Phosphate as a control on schwertmannite stability, iron mineralogy and anaerobic biogeochemistry in acid-sulfate systems

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Phosphate as a control on schwertmannite stability, iron mineralogy and anaerobic biogeochemistry in acid-sulfate systems

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November 2018
I certify that the work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and that the material has not been submitted, either in whole or in part, for a degree at this or any other university.

I acknowledge that I have read and understood the University's rules, requirements, procedures and policy relating to my higher degree research award and to my thesis. I certify that I have complied with the rules, requirements, procedures and policy of the University (as they may be from time to time).

Signed ……

………… Date ...07 November 2018…
Abstract

Schwertmannite is a common iron (Fe) mineral in acid sulfate soils and acid mine drainage systems, where it plays an important role in controlling acidity dynamics, iron and sulfur biogeochemistry, and trace metal mobility. Phosphate (PO$_4^{3-}$) is commonly found in schwertmannite-bearing systems (i.e. from runoff or internal phosphorus cycling) and readily sorbs with schwertmannite.

The overarching objectives of this thesis were 1) to examine the effects of PO$_4^{3-}$ on schwertmannite stability and its transformation to secondary iron minerals under different environmental scenarios, and 2) to determine the fate of PO$_4^{3-}$ as schwertmannite undergoes mineralogical and biogeochemical transformations.

To address these objectives, schwertmannite was dosed with varying PO$_4^{3-}$ loadings and exposed to (1) a natural assemblage of microorganisms to examine schwertmannite stability under acidic Fe(III)-reducing conditions, (2) strongly reducing conditions to assess schwertmannite stability and mineralogical transformations in the presence of microbial Fe(III)- and sulfate (SO$_4^{2-}$)-reduction, and (3) abiotic Fe(II)-rich conditions which drive the Fe(II)-accelerated transformation of schwertmannite. In these experiments, aqueous- and solid-phase properties were monitored using standard analytical techniques and selective extractions, with Fe solid-phase speciation evaluated using x-ray diffraction, Mössbauer spectroscopy and synchrotron x-ray absorption spectroscopy.

Phosphate stabilized schwertmannite under acidic, Fe(III)-reducing conditions, with the most microbial Fe(III)-reduction occurring at intermediate PO$_4^{3-}$ loadings. This discovery reflects a balance between the rapid transformation of schwertmannite to less bioavailable goethite in the absence of PO$_4^{3-}$, and PO$_4^{3-}$ stabilization of schwertmannite against microbial Fe(III)-reduction at higher PO$_4^{3-}$ loadings.

Following extensive Fe- and SO$_4^{2-}$ -reduction (i.e. the second study), secondary mineral precipitation was dictated by PO$_4^{3-}$ loading. The presence of PO$_4^{3-}$ facilitated formation of sulfate green rust. The formation of mackinawite, a product of microbial SO$_4^{2-}$ -reduction, decreased at higher loadings of PO$_4^{3-}$ due to PO$_4^{3-}$ stabilizing schwertmannite against transformation to goethite,
Abstract

which thereby causes microbial Fe(III)-reduction to outcompete microbial SO$_4^{2-}$-reduction. Vivianite became increasing important as PO$_4^{3-}$ loading increased.

In the abiotic, Fe(II) rich schwertmannite system, sorption of PO$_4^{3-}$ triggered rapid partial transformation of schwertmannite to a micro-crystalline Fe(III) oxyhydroxide. Following Fe(II) addition, PO$_4^{3-}$ loading determined the extent of more crystalline mineral formation (i.e. lepidocrocite and goethite), with PO$_4^{3-}$ inhibiting schwertmannite transformation. This experiment shows, for the first time, that PO$_4^{3-}$ has contrasting effects on schwertmannite stability, whereby it both induces and inhibits schwertmannite transformation depending on environmental conditions. Under all three experimental settings, PO$_4^{3-}$ remained bound to the solid-phase, reflecting its strong affinity for schwertmannite.
Acknowledgements

This PhD has been a journey. A journey not only across the world, but in understanding myself, new cultures, and a bit more about the scientific method. This particular journey could not have been carried out alone. Throughout it, I relied on several people for their unwavering support. In particular, both my family and Beth, who have supported me more than they probably realize. Without any of them, this journey would have been left incomplete.

I had to leave my home and my friends in order to do this. Although I acknowledge it was difficult for each one of them, in their own ways, to let me leave the country for an indeterminate amount of time, they each allowed me that freedom, and for that, I am grateful.

I want to thank my supervisors, who also supported me throughout the process. Ed, who let me explore concepts and never doubted me, even when I doubted myself. And Scott and Peter, always there to lend a hand and push me further along. The rest of GeoScience- you all created an environment where I wanted to be each day. Always a welcoming “hello” and a smile. Always a random story from Mark to brighten my day.

And Project Oceanology, where my passion for chemistry all began. Even though I never listened when I was hassled to branch out from chemistry to study lobsters, I was supported all the same. And the same goes for the Connecticut State Science Fair- always supported and challenged by judges and teachers, pushing me to learn more and develop an understanding for the scientific method at a very young age. The support, reassurance, challenges, and cheerleading I received during those years helped solidify this love for chemistry, which turned into so much more than a simple science fair project.

One quote in particular drove me through my travels- it kept me going when the times were tough, from the moment I started to the day I finished. A quote summarized by my Grandpa the night before I left to travel the world:

If ever there is a tomorrow when we’re not together,
there is something you must always remember.
You are braver than you believe, stronger than you seem,
and smarter than you think.
But the most important thing is, even if we are apart,
I’ll always be with you.

