<td>0.28</td>
<td>-</td>
<td>0.12</td>
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<td><strong>Shelburne Pond</strong></td>
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<td>0.16</td>
<td>0.10</td>
<td>0.73</td>
<td>0.0004</td>
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</table>
Figure 1: Phosphorus K-edge XANES spectra and associated best fits in dashed red lines for all experimental, Lake Carmi, and Missisquoi Bay sediment samples as well as P reference compounds used in linear combination fitting. The grey bar indicates the position of the pre-edge feature.

In order to ensure that the differences in P speciation among samples were being accurately captured by LCF, numerous mixtures of P reference compounds were analyzed and fit using the same methods described for sediment samples. This provides a more robust index of fitting error than goodness-of-fit criteria alone, and helps to validate
the results of other best fits that use combinations of the same reference compounds (Ajiboye et al. 2007). As preliminary XANES results showed that a large fraction of P in these sediments was adsorbed on ferrihydrite and calcium carbonate, nine mixtures of these P reference compounds in varying proportions were analyzed. The resulting spectra displayed a systematic shift in spectral shape as the proportion of P associated with ferrihydrite and calcium carbonate changed (SI Fig. 1). Mixtures with larger fractions of ferrihydrite-P yielded spectra with more intense pre-edge features, while mixtures with increasing calcium carbonate contributions exhibited greater spectral broadening on the high-energy side of the absorption edge as well as a more pronounced feature at 2160 eV (SI Fig. 1). These results are consistent with spectral trends observed in other studies that analyzed mixtures of Fe-P and Ca-P reference compounds (Ajiboye et al. 2007; Werner and Prietzel, 2015). Linear combination fitting of these XANES spectra resulted in good quality fits (mean R-factor = 0.0008) with accurate quantification of P species (mean error = 0.8%; SI Table 1). Although these two-component mixtures are a simplification of our sediment samples, the accuracy of our fitting method in quantifying P sorbed to ferrihydrite and CaCO$_3$ in mixed phases should lend confidence towards the ability of our method to quantify these species in natural samples as well.

2.3.2 Long-Term Reduction Experiment

For the long-term reduction experiment, the SWI and overlying water were kept anoxic for 47 days. During this time, SRP was gradually released from sediment before aqueous concentrations stabilized around Day 35, while dissolved Fe concentrations generally increased in the water column throughout the course of the experiment (Fig. 2).
Following 47 days of reducing conditions, mean dissolved Fe concentration in the water column was 1.43 µg L\(^{-1}\) and mean SRP concentration was 1.16 µg L\(^{-1}\) (Fig. 2). Prior to the onset of reducing conditions, mean dissolved Fe and SRP concentrations were 2 µg L\(^{-1}\) and 85 µg L\(^{-1}\), respectively. These results made it clear that our imposed redox manipulations were impacting the mobility of P and Fe in this mesocosm sediment-water system. The concomitant release of P and Fe from sediment under imposed anoxia and the strong positive correlation between SRP and dissolved Fe concentrations in water (\(R^2 = 0.87\)) suggested that P release from these sediments was governed by the reductive dissolution of Fe oxyhydroxides (Rozan et al. 2002; Sundby et al. 1992).

**Figure 2:** Median concentrations of soluble reactive phosphorus and total dissolved iron just above the SWI over time in the long-term reduction and redox oscillation mesocosm experiments. Red triangles indicate when a replicate sediment core was destructively sampled. The dashed grey line indicates when nitrogen bubbling began on Day 21. The vertical grey bars in the redox oscillation plot highlight 90-minute oxidation events.
End-member sediment samples were collected by destructively sampling one replicate sediment core following 19 days of pre-treatment oxidizing conditions, just before the initiation of nitrogen bubbling (‘initial oxidized’) and one at the end of the experiment, following 47 days of anoxia (‘extended reducing’). Bulk P K-edge XANES spectra for the two end-member sediment samples appeared to be very similar, however, LCF indicated that P speciation was actually quite different (Fig. 1; Table 1). In the initial oxidized sediment sample, 64% of P was sorbed to Fe oxyhydroxides (Table 1). Following extended reducing conditions the fraction of P sorbed to Fe oxyhydroxides fell to 38% and the formation of vivianite ($\text{Fe}^{2+}_3(\text{PO}_4)_2$) accounted for 28% of surface sediment P (Table 1). These results suggest that under anoxia, P-bearing Fe(III)-oxyhydroxides were reductively dissolved and some of the released Fe and P re-precipitated as Fe(II) phosphate minerals such as vivianite. The presence of vivianite in anoxic sediments has also recently been reported in various sediment-water systems (Cosmidis et al. 2014; Dijkstra et al. 2016; Egger et al. 2015; Li et al. 2015; Slomp et al. 2013), suggesting that it may represent an important P sink under sustained anoxic conditions. Following µ-XRF mapping of the extended reducing sediments, the presence of vivianite was confirmed via µ-XANES (Fig. 3) and was found to co-occur with Mn, suggesting that Mn was incorporated into the vivianite structure, consistent with the findings of Djikstra et al. (2016) and Egger et al. (2015).
Figure 3: Phosphorus K-edge μ-XANES spectra collected from the extended reducing and cyclic oxidized experimental sediment samples. Locations for the spots from the cyclic oxidized sample are indicated in Figure 6. The reference spectra for vivianite, phosphate sorbed to ferrihydrite, and biogenic apatite are included for reference. Vertical dashed lines highlight distinguishing spectral features in sample and reference spectra.