-Winnie the Pooh
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## Abbreviations

### General

<table>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>Acid mine drainage</td>
<td>AMD</td>
</tr>
<tr>
<td>Acid sulfate soils</td>
<td>ASS</td>
</tr>
<tr>
<td>Acid sulfate waters</td>
<td>ASW</td>
</tr>
<tr>
<td>Acid volatile sulfide</td>
<td>AVS</td>
</tr>
<tr>
<td>Celsius</td>
<td>C</td>
</tr>
<tr>
<td>Extended x-ray absorption fine structure</td>
<td>EXAFS</td>
</tr>
<tr>
<td>Grams</td>
<td>g</td>
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<tr>
<td>Inductively coupled plasma mass spectrophotometer</td>
<td>ICP-MS</td>
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<tr>
<td>Linear combination fit</td>
<td>LCF</td>
</tr>
<tr>
<td>Liters</td>
<td>L</td>
</tr>
<tr>
<td>Meters</td>
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</tr>
<tr>
<td>Micrometers</td>
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</tr>
<tr>
<td>Milliliters</td>
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</tr>
<tr>
<td>Most probable number</td>
<td>MPN</td>
</tr>
<tr>
<td>National Synchrotron Radiation Research Center</td>
<td>NSRRC</td>
</tr>
<tr>
<td>Weight/weight</td>
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<tr>
<td>X-ray absorbance near edge structure</td>
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<td>X-ray diffractometry</td>
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### Chemical formulas

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<tr>
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<tr>
<td>Bicarbonate</td>
<td>HCO₃⁻</td>
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<tr>
<td>Carbon</td>
<td>C</td>
</tr>
<tr>
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<td>Co</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>H⁺</td>
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<tr>
<td>Hydroxide</td>
<td>OH⁻</td>
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<tr>
<td>Iron</td>
<td>Fe</td>
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<tr>
<td>Phosphate</td>
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<tr>
<td>Sulfate</td>
<td>SO₄²⁻</td>
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### Minerals

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<tr>
<td>Akaganeite</td>
<td>$\beta$-FeOOH</td>
</tr>
<tr>
<td>Ferrihydrite</td>
<td>Fe$_2$O$_3$</td>
</tr>
<tr>
<td>Goethite</td>
<td>$\alpha$-FeOOH</td>
</tr>
<tr>
<td>Lepidocrocite</td>
<td>$\gamma$-FeOOH</td>
</tr>
<tr>
<td>Mackinawite</td>
<td>FeS</td>
</tr>
<tr>
<td>Pyrite</td>
<td>FeS$_2$</td>
</tr>
<tr>
<td>Schwertmannite</td>
<td>Fe$_8$O$<em>8$(OH)$</em>{8-2x}$(SO$_4$)$_x$</td>
</tr>
<tr>
<td>Siderite</td>
<td>FeCO$_3$</td>
</tr>
<tr>
<td>Strengite</td>
<td>FePO$_4$</td>
</tr>
<tr>
<td>Sulfate green rust</td>
<td>$[\text{Fe}^{2+}\text{Fe}^{3+}(\text{OH})^+][\text{SO}_4^{2-} * 2\text{H}_2\text{O}]^{2-}$</td>
</tr>
<tr>
<td>Vivianite</td>
<td>Fe$_3$(PO$_4$)$_2$</td>
</tr>
</tbody>
</table>
Chapter 1 - Introduction

1.1 General Introduction

The iron (Fe) oxyhydroxysulfate mineral, schwertmannite, is a common Fe(III) mineral in and around acidic mine drainage systems and acid sulfate soils (España, 2007; Fitzpatrick et al., 2017a, 2017b; Gagliano et al., 2004; Jönsson et al., 2005). Schwertmannite is a yellowish-orange, poorly crystalline mineral with a high surface area that can readily sorb nutrients and contaminants (Bigham et al., 1990; Regenspurg and Peiffer, 2005). One such nutrient, ubiquitous in natural schwertmannite containing environments, is phosphate (PO$_4^{3-}$) (Collins et al., 2010; Fitzpatrick et al., 2017a).

Phosphate can be a limiting nutrient or pollutant, depending on the aqueous concentration (Hart et al., 2004). As a nutrient, PO$_4^{3-}$ is essential for plant and microbial life (Bachmann, 1980). However, excess aqueous PO$_4^{3-}$ will often cause eutrophication in freshwater systems (Correll, 1998) and can also strongly influence estuarine and salt water system microbial productivity (Rozan et al., 2002; Sundareshwar et al., 2003).

Although schwertmannite and PO$_4^{3-}$ are known to co-exist in the natural environment (Collins et al., 2010; Fitzpatrick et al., 2017a), little work has documented their interactions (Khamphila et al., 2017). Previous work supports that schwertmannite has a high sorption capacity for PO$_4^{3-}$, both within and around its tunnel-like structure (Eskandarpour et al., 2006). Additional studies on schwertmannite and sorbed compounds other than phosphate (i.e. arsenate, silica, or copper) suggest an increased stability of schwertmannite in the presence of these compounds (Antelo et al., 2013a; Burton and Johnston, 2012; HoungAloune et al., 2015; Paikaray et al., 2012). Stabilization in the presence of sorbed compounds could explain how the original schwertmannite is maintained even under the extensive reducing conditions that are often found in wetlands (Collins et al., 2010; Fitzpatrick et al., 2017a; Regenspurg et al., 2004).

In support of potential schwertmannite surface stabilization by PO$_4^{3-}$, interactions between PO$_4^{3-}$ and the hydrous ferric oxide ferrihydrite have been investigated (Borch et al., 2007; Borch and Fendorf, 2008), showing initial PO$_4^{3-}$-induced stabilization of ferrihydrite under reducing conditions, but also the precipitation of several secondary minerals and near-complete PO$_4^{3-}$
Phosphate as a control on schwertmannite stability, iron mineralogy and anaerobic biogeochemistry in acid-sulfate systems

retention throughout the reduction process. Although these studies of the ferric oxyhydroxide ferrihydrite provide some indirect insight into the biogeochemical and mineralogical transformation processes of schwertmannite, they do not investigate schwertmannite itself, one of the most common minerals associated with acid sulfate waters which has a strong impact on the mobility of nutrients and contaminants (Bigham et al., 1990; Fitzpatrick et al., 2017a, 2017b; Jönsson et al., 2005; Regenspurg and Peiffer, 2005).