Bulk sediment Fe speciation, quantified using LCF in the EXAFS and XANES regions, indicated that Fe oxyhydroxides and mixed-valence silicates were the dominant Fe species present in these sediments (SI Table 2). LCF results from XANES and EXAFS displayed the same trends, although EXAFS LCF systematically attributed a larger
portion of Fe to oxyhydroxides than did XANES LCF. In response to the extended reducing conditions in the long-term reduction experiment, both XANES and EXAFS LCF indicated that ~ 10% of Fe oxyhydroxides initially present in surface sediments were depleted (SI Table 2). The observed depletion of Fe oxyhydroxides is consistent with the increasing Fe concentrations in the water column and occurs due to the dissolution of Fe following dissimilatory Fe reduction in sediments (Bonneville et al. 2004; Lovley, 1991). Pre-edge analysis of Fe XANES spectra adapted from Wilke et al. (2001) indicated that Fe in both samples was predominantly present as octahedrally coordinated Fe(III) (Fig. 4). The energy position of the centroid was shifted 0.12 eV lower for the extended reducing sample relative to the initial oxidized sample, signifying a slightly lower Fe$^{3+}$/Fe$_{total}$ ratio in the reducing sediments (Fig. 4). This is consistent with the formation of vivianite, as indicated by P XANES LCF, and suggests that under anoxic conditions, some of the reduced Fe was redistributed (or remained) within the solid phase, as has been reported previously (Parsons et al. 2017). Wavelet transform analysis of EXAFS spectra depicted features in the high-energy range ($k > 8$ Å$^{-1}$) for both the initial oxidized and extended reducing samples, indicative of heavier backscattering atoms in higher coordination shells ($R = 2.5$ to 4 Å, not phase-corrected; Fig. 5). These features show significant overlap with Fe-Fe scattering paths in the WT plots for lepidocrocite and illite and are thus likely due to Fe backscattering in Fe oxyhydroxides and silicate minerals. This is in general agreement with LCF of Fe XANES spectra and indicates that a large portion of Fe oxyhydroxides in surface sediments remained stable under prolonged anoxia. The persistence of Fe oxyhydroxides in sediments under
extended anoxic conditions has been reported previously (Li et al. 2015; Slomp et al. 1996b), and may be due to the inhibition of microbial Fe(III) reduction by the sorption of biogenic Fe(II) onto Fe oxyhydroxide surfaces (Roden and Urrutia, 2002). This would account for the decrease in average Fe oxidation state despite the persistence of a large portion of the Fe oxyhydroxide pool.

Figure 4: Integrated intensities vs. centroid energy position for Fe XANES pre-edge features for (a) Initial oxidized, (b) Cyclic reducing, (c) Cyclic oxidized, (d) Extended reducing, (e) Lake Carmi Stratified, (f) Lake Carmi Post-turnover, and (g) Shelburne Pond. The grey circles represent areas of pure Fe coordination chemistry. Adapted from Wilke et al. (2001).
Figure 5: Morlet wavelet transform plots ($\eta = 8$, $\sigma = 1$) for (a) Initial oxidized, (b) Cyclic reducing, (c) Cyclic oxidized, (d) Extended reducing, (e) Lake Carmi Stratified, (f) Lake Carmi Post-turnover, and (g) Shelburne Pond. The WT plots for select Fe standards are also shown for reference. The k-space is represented on the x-axis from 2 to 12 Å⁻¹ while the R-space is represented on the y-axis from 2 to 4 Å.

2.3.3 Redox Oscillation Experiment

During the redox oscillation experiment, water column SRP and dissolved Fe concentrations varied systematically with imposed redox conditions, gradually increasing during 7-day reducing half-cycles and rapidly decreasing during 90-minute oxidizing half-cycles (Fig. 2). Dissolved Mn concentrations followed the same trends but peak Mn concentrations occurred before peak Fe concentrations under anoxic half-cycles, implying that terminal electron acceptors were being reduced in order of decreasing energetic yield. During the four oxidation events, the concentrations of dissolved Fe and
SRP in the water column decreased by an average of 95.4% and 83.1%, respectively, after just 90 minutes. By contrast, reducing conditions drove gradual P and Fe release from sediment, with aqueous concentrations returning to previous peak values after ~ 7 days of anoxia. The rapid decline in aqueous Fe and SRP concentrations under oxic conditions suggests that Fe oxyhydroxides were precipitating much faster in the presence of dissolved oxygen than they were dissolving in the absence of dissolved oxygen (Shukle et al. 2019). Furthermore, once reducing conditions were resumed following each oxidation event, P release from sediment occurred at a faster rate than during the long-term reduction experiment (Fig. 2; Shukle et al. 2019). These results imply that redox oscillations near the SWI actually mobilize P more efficiently than uninterrupted anoxia. The behavior of Fe and P was tightly coupled throughout the experiment, as indicated by the strong positive correlation between dissolved Fe and SRP concentrations (R² = 0.97), further suggesting that Fe oxyhydroxide dissolution and precipitation was controlling P mobility (Bonneville et al. 2004; Penn et al. 2000). These results are consistent with previous work at Missisquoi Bay demonstrating sediment Fe : P molar ratios ranging from 28 to 36 (Joung et al. 2017), far exceeding the threshold of 15, above which Fe is thought to exert significant control on P mobility (Jensen et al. 1992).

2.3.3-1 Phosphorus Speciation in Sediment

Despite their spectral similarity, differences in sediment sample P speciation, determined by LCF of bulk P K-edge XANES spectra, showed systematic variation with imposed redox conditions. While mass balance was not performed for these experiments, LCF indicated more Fe oxyhydroxide-associated P in the oxidized samples when water
column Fe and P concentrations were near-minimum, than in the reducing samples, when water column concentrations of Fe and P were much higher (Table 1; Fig. 2). These results indicate that redox-driven P cycling in these sediments may be reversible on very short timescales, with dissolved P rapidly sorbing to Fe oxyhydroxides during 90-minute oxidation events and slowly being released back into bottom water and pore water under reducing conditions. Some of this released Fe and P may be reincorporated into Fe(II)-phosphate minerals, as LCF determined that 7% of sediment P in the cyclic reducing sample was present as vivianite, which is consistent with results of our long term reduction experiment.

To complement bulk XANES analysis and further constrain the sediment distribution of P under contrasting redox regimes, synchrotron-based μ-XRF mapping was performed on the cyclic reducing and oxidized sediment samples (SI Fig. 2). These maps revealed three distinct pools of P; 1) homogeneously dispersed “background” P that was ubiquitous in sediments and present at low concentrations, 2) P hot spots that showed strong spatial correlation with Fe, and 3) P hot spots that showed strong spatial correlations with Ca. Spatially resolved XANES spectra collected from various P-rich spots showed that both samples contained Ca-phosphate minerals that closely resembled the standard spectrum for biogenic apatite (Fig. 3). One of these spectra, collected at point 2 in Fig. 6, contains the post-edge shoulder typical of all apatite minerals (Ingall et al. 2011) and the slight shift in absorption edge characteristic of Ca-phosphate minerals (Kraal et al. 2017). The spatial co-occurrence of P and Ca did not appear to vary with redox conditions,
suggesting that apatite-like minerals may be a more permanent P sink in these sediments, as is the case in marine environments (Anderson et al. 2001; Ruttenberg, 2003; Slomp et al. 1996a).