Microbial schwertmannite reduction can raise the pH from ~3.0 to circumneutral, making the once acidic environment more hospitable for biota (Peine et al., 2000). In addition, this pH increase can facilitate the precipitation of toxic metals and metalloids (Borch et al., 2010), further enhancing the suitability of the environment for biota. However, schwertmannite stability, induced by anion sorption, retards reduction and the consequent pH increase (Regenspurg et al., 2004). Consequently, schwertmannite stability and schwertmannite transformation have vastly different end results and implications with regard to acid sulfate waters. Although PO$_4^{3-}$ and schwertmannite dynamics can be approximated with evidence from other anions and minerals, the exact mechanisms and interactions of schwertmannite and PO$_4^{3-}$ under a variety of environmental conditions are unknown. Furthermore, the mobility or sequestration of aqueous PO$_4^{3-}$ under schwertmannite evolution is unknown, and this could have major implications for the eutrophication dynamics of acid sulfate waters.

Phosphate can co-occur with schwertmannite as a result of internal phosphorus cycling in acid sulfate waters or nutrient rich runoff to acidic lowlands. Furthermore, several studies have suggested PO$_4^{3-}$ could be used to artificially induce and control eutrophication events in acid sulfate systems in an attempt to introduce reducing conditions (Fyson et al., 1998; Kalin et al., 2006b) resulting in PO$_4^{3-}$-rich, schwertmannite-based sediments, where the interactions between the two would be magnified. However, the effects of PO$_4^{3-}$ on schwertmannite dynamics, as well as the effects of schwertmannite transformation on aqueous PO$_4^{3-}$ dynamics have not yet been assessed. As a result of this need for information, the following studies are aimed to increase our process-based understanding of the interactions between schwertmannite and PO$_4^{3-}$ in regards to schwertmannite stability and mineralogical transformation, as well as the effects on aqueous PO$_4^{3-}$ behavior.
1.2 Thesis Scope
This work examines the dynamic aqueous and mineralogical interactions between phosphate and schwertmannite under varying environmentally relevant conditions such as are found in many mining-impacted lakes or acid sulfate waters. It investigates PO$_4^{3-}$ as a control on schwertmannite stability, anaerobic biogeochemistry, and the Fe mineralogy resulting from schwertmannite transformations.

Specific objectives include:

- Examining the occurrence of schwertmannite stability under acidic reducing conditions and varying PO$_4^{3-}$ loadings.
- Exploring the secondary mineral evolution following extensive schwertmannite –Fe and –SO$_4^{2-}$ reduction under these same PO$_4^{3-}$ loadings.
- Investigating 1) the short-term effects of PO$_4^{3-}$ loading on schwertmannite transformation or stability, as well as 2) the effects of PO$_4^{3-}$ on the Fe(II)-induced transformation of schwertmannite and 3) the associated mineral products of both reactions.
- Assessing the release or sequestration of PO$_4^{3-}$ under all tested environmental scenarios.

1.3 Thesis structure
The thesis is comprised of a literature review and three publication outputs stemming from the work conducted during this research program, as well as a conclusion. Chapters 3, 4 and 5 are peer-reviewed, published manuscripts. As such, each chapter is an independent entity and contains relevant methods, data, and references to support the discussion within.

A cover page is included at the beginning of each chapter to detail study objectives and justifications.

The following is a brief outline of each chapter.
Chapter 1. Introduction
This chapter outlines the aims and motivation for the research conducted within.

Chapter 2. Background
This chapter gives an overview on acid sulfate waters, including background on schwertmannite and phosphate. Additionally, information about the existing knowledge on schwertmannite under reducing conditions is included.

Chapter 3. Phosphate-imposed constraints on schwertmannite stability under reducing conditions.
In some environments, extensive Fe(III) reduction does not occur due to microbial community structure or other non-ideal environmental conditions, resulting in the continuity of acidic sediments. As such, this chapter describes the effect of phosphate on schwertmannite stability under medium term (41 day) acidic reducing conditions.


Chapter 4. Phosphate loading alters schwertmannite transformation rates and pathways during microbial reduction.
When Fe(III) reduction does proceed to its fullest extent, the pH of the sediment can increase to circumneutral and sulfate reduction is favored. A larger variety of secondary minerals can then precipitate. This chapter largely examines the mineralogical products of extensive schwertmannite -Fe and – SO$_4^{2-}$ reduction after 82 days following phosphate addition.

Chapter 5. Contrasting effects of phosphate on the rapid transformation of schwertmannite to Fe(III) (oxy)hydroxides at near-neutral pH

This chapter explores the effects of PO$_4^{3-}$ on short term (10 day) schwertmannite transformations. Schwertmannite was exposed to phosphate alone, and subsequently phosphate and Fe(II) to prompt the Fe(II) induced transformation, which is known to rapidly transform poorly ordered ferric oxyhydroxides to more stable iron oxides.


Chapter 6. Conclusions

This chapter summarizes the individual findings and outlines key conclusions of this research. Four broad conclusions are drawn as a summary of the three studies and future work is suggested.

Appendix 1. Author contribution statements

The contributions of each author are outlined.

Appendix 2. Published articles

Citations for each published article are included.
Chapter 2 - Literature Review

2.1 Acid sulfate waters

2.1.1 Definition

Acid sulfate waters (ASW) are broadly classified as waters influenced by the oxidation of sulfide minerals including mackinawite (FeS) and pyrite (FeS$_2$) (Bigham and Nordstrom, 2000a; Burton et al., 2006b; Johnson and Hallberg, 2005). Acid sulfate waters are found globally (Fanning et al., 2017), however are most common in mining localities (Blodau, 2006a; Johnson and Hallberg, 2005) or adjacent to the coastline as a result of historic saltwater-sulfate deposition, reduction, and precipitation (Fitzpatrick, 2003). The sulfur oxidation process can be a purely natural process, such as a result of drought (Fitzpatrick et al., 2017b), but is often greatly accelerated and intensified by human activities such as wetland drainage, mining, or land development (Akcil and Koldas, 2006; Bigham and Nordstrom, 2000a; Singh et al., 1997).

2.1.2 Acid Mine Drainage (AMD)

Lignite mine effluent is the most common source of AMD, although mining of metals and metalloids such as iron (Fe) and arsenic can also produce acidic drainage (Blodau, 2006a; Burton et al., 2006b; Johnson and Hallberg, 2005). Lignite is a reduced inorganic sulfur-bearing coal material formed from the historic compaction of peat (Blodau, 2006a; Johnson and Hallberg, 2005; Küsel, 2003). Coal is mined around the world, and exposure and oxidation of the contained reduced sulfur species occur regularly during the mining process (Singh et al., 1997). In coal and metal mining practices, only ~2% of the excavated material is retained as the desired ore (Bigham and Nordstrom, 2000a), while the residue is discarded as mine tailings (Küsel, 2003). When buried and in their reduced form, these minerals are usually harmless. However when excavated and exposed to the atmosphere or to oxygenated water, rapid oxidation of the reduced inorganic sulfur species can occur (Singh et al., 1997).

The mining process can create excavated pits and/or waste heaps that can form abandoned mine lakes and influence the chemistry of percolating water. In these locations, the generated acidity from sulfide oxidation is greater than the buffering capacity of the sediment. The result is a characteristic pH around 3.0 (Akcil and Koldas, 2006; Blodau, 2004; Johnson and Hallberg, 2005;
Küsel, 2003). At this low pH, many toxic metals and metalloids such as arsenic, copper, chromium and cadmium dissolve (Johnson and Hallberg, 2005; Küsel, 2003; Singh et al., 1997). These conditions often last long after the mine is abandoned (Akcil and Koldas, 2006; Blodau, 2006a; Johnson and Hallberg, 2005; Peretyazhko et al., 2009), stretching the impact of a mine far beyond its physical and temporal footprint.

2.1.3 **Acid Sulfate Soils (ASS)**

In addition to AMD, an alternate source of ASW are waters originating from acid sulfate soils (ASS). Acid sulfate soils are most commonly historic organic-rich wetlands in Fe-laden sediments (Powell and Martens, 2005). During the Holocene Epoch, sea levels rose and SO$_4^{2-}$-rich sea water flooded these wetlands (Powell and Martens, 2005). Oxygen is rapidly depleted in organic-rich submerged soils, facilitating microbial reduction of Fe(III) minerals and SO$_4^{2-}$. Reduced Fe(II) and sulfide can precipitate as FeS$_x$ minerals and persist when the soils are undisturbed.

Much like in AMD scenarios, the reduced species are potentially hazardous and become problematic upon oxidation. Oxidation can occur as a result of soil dredging, anthropogenic land development, or any form of wetland disturbance including hydrological disturbance (Burton et al., 2006b; Fitzpatrick et al., 2017b; Knorr and Blodau, 2007a; Simpson et al., 2008), and result in the release of acidity, metals and nutrients (Simpson et al., 2008). Although the soil formation processes differ, AMD and ASS chemistry and mineralogical evolution behave similarly.

In both AMD and ASS systems, Fe, carbon (C), and S cycling are intricately linked to the pH and buffering capacity of the sediment through microbial redox cycling and mineral precipitation (Blodau, 2006a, 2004; Peretyazhko et al., 2009). Iron minerals include a variety of Fe oxides (e.g. goethite and ferrihydrite), Fe(III) sulfates (e.g. schwertmannite), Fe(II) sulfides (e.g. pyrite and mackinawite), and Fe(II) carbonates (e.g. siderite). Consequently, while each system is unique, the biogeochemical cycling is controlled by redox-dependent C-Fe-S dynamics.

2.1.4 **Pyrite oxidation process**

The process of ASW generation occurs as a combination of abiotic and biotic reactions irrespective of the origin of the water (Bigham and Nordstrom,
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2.1.5 Redox changes and mackinawite formation- completing the cycle

Acid sulfate waters can be exposed to reducing conditions if or when the original sediment oxidizing event dissipates (Fitzpatrick et al., 2009). Microbial Fe(III) oxide reduction (Eq. 2.4) (Blodau, 2006a) occurs before...

2Fe$_{2+}$ + $\frac{1}{2}$ O$_2$ + 2H$^+$ $\rightarrow$ 2Fe$^{3+}$ + 2H$_2$O  

Eq. 2.2

This oxidation spurs a catalytic propagation cycle, where the newly produced Fe(II) can undergo microbial (re)oxidation (Eq. 2.2) (Johnson and Hallberg, 2005; Küsel, 2003; Silverman, 1967; Singh et al., 1997; Taylor et al., 1984). The oxidation of pyrite, subsequent precipitation of Fe(III) oxide species, and the subsequently lowered pH proceeds at a progressively faster rate with the increasing abundance of Fe(III) (Küsel, 2003). Consequently, acid mine lakes and ASS are some of the world’s most acidic environments (Dent and Pons, 1995).

FeS$_2$ + 14Fe$^{3+}$ + 8H$_2$O $\rightarrow$ 15Fe$^{2+}$ + 2SO$_4^{2-}$ + 16H$^+$  

Eq. 2.3

The Fe(III) produced during this step can precipitate as Fe(III) oxides and/or react with the pyrite surface in an important abiotic oxidation step (Knorr and Blodau, 2007a) that produces Fe(II), SO$_4^{2-}$ and H$^+$ (Eq. 2.3).

2Fe$^{2+}$ + $\frac{1}{2}$ O$_2$ + 2H$^+$ $\rightarrow$ 2Fe$^{3+}$ + 2H$_2$O

Eq. 2.2

Bacterial oxidation of Fe(II) can occur, resulting in aqueous Fe(III), which is soluble in the acidic conditions (Eq. 2.2) (Singh et al., 1997). The most well-known pyrite-oxidizing bacteria (Thiobacillus ferrooxidans) thrives under pH 1.0-3.5 conditions (Bigham and Nordstrom, 2000a; Knorr and Blodau, 2007a; Küsel, 2003; Nordstrom, 1982; Singh et al., 1997). This bacterial oxidation step is often the rate limiting step in pyrite oxidation (Küsel, 2003), although the pH, temperature, oxygen content, and surface area of the FeS$_x$ species also play a role in controlling reaction rates (Akcil and Koldas, 2006; Knorr and Blodau, 2007a).