Figure 6. Fine-scale μ-XRF maps (10x10 μm step size) depicting P, Fe and Ca distribution near discrete P hot spots in the cyclic reducing sediment sample (top) and the cyclic oxidized sediment sample (bottom). Labeled points indicate where μ-XANES spectra were collected, shown in Figure 3. Note the difference in scale between maps.

The distribution of Fe-bearing particles in these sediments showed far greater variation between samples and appeared to change rapidly and significantly in response to the 90-
minute oxidation event that distinguished the cyclic oxidized sample from the cyclic reducing sample. In the cyclic oxidized sample, Fe and P exhibited a strong spatial correlation, with few points showing variation in Fe counts without covariation in P counts (Table 2; SI Fig. 4). This relationship was much less pronounced in the cyclic reducing sample, where average Fe counts (and thus concentration) were much lower (SI Fig. 4). These observations support the bulk XANES LCF results, which indicated that a larger fraction of P was sorbed to Fe oxyhydroxides in the oxidized sample (69%) than in the reducing sample (62%; Table 1).

**Table 2:** Spearman’s correlation coefficients (ρ) for phosphorus and other elements of interest from 2x2 mm μ-XRF maps collected at 50x50 μm resolution. All correlations are statistically significant except where otherwise noted (p < 0.001; n = 1681)

<table>
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<tr>
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<th>P vs. Fe</th>
<th>P vs. Ca</th>
<th>P vs. Mn</th>
<th>P vs. Al</th>
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<td>-0.09</td>
<td>0.73</td>
</tr>
<tr>
<td>Extended Reducing</td>
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<td>0.85</td>
<td>N.S.</td>
<td>0.78</td>
</tr>
<tr>
<td>Cyclic Reducing</td>
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<td>0.56</td>
<td>0.11</td>
<td>0.46</td>
</tr>
<tr>
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<td>0.69</td>
<td>N.S.</td>
<td>0.62</td>
</tr>
<tr>
<td>LC Stratified</td>
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<td>0.73</td>
<td>0.17</td>
<td>0.63</td>
</tr>
<tr>
<td>LC Post-turnover</td>
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<td>0.67</td>
<td>0.32</td>
<td>0.64</td>
</tr>
<tr>
<td>Shelburne Pond</td>
<td>0.75</td>
<td>0.85</td>
<td>0.23</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Fine-scale μ-XRF maps, collected in regions where P counts were at or near their maximum values in the coarse maps (SI Fig. 2), provided insight into P speciation at the microscale (Fig. 6). Phosphorus K-edge μ-XANES spectra collected at points where P
and Fe co-occurred in the cyclic oxidized sample closely resembled the standard spectrum for phosphate sorbed to ferrihydrite (Fig. 3), containing the pre-edge feature characteristic of phosphate adsorbed on Fe oxides (Khare et al. 2005; Prietzel et al. 2016). These Fe-P enrichments were not found in the cyclic reducing sample, suggesting that they are being reductively dissolved under anoxic conditions, which supports the observed increase in aqueous SRP and Fe and simultaneous decrease in sediment Fe(III)-P, per XANES LCF.

Attempts to collect µ-XANES spectra at various background spots were unsuccessful, as this diffuse P was too dilute to acquire spectra with acceptable resolution. This background P should not be ignored, however, as it makes up the majority of P in these sediments. As such, the bulk XANES signal essentially arises from this background P, because bulk P-XANES probes the average P bonding environment for all P atoms in the sample (Beauchemin et al. 2003; Rivard et al. 2016). This may explain why all of our experimental XANES spectra were so similar to one another, despite significant P exchange between sediment and water and observed differences in P association at the microscale. It follows that some of this diffuse background P may be associated with finely divided Fe oxyhydroxides, as XANES LCF analysis indicated that 38 to 69% of P was sorbed on ferrihydrite or goethite in all experimental samples (Table 1). This is in good agreement with previous work which has demonstrated that 35 to 80% of P in Missisquoi Bay surface sediments is redox-sensitive (Giles et al. 2016; Joung et al. 2017; Schroth et al. 2015; Smith et al. 2011). It is also likely, however, that a significant portion
of this background P is organic P, as Giles et al. (2015) demonstrated that organic P constitutes approximately 18% of P in surface sediments of Missisquoi Bay. The presence (and speciation) of organic P is difficult to determine with bulk P XANES alone, especially in the presence of Fe-P species (Werner and Prietzel, 2015), as spectra for common organic P compounds are relatively featureless (Ajioboye et al. 2007; Kraal et al. 2015a) and, other than the lack of a pre-edge feature, closely resemble the spectrum for phosphate sorbed on ferrihydrite (Rivard et al. 2016). Spectroscopic analysis of DOM and phosphate bound to DOM by Rivard et al. (2016) also resulted in spectra similar to those from our experimental sediment samples, and thus may constitute a portion of the diffuse background P present in all samples.

2.3.3-2 Iron Speciation in Sediment

Linear combination fitting of Fe XANES spectra revealed that, as in the manipulated sediments from the long-term reduction experiment, Fe in these sediments was predominantly present in mixed-valence silicate minerals and Fe oxyhydroxides (SI Table 2). Iron XANES pre-edge analysis, following the procedure outlined in Wilke et al. (2001), indicated that Fe(III) was the dominant Fe oxidation state in all experimental sediment samples (Fig. 4). The centroid position for the cyclic reducing sample, however, was shifted 0.4 eV lower than the initial oxidized sample, indicating a lower Fe$^{3+}$/Fe$_{total}$ ratio in reducing sediments following successive redox oscillations. Additionally, the centroid energy position for the cyclic oxidized sample was shifted ~ 0.7 eV higher than that of the cyclic reducing sample, suggesting that the average Fe oxidation state in surface sediments increased significantly following the 90-min oxidation event that was
administered between collection of these samples. These results are consistent with LCF from bulk P-XANES, which showed a larger fraction of P associated with Fe(III)-oxyhydroxides in the oxidized samples than in the reducing samples and the presence of Fe(II)-phosphate (vivianite) in both reducing samples (Table 1). Integrated pre-edge intensities were similar and consistent with 6-coordination Fe for all experimental samples although the cyclic reducing sample exhibited a slightly greater integrated intensity, indicative of a shift towards less centrosymmetric (tetrahedral) Fe coordination (Wilke et al. 2001). This is corroborated by LCF of the Fe XANES region, which indicated the presence of magnetite (Fe$^{2+}$Fe$^{3+}_2$O$_4$) in the cyclic reducing sample, likely an ultrafine-grained amorphous phase forming from the microbial reduction of Fe oxyhydroxides under anoxic conditions (Lovley et al. 1987; Cummings et al. 2000; Bell et al. 1987).