FeS$_2$ + 3/5 O$_2$ + H$_2$O $\rightarrow$ Fe$^{2+}$ + 2SO$_4^{2-}$ + 2H$^+$  

Eq. 2.1

Initially, pyrite is exposed to molecular oxygen either through exposure to the atmosphere, such as during a drought, or to oxygenated water (Fitzpatrick et al., 2017b; Knorr and Blodau, 2007a). Pyrite oxidizes to form ferrous Fe(II), sulfate (SO$_4^{2-}$) and hydrogen (H$^+$) ions (Blodau, 2004) (Eq. 2.1).

FeS$_2$ + 3/5 O$_2$ + H$_2$O $\rightarrow$ Fe$^{2+}$ + 2SO$_4^{2-}$ + 2H$^+$  

Eq. 2.1

This oxidation spurs a catalytic propagation cycle, where the newly produced Fe(II) can undergo microbial (re)oxidation (Eq. 2.2) (Johnson and Hallberg, 2005; Küsel, 2003; Silverman, 1967; Singh et al., 1997; Taylor et al., 1984). The oxidation of pyrite, subsequent precipitation of Fe(III) oxide species, and the subsequently lowered pH proceeds at a progressively faster rate with the increasing abundance of Fe(III) (Küsel, 2003). Consequently, acid mine lakes and ASS are some of the world’s most acidic environments (Dent and Pons, 1995).
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\[ \text{SO}_4^{2-} \text{ reduction due to a greater yield of free energy (Megenigal and Neubauer, 2009).} \]

\[ \text{Fe(OH)}_3 + \frac{1}{4} \text{CH}_2\text{O} + 2\text{H}^+ \rightarrow \frac{1}{4} \text{CO}_2 + \text{Fe}^{2+} + 11/4 \text{H}_2\text{O} \quad \text{Eq. 2.4} \]

One consequence of Fe(III) reduction is an increased pH due to the consumption of H\(^+\). Sulfate reducers thrive under the slightly higher pH as compared to low pH (Peine et al., 2000), and therefore, the progression to \(\text{SO}_4^{2-}\) reduction becomes favorable (Eq. 2.5).

\[ 2 \text{SO}_4^{2-} + \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{H}_2\text{S} + 6\text{CO}_2 + 2\text{H}_2\text{O} + 4\text{H}^+ \quad \text{Eq. 2.5} \]

Fe(II) has a high affinity for sulfides, leading to the rapid precipitation of Fe(II) monosulfides such as mackinawite in the anoxic waters (FeS, Eq. 2.6) (Rickard and Luther, 2007).

\[ \text{Fe}^{2+} + \text{H}_2\text{S} \rightarrow \text{FeS} + 2\text{H}^+ \quad \text{Eq. 2.6} \]

Mackinawite is a black, X-ray amorphous Fe(II) sulfide mineral commonly found in reduced AMD or ASS environments (Rickard and Luther, 2007). Mackinawite can be replaced by end member pyrite (FeS\(_2\)) through the dissolution of FeS and the reaction with additional H\(_2\)S (Burton et al., 2006a; Kraal et al., 2013). Pyrite is the principle long-term sink for Fe and sulfur minerals under anoxic conditions (Hines et al., 1989). Many metals, metalloids and nutrients can be associated with the FeS\(_x\) mineral structures (Burton et al., 2013; Morse et al., 1987; Niazi and Burton, 2016a), and may be sequestered or alternatively released during FeS\(_x\) mineral dissolution, as the low pH of the environment renders many of these compounds soluble (Küsel, 2003; Ljung et al., 2009; Singh et al., 1997; Wong et al., 2010). Toxic metals have consequently been observed in very high concentrations in some ASW (Burton et al., 2006a; Johnston et al., 2010). These waters are not often mitigated (Akcil and Koldas, 2006), and the toxic and acidic overland flow can result in large scale fish kills or land scalds (Akcil and Koldas, 2006; Ljung et al., 2009; Powell and Martens, 2005).

### 2.2 Schwertmannite characteristics and behavior

#### 2.2.1 Schwertmannite discovery and features

Often, the first mineral to precipitate under conditions created by bacterial pyrite oxidation is schwertmannite, a ferric oxyhydroxysulfate mineral with the
idealized formula $\text{Fe}_8\text{O}_8(\text{OH})_8(\text{SO}_4)_x \cdot n\text{H}_2\text{O}$, where $1<x<1.75$ (Bigham et al., 1996; Bigham and Nordstrom, 2000a; Jönsson et al., 2005). Schwertmannite is an abundant yellowish-orange mineral in AMD and ASS environments (Bigham et al., 1996; Fitzpatrick et al., 2017a; Regenspurg and Peiffer, 2005). Schwertmannite precipitation occurs most readily between pH 2.8 and 4.0 (Bigham et al., 1996). Its precipitation controls the concentration of aqueous $\text{SO}_4^{2-}$ (Singh et al., 1997) and consumes $\text{H}^+$, thereby raising and modulating pH (Peine et al., 2000), which can control contaminant and nutrient mobility (Bigham et al., 1990; Regenspurg and Peiffer, 2005). Schwertmannite dissolution and equilibration with porewaters often will buffer the pH to ~3.0 (Knorr and Blodau, 2007a; Regenspurg, 2003).

Despite the abundance of schwertmannite in ASW, schwertmannite was only recently identified as a distinct mineral (Bigham et al., 1994, 1990). Before this, it was identified simply as an amorphous solid (Brady, 1986) and as the mine drainage mineral (AMD Mineral) by Bigham et al. (1992). This is in part due to its metastable nature with regard to solution pH, dissolved ions and temperature (Bigham et al., 1996; Jönsson et al., 2005, 2006). However, a major contributing factor in its late identification and accurate characterization is its poorly crystalline and X-ray amorphous nature (Bigham et al., 1994; Regenspurg and Peiffer, 2005), as well as the uncertainties in the $\text{SO}_4^{2-}$ coordination behavior (Fernandez-Martinez et al., 2010). This relates largely to its variable $\text{SO}_4^{2-}$ content, characteristic of the mineral (Regenspurg and Peiffer, 2005).

Bigham et al., (1990) synthesized a ferric oxyhydroxysulfate mineral with unit cell dimensions of $a_0 = 1.065$ and $c_0 = 0.604$, which was isostructural to the mineral akaganeite, both hosting a tunnel structure. Bigham et al. (1996, 1994) identified this synthesized ferric oxyhydroxysulfate mineral to be the mineral schwertmannite. Rather than chloride inside the tunnel structure, however, schwertmannite contains tetragonal $\text{SO}_4^{2-}$ (Bigham et al., 1996). The tunnel $\text{SO}_4^{2-}$ is thought to exchange with other similarly sized and shaped anions such as arsenate and chromate (Regenspurg, 2003). Schwertmannite additionally contains up to 30% of its $\text{SO}_4^{2-}$ as surface bound $\text{SO}_4^{2-}$, which can also readily dissolve or exchange with other anions (Bigham et al., 1996; Regenspurg, 2003; Regenspurg et al., 2002).
Schwertmannite has a large surface area, upwards of 220 m$^2$ g$^{-1}$ (Regenspurjg and Peiffer, 2005), and is typically arranged in a pincushion surface morphology (Bigham et al., 1994). The pincushion formation and its positive surface charge allows for great anion sorption on its surface (e.g. arsenate, chromate, and phosphate), which has major implications for contaminant and nutrient dynamics (Antelo et al., 2012; Bigham et al., 1996; Fernandez-Martinez et al., 2010; Fitzpatrick et al., 2017a; Jönsson et al., 2005; Khamphila et al., 2017; Regenspurjg and Peiffer, 2005).

2.2.2 Schwertmannite metastability

Schwertmannite is metastable with respect to the more crystalline Fe(III) oxide goethite (Bigham et al., 1996; Regenspurjg and Peiffer, 2005).

\[
\text{Fe}_8\text{O}_8(\text{OH})_8\text{SO}_4 + 2\text{H}_2\text{O} \rightarrow 8\text{FeOOH} + \text{SO}_4^{2-} + 2\text{H}^+ \quad \text{Eq. 2.7}
\]

The transformation of schwertmannite to goethite by a dissolution-precipitation reaction requires months to years for completion under Fe(II)-poor conditions (Bigham et al., 1994; Burton et al., 2008b; Knorr and Blodau, 2007a). Low pH and temperatures additionally inhibit transformation, with pH exerting the greatest control over this reaction (Boland et al., 2014; Burton et al., 2008b; Liu et al., 2007; Paikaray et al., 2017a; Paikaray and Peiffer, 2014; Pedersen et al., 2005; Schwertmann and Taylor, 1972). The extent of the transformation can be documented either through the progressive formation of goethite or the release of SO$_4^{2-}$ from the schwertmannite structure (Collins et al., 2010; Paikaray and Peiffer, 2014; Regenspurjg et al., 2004).

Low pH (Bigham et al., 1996; Collins et al., 2010; Jönsson et al., 2005; Knorr and Blodau, 2007a) and excess SO$_4^{2-}$ (Jönsson et al., 2005; Knorr and Blodau, 2007a) stabilize schwertmannite and thereby inhibit transformation to goethite. Additional compounds, such as are common in natural schwertmannite-containing environments (Collins et al., 2010), also help retain the original schwertmannite. For example, dissolved organic carbon (Collins et al., 2010; Knorr and Blodau, 2007a) and arsenate (Peine et al., 2000; Regenspurjg et al., 2002) lower the ability of schwertmannite to dissolve to its elemental components, thereby retaining its original structure.
2.2.3 Fe(II) induced transformation of Fe oxides

2.2.3.1 Reaction pathway

Similar to the transformation of other Fe(III) oxides (such as ferrihydrite) to more crystalline Fe(III) oxide species, the conversion of schwertmannite into goethite is accelerated under high Fe(II) content and circumneutral pH (Boland et al., 2014; Burton et al., 2008b; Liu et al., 2007; Paikaray et al., 2017a; Paikaray and Peiffer, 2014; Pedersen et al., 2005).

Boland et al. (2014) discuss the mechanism of transformation in a ferrihydrite system, which is similar to a schwertmannite system, as they are both poorly ordered Fe(III) phases. The authors establish a reaction pathway for the Fe(II)-induced recrystallization, where an Fe(II) atom reacts with the Fe(III) oxide surface and is immediately oxidized. Simultaneously, the resulting electron is transferred to another Fe(III), which is released as Fe(II). The resulting oxidized Fe(III) acts as a nucleus for secondary Fe(III) oxide minerals and facilitates the uptake of Fe(II) through the production of new surface sites. In several isotopic studies describing this pathway, complete isotopic exchange between Fe(III) and aqueous $^{57}$Fe(II) occurred within several days (Crosby et al., 2005; Gorski et al., 2012; Gorski and Fantle, 2017; Pedersen et al., 2005). The crystal structure dissolves and immediately precipitates as a new, more crystalline mineral, with homogenization of labelled and non labelled Fe (Handler et al., 2009).

The determining factor controlling the rate of this pathway is often the amount of Fe(II) sorption to the Fe(III) oxide mineral surface (Paikaray et al., 2017a; Paikaray and Peiffer, 2014) and the resulting electron transfer process (Boland et al., 2013; Burton et al., 2008b; Handler et al., 2009; Jones et al., 2009; Williams and Scherer, 2004a). The solution pH frequently controls this sorption, as a higher pH will increase the negative surface charge of the oxide and allow for greater Fe(II) sorption (Boland et al., 2014; Liu et al., 2007; Paikaray et al., 2017a; Paikaray and Peiffer, 2014; Pedersen et al., 2005; Schwertmann and Taylor, 1972). This has been demonstrated by Burton et al. (2008b), where under low pH (<pH 5.0), little to no sorption of Fe(II) occurred and transformation to goethite was inhibited within the eight hours of the study. However, above pH 5, the reaction rate progressively increased with each pH unit (Burton et al., 2008b).
Furthermore, pre-sorption of compounds to the Fe(III) oxide surface can slow or inhibit the Fe(II)-induced transformation (Jones et al., 2009), with Hinkle et al. (2015) hypothesizing that this retardation was due to the external compound occupying the available binding sites of Fe(III). Inhibition of the Fe(II)-induced crystallization has been supported in several studies of both ferrihydrite and schwertmannite. For example, the pre-sorption of silicate and natural organic matter to schwertmannite inhibit Fe(II) sorption, which further inhibits the Fe(II)-induced reaction (Jones et al., 2009). In this study, silicate ranged from ~0-90% surface site coverage and resulted in ~80-5% isotopic exchange, decreasing exponentially (Jones et al., 2009). Phosphate (PO$_4^{3-}$) surface coverage (0, 50, and 100%) has also been demonstrated to inhibit ferrihydrite transformation (resulting in ~30, 20, and 10% transformation, respectively) induced by Fe(II) (Borch et al., 2007). Arsenic (As) pre-sorption inhibits the extent of the Fe(II)-induced transformation of schwertmannite (Paikaray et al., 2017a), while As redox speciation can also play an important role. In the presence of 1 mM As(III), 72% transformation of schwertmannite occurred, compared to only 6% transformation in the presence of 1 mM As(V) (Burton et al., 2010). Together, these studies suggest compound pre-sorption inhibits the Fe(II)-induced transformation pathway in both ferrihydrite and schwertmannite.