From a visual standpoint, the XANES spectra for the oxidized samples were virtually indistinguishable from one another, implying similar Fe mineralogy (SI Fig. 3). The spectrum for the cyclic reducing sample exhibited lower white line absorption intensity and a pronounced pre-edge feature characteristic of magnetite (O’Day et al. 2004). Wavelet transform analysis of EXAFS spectra was performed to differentiate heavy and light backscattering atoms in higher coordination shells (Karlsson et al. 2008). In general, WT revealed differences in Fe mineralogy between experimental sediment samples subjected to different redox treatments (Fig. 5). These WT plots share some of the same features with previously published WT plots for Fe in soils (Hesterberg and Morris, 2012;
Karlsson and Persson, 2010; Sundman et al. 2014) and river water samples (Herzog et al. 2017), but also exhibit unique features arising from backscattering (or lack thereof) in high-shell coordination. Many of the features present in the WT plots for goethite, lepidocrocite, and illite were also apparent in the WT plots for the manipulated sediment samples.

In the oxidized samples, the bright colored feature between R = 2 to 2.5 Å and k < 5 Å⁻¹ corresponds to first-shell O (Fig. 5; Morris and Hesterberg, 2012). This feature was absent in the cyclic reducing sample, implying a lack of Fe hydrolysis in this sample. The bright feature in the goethite WT, which corresponds to Fe-Fe scattering paths at distances of 3.03, 3.26, and 3.43 Å (O’Day et al. 2004), was present in three of the four experimental samples and appeared to vary with average Fe oxidation state as indicated by pre-edge centroid positions (Fig. 5; Fig. 4). This feature was most prominent in the cyclic oxidized sample, which showed the largest proportion of Fe oxyhydroxides in Fe XANES LCF and the highest average Fe oxidation state as determined by centroid energy position (SI Table 2; Fig. 4). This Fe-Fe feature was completely absent in the cyclic reducing sample, indicating no Fe in the second shell. This further supports there being very little or no Fe hydrolysis in this sample and could be interpreted as Fe speciation being dominated by monomeric organic complexes (Karlsson et al. 2008; Karlsson and Persson, 2010; Sjöstedt et al. 2013). This explanation seems unlikely, however, as the bright feature depicted in the WT plot is centered at 8 Å⁻¹ and is therefore the result of backscattering from heavier atoms. Furthermore, none of our experimental
samples showed features in the $R = 3$ to $4 \text{ Å}$ and $k = 3 \text{ Å}^{-1}$ range, which have previously been attributed to Fe-C/O multiple scattering from Fe-OM complexes (Fig. 5; Herzog et al. 2017; Karlsson and Persson, 2012). The intensity of this feature is likely highly variable between different Fe-OM complexes, as Karlsson and Persson (2010) demonstrated that it was prominent in WT plots for 50:50 mixtures of goethite and trisoxalatoferric(III) but weak for iron(III) desferrioxamine B. Regardless, the absence of any features between $R = 3$ to $4 \text{ Å}$ and $k < 4 \text{ Å}^{-1}$ for our experimental samples indicates that Fe-OM complexes do not constitute a major fraction of Fe in these sediments (Fig. 5).

Combined analysis of Fe XANES, XANES pre-edge centroids, wavelet transformations, P XANES, and aqueous Fe chemistry provides a comprehensive perspective of the behavior of Fe under short-term redox fluctuations near the SWI. During short (7-day) periods of anoxia, a portion of Fe oxyhydroxides in sediment are slowly reduced, releasing Fe(II) and previously sorbed P into the water column and sediment pore water (Fig. 2). Some of this released Fe(II) re-precipitates as vivianite and possibly other Fe(II) minerals, lowering the average Fe oxidation state in sediment (Fig. 4). Upon oxidation, dissolved Fe rapidly precipitates in surface sediments, replenishing Fe oxyhydroxides, increasing the average Fe oxidation state in sediment (Fig. 4), and removing P from the aqueous phase through adsorption. The rapid shift in surface sediment Fe speciation during oxygenation events (90 minutes) and simultaneous sequestration of P
demonstrates that Fe cycling has a substantial impact on P mobility near the SWI in this system on the order of minutes to hours (Fig. 2).

2.3.4 Natural Systems

The redox manipulation experiments performed in this study demonstrate that internal loading of P from Missisquoi Bay sediments is a redox-sensitive process, and that P release occurs more rapidly under redox fluctuations relative to persistent reducing conditions. To complement these results, it was logical to examine P dynamics in natural settings, where internal loading of P from sediments may manifest differently due to different site-specific characteristics. To this end, three different lake systems, which vary in watershed to lake area ratio, lake depth, and sediment organic content, were chosen.

2.3.4-1 Lake Carmi

Lake Carmi is a dimictic seasonally anoxic eutrophic lake, and as such, was chosen to represent a natural analog of our long-term reduction experiment. The lake was thermally stratified from the onset of our monitoring (June 15) until late September, with mean difference in surface and bottom water temperature ($\Delta T$) = 6.1 °C and mean difference in surface and bottom water dissolved oxygen concentration ($\Delta DO$) = 8.5 mg L$^{-1}$ (SI Fig. 5). High frequency sensor data show that the lake completely mixed around September 21. From September 21 until the sensors were pulled on October 31, mean $\Delta DO = 0.3$ mg L$^{-1}$ and mean $\Delta T = 0.2$ °C. Under the stratified water column, mean concentrations of total dissolved Fe, Mn, and TP in bottom water were 458, 643, and 82 µg L$^{-1}$, respectively (SI Fig. 7). By contrast, mean bottom water concentrations of Fe, Mn, and TP measured on
the three sampling dates following lake mixing were 67, 32, and 36 µg L⁻¹, respectively. For the 12 sampling dates of the 2018 season total dissolved Fe and TP concentrations in bottom water were strongly correlated with $R^2 = 0.99$. Differences observed in DO, Fe, and Mn concentrations near the SWI between the stratification period and following lake turnover are indicative of highly contrasting redox regimes. To investigate the impact of these contrasting redox states on P and Fe species in sediment, sediment cores were collected during peak stratification (stratified sample) and shortly after lake turnover (post-turnover sample) at the dates indicated in SI Fig. 5. Sediment subsamples were sectioned, preserved, and analyzed following the same procedures described above for experimental sediment samples.