### 2.2.3.2 Additional mineral formation

Although the secondary mineral formed from the Fe(II) induced reaction of schwertmannite is often goethite, the platy mineral lepidocrocite ($\gamma$-FeOOH) can form as an intermediary mineral (Boland et al., 2014; Hansel et al., 2005; Paikaray et al., 2017a; Pedersen et al., 2005). This formation is most commonly observed in systems with low Fe(II) sorption or early in the reaction from schwertmannite to goethite, as lepidocrocite may often transform into its isomorph, goethite ($\alpha$-FeOOH) (Boland et al., 2014; Liu et al., 2007; Paikaray et al., 2017a; Paikaray and Peiffer, 2014; Schwertmann and Taylor, 1972). Lepidocrocite is a reddish orange mineral commonly found in schwertmannite systems and commonly associated with the Fe(II) driven schwertmannite crystallization (Liu et al., 2007; Tomaszewski et al., 2016). Lepidocrocite has been additionally theorized to be partially reduced to green rust, a mixed valence mineral (Borch et al., 2007; Ona-nguema et al., 2002).
Green rusts are reactive, mixed valence Fe minerals that are often difficult to characterize due to their tendency to rapidly oxidize (Genin et al., 2001; Onanguema et al., 2002; Ruby et al., 2006). They form as double layered minerals, incorporating ions such as $\text{SO}_4^{2-}$, carbonate or chloride (Genin et al., 2001). Carbonate and chloride are planar ions, while $\text{SO}_4^{2-}$ is a tetrahedral ion located within the plate-like green rust structure (Genin et al., 2001). Sulfate green rust is the most likely green rust species to precipitate in schwertmannite bearing systems, due to the abundance of the $\text{SO}_4^{2-}$ ions in solution following schwertmannite dissolution and partial reduction.

2.2.4 Schwertmannite reduction

Schwertmannite reductive dissolution occurs readily in reducing environments rich in bacterial Fe(III)- and $\text{SO}_4^{2-}$ reducers (Burton et al., 2007; Jones et al., 2006). The rate limiting step in schwertmannite reduction is often the dissolution of schwertmannite to its elemental components (Küsel, 2003). Dissolution of schwertmannite liberates both Fe(III) and $\text{SO}_4^{2-}$ ions to solution (Regenspurg et al., 2002), as well as the many trace elements often associated with the schwertmannite structure (Burton and Johnston, 2012).

2.2.4.1 Fe reduction

Iron(III) is typically the first of the two abundant and available schwertmannite-derived electron acceptors to undergo dissimilatory microbial reduction (Jones et al., 2006; Peine et al., 2000).

$$3\text{Fe}_8\text{O}_8(\text{OH})_6\text{SO}_4 + \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} \rightarrow 24\text{Fe}^{2+} + 3\text{SO}_4^{2-} + 6\text{CO}_2 + 42\text{OH}^-$$

Eq. 2.8

The majority of microbes responsible for this reduction process are acidophiles, as the initial Fe(II) oxidation lowers the pH of the surrounding environment (Peine et al., 2000). Several important genera of Fe(III) reducing bacteria include *Geobacter* and *Shewanella* (Küsel et al., 1999). Bacterial reduction often occurs through the direct contact of these microbes with the schwertmannite mineral surface, however intermediaries can also play a role (Nevin and Lovley, 2002). For example, humic acid can act as an electron shuttle, eliminating the need for direct contact between the bacteria and the Fe(III) surface (Fredrickson et al., 1998; Nevin and Lovley, 2000).
Schwertmannite reduction can raise the pH of an acidic environment to circumneutral (Eq. 2.8). This not only has obvious benefits for biota, but also for contaminant sorption and mobility dynamics. Many metals and other contaminants are mobile at the low pH values observed in acid sulfate systems (Nystrand et al., 2016; Simpson et al., 2008). Upon Fe(III) reduction and the consequent increase in pH, these metals and contaminants can precipitate, removing them from the water column (Borch et al., 2010; Burton et al., 2008a; Simpson et al., 2008).

A key complicating factor in the Fe(III) oxide reduction process, especially in schwertmannite reduction, is the characteristic production of aqueous Fe(II). As previously mentioned, Fe(II) addition to the schwertmannite mineral can induce a rapid transformation to goethite (Burton and Johnston, 2012). Schwertmannite is less crystalline and more reactive than goethite (Küsel, 2003; Peine et al., 2000), and consequently, goethite will not reductively dissolve as easily as schwertmannite (Burton and Johnston, 2012; Fredrickson et al., 1998; Knorr and Blodau, 2007a; Peine et al., 2000).

### 2.2.4.2 Sulfate (SO₄²⁻) reduction

Low pH is also inhibitory of SO₄²⁻ reduction. Although SO₄²⁻ could be abundant, microbial SO₄²⁻ reducers do not thrive under low pH. Once the Fe(III) pool has become depleted through Fe(III) reduction, however, and the pH increases from ~3.0 to 4-5, SO₄²⁻ reduction is favored (Peine et al., 2000). Sulfate reduction frequently will not produce free sulfide into solution, especially in Fe(II) rich environments (Burton et al., 2007). As such, the Fe(II) monosulfide mackinawite (FeS) can precipitate (Eq. 2.6).