Like the Missisquoi Bay sediments, LCF of Fe K-edge XANES spectra suggested that Fe in Lake Carmi sediments was present as mixed-valence silicate minerals and Fe oxyhydroxides (SI Table 2). Wavelet transform analysis of the EXAFS spectra also depicted features similar to those found in the experimental sediments (Fig. 5). Both samples contained the small feature between $R = 2$ to $2.5$ Å and $k < 5$ Å⁻¹ corresponding to first-shell O (Morris and Hesterberg, 2012), and the bright feature between $R = 2.5$ to $3$ Å and $k = 6$ to $10$ Å⁻¹ arising from higher shell Fe-Fe backscattering in Fe-oxides (Karlsson et al. 2008; Sundman et al. 2014). The WT plots for both samples contained most of the same features but the stratified sample showed diminished intensity in the high k-range relative to the post-turnover sample (Fig. 5). This suggests that the stratified sediment sample contained less second shell Fe, which is consistent with XANES LCF
results showing a smaller Fe oxyhydroxide contribution in the stratified sample compared to the post-turnover sample (SI Table 2). Interestingly, the WT for the stratified sample closely resembled the WT for the cyclic oxidized experimental sample from Missisquoi Bay (Fig. 5), implying similar average Fe speciation in these samples. These results, along with LCF of Fe XANES, suggest that oxyhydroxides make up a larger fraction of the Fe in Lake Carmi sediments relative to Missisquoi Bay sediments (SI Table 2). Like the Missisquoi Bay sediments, the WT plots lack the features at R = 3 to 4 Å and k = 3 Å\(^{-1}\), arising from multiple scattering paths in Fe-OM complexes (Herzog et al. 2017; Karlsson and Persson, 2012). Pre-edge analysis revealed that Fe in both samples was predominantly octahedrally-coordinated Fe\(^{3+}\) (Fig. 4). The centroid energy position for the post-turnover sample was shifted 0.1 eV higher than the centroid of the stratified sample, indicating that surface sediments contained more Fe\(^{3+}\) following SWI oxygenation via lake turnover than under stratification (Wilke et al. 2001). This shift in average Fe oxidation state is comparable to that observed in the long-term reduction experiment (Fig. 4). The depletion of Fe oxyhydroxides under SWI anoxia, relative to oxygenated conditions, is also consistent with the results of the long-term reduction experiment and indicates that Fe redox cycling manifests similarly in sediments of Lake Carmi and Missisquoi Bay (SI Table 2).

Bulk P K-edge XANES spectra were very similar to those acquired from Missisquoi Bay sediments, with a slight shift in absorption edge energy characteristic of an increased contribution from Ca-P bonding (Kraal et al. 2017; Fig. 1). Linear combination fitting
indicated that P in these sediments was predominantly associated with Fe(III) and Ca through sorption onto ferrihydrite and goethite and through incorporation into calcium-phosphate minerals or sorption onto calcium carbonate (Table 1). While containing a relatively smaller proportion of P associated with Fe oxyhydroxides, these sediments contained significantly more P sorbed to calcium carbonate than the organic-poor experimental sediments from Missisquoi Bay (Table 1). Chemical extractions for these sediments revealed that the post-turnover sample contained 33% more total P than the stratified sample (2.71 ± 0.03 vs. 2.04 ± 0.03 mg g⁻¹) suggesting that lake mixing had a similar effect on P mobility as the oxidation events implemented in our mesocosm experiments. This deposition of P in surface sediments can likely be attributed to the oxidative precipitation of Fe oxyhydroxides and simultaneous sorption of P—as the fraction of P adsorbed to Fe oxyhydroxides increased from 53% to 57% between the stratified sediments and the post-turnover sediments, per XANES LCF (Table 1).

Following the same procedure described for experimental samples, randomly selected 2x2 mm “coarse maps” were acquired for each sample using µ-XRF (SI Fig. 8). In good agreement with bulk XANES, coarse maps revealed that P speciation was dominated by finely dispersed P that covaried with Fe and Ca under both redox regimes (Table 2). The strong spatial correlation between P and Fe in both samples, along with their respective bulk XANES fits, indicates that Fe sequesters P in these sediments even under prolonged anoxia. These results are consistent with our experimental results as well as recent work by Li et al. (2015), which demonstrated that ferric Fe was a persistent P sink in anoxic
sediments of the Chesapeake Bay. The main difference between μ-XRF maps of Lake Carmi samples was the presence of discrete P enrichments (2-15x background P counts) in the post-turnover sample that were absent in the stratified sample (SI Fig. 6). These P enrichments showed strong covariation with Fe and Mn, suggesting that they were co-precipitating with or sorbing to freshly formed Fe/Mn oxyhydroxides following SWI reoxygenation (SI Fig. 5). This is consistent with P behavior in our redox oscillation experiment and with Fe XANES pre-edge, Fe XANES LCF, and P XANES LCF results, which indicated that the post-turnover sediments contained a larger fraction of Fe(III), Fe oxyhydroxides, and P sorbed to Fe oxyhydroxides, respectively, relative to the stratified sediments.

2.3.4-2 Missisquoi Bay

As a natural follow up to our redox manipulation experiments, P K-edge XANES was performed on a series of freeze-dried surface sediment samples collected from Missisquoi Bay in 2015. These samples span a range of lake conditions and represent distinct geochemical events (i.e. under-ice stratification, spring melt, summer stratification), which may be impacting sediment P speciation in different ways. Bottom water geochemical data and XANES LCF results for these samples are summarized in SI Table 3. In general, the fraction of inorganic P associated with Fe oxyhydroxides was higher in these unaltered sediments (69 to 75%) than in experimentally manipulated sediments from the same site (38 to 69%). This difference is visible in Fig. 1, where the natural sample spectra generally have more intense pre-edge features than the experimental sample spectra and exhibit less spectral broadening on the high-energy side of the
absorption edge due to a smaller contribution from apatite minerals. This difference may be a reflection of our experimental treatments, which consisted of a 47-day reduction and a 25-day reduction interrupted by four 90-minute oxidations, or it may be the result of post-sampling oxidation of sediments prior to lyophilization. In either case, P speciation varied throughout the sampling period and appeared to respond to different watershed events.

The first sediment sample was collected at the onset of under-ice stratification on JD 15 when DO concentrations were declining in bottom water and TP concentrations were at their winter maximum (SI Table 3). Linear combination fitting of XANES spectra indicated that 70% of inorganic P was associated with Fe oxyhydroxides in this sample. Total P content, determined by chemical extraction was 1.73 mg g\(^{-1}\). Following sustained cold and increasing under-ice stratification, the total P content of sediments was similar on JD 48, with TP = 1.62 mg g\(^{-1}\), but the fraction of P associated with Fe oxyhydroxides increased to 75%. This is consistent with the findings of Schroth et al. (2015), which demonstrated that redox-sensitive Fe, Mn and P accumulated in surface sediments at this site under ice throughout the 2015 winter.

By JD 96, at the peak of spring snowmelt, DO concentrations were significantly higher in bottom water relative to the under-ice period. Total phosphorus content in surface sediments was 13% higher than on JD 48 with TP = 1.83 mg g\(^{-1}\). This is likely due to the deposition of watershed-sourced P in surface sediments, as other sampling efforts
indicated that the thaw delivered a large pulse of particulate P to the water column (Joung et al. 2017; Rosenberg and Schroth, 2017). In sediments sampled during this event, 72% of P was associated with Fe oxyhydroxides while 11% was in biogenic apatite, per XANES LCF, indicating a relative enrichment in amorphous Ca-P relative to the previous sample from the under-ice period. On JD 120 during the falling limb of spring snowmelt, the fraction of P associated with Fe oxyhydroxides was 73% while the total P content of sediments fell to 1.48 mg g⁻¹. This decrease in total sediment P is likely due to the depletion of P sorbed on calcium carbonate, as LCF indicated that this sample contained the least CaCO₃-P of all sediment samples in the Missisquoi Bay time series (Table 1). Although it is not clear why this particular sample is depleted in CaCO₃-P, this would explain the increase in the proportion of P sorbed on Fe oxyhydroxides despite the overall decrease in sediment P, relative to the previous sample.

By contrast, during peak stratification on JD 210 when the lowest DO and highest Fe, Mn, and TP concentrations of the season were measured in bottom water, the fraction of P associated with Fe oxyhydroxides fell to 69%. This sample also contained the lowest total P content of any sediment sample from this time series with TP = 1.32 mg g⁻¹. In good agreement with past work at Missisquoi Bay, this implies that the fraction of P associated with Fe oxyhydroxides was being depleted and released to the water column under stratified anoxic conditions (Giles et al. 2016; Smith et al. 2011). Following reoxygenation of the SWI, on JD 230 total P content increased to 1.54 mg g⁻¹ while total dissolved Fe and Mn concentrations in bottom water fell significantly and the fraction of
P associated with Fe oxyhydroxides in sediment rose to 73%. These results support the trends observed in mesocosm experiments and suggest that Fe redox cycling is in fact governing P mobility near the SWI in Missisquoi Bay. This is consistent with work by Joung et al. (2017) demonstrating that molar Fe : P ratios in Missisquoi Bay sediments ranged from 28 to 36, far exceeding the threshold of 15, above which Fe may exert significant control on P mobility (Jensen et al. 1992). Synchrotron-based P K-edge XANES appears to be a useful tool to complement aqueous and solid-phase geochemical data in sediment-water systems, as the fluctuations observed in P speciation generally agreed with the conclusions of previous work at Missisquoi Bay (Giles et al. 2016; Joung et al. 2017; Schroth et al. 2015; Smith et al. 2011).

2.3.4-3 Shelburne Pond

Unlike Missisquoi Bay and Lake Carmi, Shelburne Pond is hypereutrophic, relatively organic-rich, and has previously been shown not to exhibit coupled Fe and P behavior (Joung et al. 2017). In August 2018 duplicate sediment cores were collected from Shelburne Pond under oxic conditions. Linear combination fitting of P XANES spectra indicated that surface sediments at Shelburne Pond contained a relatively smaller fraction of P sorbed to Fe oxyhydroxides and a larger fraction of P sorbed to CaCO₃ than the surface sediments of Lake Carmi or Missisquoi Bay (Table 1). In general, the proportion of P associated with CaCO₃ increased with increasing sediment organic carbon content while the proportion of P associated with Fe oxyhydroxides decreased with increasing organic carbon content. This trend can be seen in the data for Missisquoi Bay (OC% = 4-5%; mean CaCO₃-P = 16%; mean Fe oxyhydroxide-P = 67%), Lake Carmi (OC% =
11.5%; mean CaCO$_3$-P = 33%; mean Fe oxyhydroxide-P = 55%), and Shelburne Pond (OC% = 20-25%; mean CaCO$_3$-P = 48%; mean Fe oxyhydroxide-P = 45%). Linear combination fitting of Fe XANES spectra indicated that Shelburne Pond surface sediments contained Fe-bearing silicates, oxides and sulfides (SI Table 2). Wavelet transformation of the EXAFS spectrum resulted in a distribution of backscattering atoms that resembled a mixture of the WT plots for illite and pyrite (Fig. 5). The WT plot depicted features in the high k-range (8 to 12 Å$^{-1}$) and high R-range (3 to 4 Å) that had significant overlap with features in the WT plot for pyrite and correspond to Fe-Fe backscattering at 3.82 Å in the pyrite structure (O’Day et al. 2004). This sample showed almost no indication of the feature arising from Fe-Fe backscattering in oxyhydroxides at (R = 2.5 to 3 Å; k = 6 to 10 Å$^{-1}$), which was consistent with results from Fe XANES pre-edge analysis showing Fe mineralogy dominated by tetrahedrally-coordinated Fe (Fig. 4).

Pre-edge analysis indicated that Fe in this sample was still predominantly Fe$^{3+}$, however, the integrated intensity of the pre-edge peak suggested that more Fe was in tetrahedral coordination than octahedral (Fig. 4). This further supports these sediments containing a relatively smaller fraction of Fe oxyhydroxides than the sediments of Missisquoi Bay or Lake Carmi, where Fe coordination is dominated by octahedrally coordinated Fe$^{3+}$ (Fig. 4). This is supported by previous work by Joung et al. (2017), which found that only ~5% of Fe in Shelburne Pond sediments was redox-sensitive, compared to ~35% at Missisquoi Bay. Chemical extractions also indicated that these sediments contained less total P than the sediments of Lake Carmi (TP = 1.64 ± 0.03 mg g$^{-1}$). The presence of
sulfide minerals in these sediments, which precipitate due to sulfate reduction under anoxic conditions, likely contributes to the dissolution of Fe oxyhydroxides (Jensen et al. 1995). The resulting aqueous Fe(II) can then be removed from solution and sequestered as FeS or FeS₂, which may diminish the extent to which Fe controls P mobility as these minerals have minimal affinity for PO₄³⁻ (Kraal et al. 2015; Rozan et al. 2002). Furthermore, anoxic conditions also increase regeneration of P from organic matter (Jilbert et al. 2011; Slomp et al. 2002), which in the presence of Fe sulfides could drive enhanced P loading to the water column (Jensen et al. 1995; Rozan et al. 2002). As these sediments are relatively organic-rich (organic carbon content = 20 to 25%; Joung et al. 2017), this could explain the smaller fraction of Fe oxyhydroxides and lower TP content observed relative to the anoxic non-sulfidic sediments of Lake Carmi.

Microprobe analysis confirmed the presence of Fe sulfides in this sample due to the strong spatial correlation between Fe and S in the 2x2 mm μ-XRF map. Phosphorus in these sediments displayed strong covariation with Ca (ρ = 0.85), Al (ρ = 0.85), Si (ρ = 0.85), S (ρ = 0.80), and Fe (ρ = 0.75; Table 2). In general, P distribution in this sediment was much more homogeneous than any sample for which μ-XRF was performed from Missisquoi Bay or Lake Carmi. While discrete spots of Ca-P and Fe-P overlap were identified, the strong correlation with the aluminosilicate background suggests that most P is finely dispersed throughout these sediments rather than enriched in large mineral grains. These results are consistent with those from Joung et al. (2017), which showed that Fe : P ratios in sediment hover around or slightly below 15 – a threshold below
which P mobility is thought to be decoupled from Fe redox cycling in sediment-water systems (Jensen et al. 1992). Furthermore, the observed presence of Fe sulfides in these sediments suggests that Fe oxyhydroxides do not play a major role in P sequestration (Mort et al. 2010). As such, we postulate that internal loading of P in this system may be driven by organic matter remineralization more so than Fe redox cycling, as has recently been shown to be the case in the Chesapeake Bay (Joshi et al. 2015) and the Baltic Sea (Jilbert et al. 2011).

2.4 Implications

Internal loading of P is a pervasive problem with negative consequences for water quality in many freshwater systems; however, phosphorus release from sediment can be governed by very different mechanisms in different lakes (Søndergaard et al. 2003). Here, we demonstrate that P is predominantly associated with Fe oxyhydroxides and calcium-bearing minerals in mineral-rich/organic poor benthic sediments of two shallow eutrophic systems, Missisquoi Bay and Lake Carmi. Despite their geographic proximity (20 km) and similar meteorological drivers, differences in physical lake configurations and sediment P speciation cause internal loading to manifest differently in these two systems. In Missisquoi Bay, our redox manipulation experiments and XAS measurements of Fe and P in sediments provide direct evidence that P mobility is governed by the formation and dissolution of Fe oxyhydroxides near the SWI. Furthermore, these experiments demonstrate that P was released from sediments more rapidly under sequences of fluctuating redox conditions than under persistent anoxia (Shukle et al. 2019). This has important implications for the internal loading of P in shallow systems,
where thermal stratification can quickly set up and break down intermittently throughout the summer months, promoting frequent changes in SWI redox status (Giles et al. 2016; Smith et al. 2011). These episodic redox fluctuations can be expected to drive rapid changes in P mobility and bioavailability, as P gradually released from sediments under anoxia can quickly re-precipitate during sudden SWI oxygenation events such as wind-driven mixing (Giles et al. 2016; Isles et al. 2017; Smith et al. 2011). Our synchrotron-based measurements of P and Fe speciation in manipulated Missisquoi Bay sediments directly demonstrate that Fe oxyhydroxides can precipitate, along with P, in surface sediments on the order of minutes to hours during the transition from anoxic to oxic conditions. As a result, Fe oxyhydroxides may be expected to rapidly switch from a P source to sink as physical mixing oxygenates the SWI.

In the deeper, lower watershed to lake area Lake Carmi, where the SWI is seasonally anoxic and less susceptible to oxygenation via physical mixing outside of spring and fall turnover, P mobility is still predominantly controlled by SWI redox status. As a dimictic lake, however, redox conditions are less dynamic than those observed in the polymictic Missisquoi Bay and P mobility is therefore also less dynamic. Although the same mechanisms of P release and sequestration govern P mobility in these two systems, P behavior is different due to the different physical lake configurations. As a result, internal P loading in Lake Carmi mimics our long-term reduction experiment, with an extended period of gradual P release from sediments while the water column is stratified followed by rapid re-accumulation in sediments during lake turnover. In both systems, XAS
measurements indicate that Fe oxyhydroxides (and sorbed P) are depleted in the end-member reducing sediments relative to the end-member oxidized sediments and aqueous chemistry and chemical extractions corroborate this result. While the same main phases dominate sediment P speciation in both systems, a larger fraction of P is associated with CaCO₃ in Lake Carmi than in Missisquoi Bay, likely due to the much higher sediment organic carbon content.

In the organic-rich Shelburne Pond, lower Fe : P ratios and the presence of Fe sulfides result in P mobility being less tightly coupled to Fe mobility. These sediments contained the most organic carbon, the smallest proportion of P sorbed to Fe oxyhydroxides, and the largest proportion associated with CaCO₃. Here, P mobility may be expected to increase under extended anoxic conditions due to the preferential regeneration of P from organic matter under anoxia (Jilbert et al. 2011; Slomp et al. 2002), however, organic matter mineralization will take place regardless of redox status. Consequently, despite receiving the same meteorological inputs, P dynamics manifest differently in these three systems due to differences in physical, chemical and biological variables including lake configuration, sediment Fe speciation, and organic carbon content. These P dynamics may help explain observed differences in cyanobacteria blooms between these systems. In Missisquoi Bay, blooms are highly dynamic and sensitive to the timing and duration of conditions that promote transient thermal stratification (Isles et al. 2015). In Shelburne Pond, where P availability is less dependent on SWI redox conditions, blooms are more consistent and have been shown to persist under ice (Joung et al. 2017). And in Lake
Carmi, where P accumulates in anoxic bottom waters during prolonged summer stratification, intense blooms have been triggered by late-summer temperature fluctuations that upset and reestablish stratification, bringing bioavailable P into surface waters (VT DEC, 2018).

The results of this study highlight the usefulness of synchrotron-based techniques for constraining P speciation in freshwater lake sediments, as well as the limitations. Using multiple complementary techniques we were able to identify systematic changes in benthic P and Fe speciation in response to changing SWI redox conditions. While changes in fractional P speciation were often small, the consilience of results from multiple XAS techniques and the robustness of our XANES LCF method, as demonstrated by best fits of the suite of P reference compound mixtures analyzed, should lend confidence towards our conclusions. While these results demonstrate the role that inorganic P cycling plays in internal loading in these systems, previous work has highlighted the significance of organic P species in these systems as well (Giles et al. 2015; Joung et al. 2017). Phosphorus K-edge XANES is relatively insensitive to organic P speciation due to the lack of distinguishing spectral features in the XANES spectra for common organic P compounds like phytic acid (Ajioboye et al. 2007; Beauchemin et al. 2003), and therefore may not be the best technique for differentiating organic P cycling from total P cycling. Future studies should look to employ techniques such as $^{31}$P NMR in conjunction with synchrotron-based techniques like P XANES to further discriminate between organic and inorganic P forms in sediment-water systems.
2.5 Supporting Information

SI Figure 1: Bulk P K-edge XANES spectra for each of the nine mixtures of P sorbed to ferrihydrite and CaCO$_3$ as well as spectra for the end member reference compounds, P sorbed to ferrihydrite (100:0) and P sorbed to CaCO$_3$ (0:100). Exact proportions for mixtures along with fitting errors can be found in SI Table 1.
**SI Table 1:** Proportions of P sorbed to ferrihydrite and CaCO$_3$ and associated linear combination fitting results for each of the nine mixtures analyzed with P K-edge XANES. Fitting error is reported as the R-factor and the difference between the actual proportion of P sorbed to ferrihydrite and the LCF-derived proportion is reported as percent error. Spectra and associated fits are presented in SI Figure 1.

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<th>Actual composition</th>
<th>LCF results</th>
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<tr>
<td>Ferrihydrite-P</td>
<td>CaCO$_3$-P</td>
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<tr>
<td>90.0%</td>
<td>10.1%</td>
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<tr>
<td>74.9%</td>
<td>25.1%</td>
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<tr>
<td>59.6%</td>
<td>40.4%</td>
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<td>54.8%</td>
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<td>60.1%</td>
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<tr>
<td>10.0%</td>
<td>90.0%</td>
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</table>
**SI Table 2:** Linear combination fitting results for all normalized Fe XANES spectra from 7100 to 7160 eV and all \( k^3 \) weighted bulk Fe EXAFS spectra from \( k = 2 \) to 10 Å\(^{-1}\). No satisfactory fit could be found for the EXAFS spectrum of the cyclic reducing sample.

<table>
<thead>
<tr>
<th></th>
<th>Fe Silicate Fe</th>
<th>Fe Oxyhydroxides</th>
<th>Fe Sulfides</th>
<th>Magnetite R-factor</th>
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<td><strong>Redox Experiments</strong></td>
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<tr>
<td>Initial Oxidized</td>
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<td>EXAFS 0.48</td>
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<tr>
<td></td>
<td>EXAFS -</td>
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<td>-</td>
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<tr>
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<td><strong>Natural Samples</strong></td>
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<td>EXAFS 0.42</td>
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**SI Figure 2**: Synchrotron µ-XRF maps of the cyclic reducing sediment (top) and the cyclic oxidized sediment (bottom) showing the distribution of P, Fe and Ca in 2x2 mm maps, collected at 50x50 µm step size. The areas shown inside the dashed boxes are where the fine-scale maps were collected, shown in Figure 6.
**SI Figure 3:** Bulk Fe K-edge XANES spectra (black) with LCF best fits (red) for the (a) initial oxidized, (b) cyclic reducing, (c) cyclic oxidized, (d) extended reducing, (e) Lake Carmi stratified, (f) Lake Carmi post-turnover, and (g) Shelburne Pond surface sediment samples.
**SI Figure 4:** Scatterplots of synchrotron μ-XRF data showing the correlations between various elements in experimental sediment samples following 7 days of reducing conditions (blue) and following a 90-minute oxidation (red). Each point represents a pixel in one of the 2x2 mm μ-XRF maps, collected at 50x50 μm step size, shown in SI. Fig. 2.
SI Figure 5: High frequency time series of dissolved oxygen concentrations with depth (in meters below the water’s surface) at our sediment-sampling site in Lake Carmi for part of the 2018 season. Dashed vertical lines indicate dates when sediment cores were collected.
SI Figure 6: Scatterplots of synchrotron µ-XRF data showing the correlations between various elements in Lake Carmi sediment samples under a stratified water column (blue) and following fall lake turnover (red). Each point represents a pixel in one of the 2x2 mm µ-XRF maps collected at 50x50 µm step size, shown in SI. Fig. 8.
SI Figure 7: Biweekly surface and bottom water concentrations of total dissolved Fe, total dissolved Mn, and total P at our sediment sampling site in Lake Carmi. Red boxes highlight the dates where sediment cores were collected. Data courtesy of Vermont DEC.
SI Figure 8: Synchrotron $\mu$-XRF maps of the stratified sediment sample (top) and the post-turnover sediment sample (bottom) from Lake Carmi showing the distribution of P, Fe and Ca in 2x2 mm maps, at 50x50 $\mu$m step size.
SI Figure 9: Scatterplots of synchrotron μ-XRF data from a 2x2 mm coarse map at 50-μm step size showing the correlations between various elements in the Shelburne Pond sediment sample from Aug. 7, 2018.
**SI Table 3**: Bottom water geochemical measurements and total extracted sediment P from surface sediments (0-1 cm) for Missisquoi Bay sampling dates in 2015. Temperature is in °C. DO and sediment TP are expressed in mg g⁻¹ while dissolved Mn and Fe and aqueous TP are expressed in µg L⁻¹.

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<tr>
<th>JD</th>
<th>Temp</th>
<th>pH</th>
<th>DO</th>
<th>Mn</th>
<th>Fe</th>
<th>TPₐq</th>
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<td>1.54</td>
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</table>
2.6 References


COMPREHENSIVE BIBLIOGRAPHY