### 2.2.4.3 Secondary mineral formation

During and following the reduction of Fe(III) and SO₄²⁻, several secondary minerals can precipitate, depending on the environmental conditions and available compounds in solution. For example, during microbial reduction in pure schwertmannite systems, Fe(II) and sulfide are the main products, along with bicarbonate from the respiration of organic matter. Therefore, siderite (FeCO₃) may precipitate (Eq. 2.9) in addition to mackinawite (FeS) or pyrite (FeS₂).
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Fe$^{2+}$ + HCO$_3^-$ \rightarrow FeCO$_3$ + H$^+$ \quad \text{Eq. 2.9}

Siderite is a common ferrous carbonate mineral in acid sulfate systems (Benner et al., 2002; Burton et al., 2006b). It has a cubic morphology and is known to precipitate slowly, even when the aqueous phase is supersaturated with respect to carbonate (Burton et al., 2006b).

### 2.3 Phosphate characteristics and interactions

#### 2.3.1 Background

Phosphate (PO$_4^{3-}$) is an anion that can act either as a nutrient or pollutant depending on its concentration in aqueous systems (Clayton et al., 2004; Hart et al., 2004). Phosphate can be introduced to ASW through phosphorus laden runoff or internal phosphorus cycling, and is often found in high concentrations in acidic environments, (i.e. up to 5.9 g/kg sediment) (Collins et al., 2010; Fitzpatrick et al., 2017a). Phosphate is necessary for crop production and soil health, for example (Barber, 2002; Sharpley et al., 2001). However, PO$_4^{3-}$ can be added to soils in excess, and may be lost from the soils to the water through runoff or leaching (Barber, 2002; Bennett et al., 2001).

Eutrophication is a process involving excess organic matter within a waterbody and often begins with excess PO$_4^{3-}$ loading (Bennett et al., 2001; Sharpley et al., 2001). Phosphate acts as a fertilizer, and excess plant or algal growth occurs. When this organic material microbially decomposes, oxygen is consumed. The consumption of oxygen in the water column is harmful to biota and has the potential to create toxic conditions (i.e. red tides or dead zones), and consequently, eutrophication surrounding agricultural lands is a major potential problem around the world (Bennett et al., 2001).

#### 2.3.2 Interactions with Fe(III) oxides and redox cycling

Phosphate is known to interact strongly with Fe(III) oxide species through both surface complexation and precipitation (Borch and Fendorf, 2008). Iron(III) oxides are efficient scavengers of PO$_4^{3-}$ in oxic sediments, resulting in the potential sequestration of PO$_4^{3-}$ in these sediments (i.e. Boers et al., 1998). Limnological studies often report the dissolution and release of PO$_4^{3-}$ to the water column under reducing conditions (P.C.M. Boers et al., 1998; Maynard et al., 2011; Sharpley et al., 2001). However, Simpson et al. (2008)
investigated the potential mobility of metals and nutrients, including $\text{PO}_4^{3-}$ from ASS and found that upon rewetting (and subsequent reduction), little $\text{PO}_4^{3-}$ was released to the water column, likely a result of Fe mineral precipitation and $\text{PO}_4^{3-}$ adsorption.

The stoichiometry between Fe and S is an important consideration in the release or sequestration of $\text{PO}_4^{3-}$, however. Excess free sulfide (over the stoichiometric reactive Fe content) will preferentially sulfidize the Fe(III) oxides and release aqueous $\text{PO}_4^{3-}$. Conversely, excess reactive Fe can accommodate both FeS$_x$ species and Fe(II)-$\text{PO}_4^{3-}$ minerals (Rothe et al., 2016).

Sedimentary P phases are usually operationally defined (Ruttenberg, 1992; Scalenghe et al., 2012), such that $\text{PO}_4^{3-}$ reservoirs are determined by chemical extractions rather than P mineral speciation. For example, a common extraction technique (SEDEX) contains a magnesium chloride extraction step (Lukkari et al., 2007; Ruttenberg, 1992), describing the $\text{PO}_4^{3-}$ loosely associated with the mineral surface. An ascorbic acid step can then identify the quantity of $\text{PO}_4^{3-}$ associated with the easily reducible Fe species such as schwertmannite, leaving the more crystalline species like goethite in place (Scicluna et al., 2015). Although useful on their own, operationally defined $\text{PO}_4^{3-}$ measurements do not identify specific minerals. Mineralogical techniques are available to identify $\text{PO}_4^{3-}$ mineral species (such as x-ray diffraction, x-ray absorbance spectroscopy, Mössbauer spectroscopy, and scanning electron microscopy), but this identification is done much less frequently than mineral extracts, especially in ecological studies or when using natural soils, as $\text{PO}_4^{3-}$ species may be of low abundance among more crystalline species.

There are two major Fe- $\text{PO}_4^{3-}$ minerals identified in Fe rich systems: the Fe(III) mineral strengite, and the Fe(II) mineral vivianite. Strengite (FePO$_4$) is not commonly identified in natural soils, especially in ASW impacted soils, due partly to its solubility at pH < 5.0 and partly because of the sheer amount of other $\text{PO}_4^{3-}$ species in natural soils (i.e. calcium $\text{PO}_4^{3-}$) (Willett, 1985; Willett and Cunningham, 1983). Vivianite (Fe$_3$(PO$_4$)$_2$) can be found in numerous environments as blue green crystal nodules, ranging from saltwater to freshwater systems (Egger et al., 2015; Rothe et al., 2016). Vivianite formation is also hypothesized to be related to Fe(III) oxyhydroxide mineral reduction, as these Fe(III) oxide minerals are important scavengers for anions such as $\text{PO}_4^{3-}$ (Rothe et al., 2016). Consequently, Fe(III) oxide mineral reduction has the
potential to either sequester $\text{PO}_4^{3-}$ in non-sulfidic environments as vivianite, a more crystalline and stable mineral (Rothe et al., 2016), or release $\text{PO}_4^{3-}$ to the water column under sulfidic conditions.

Despite $\text{PO}_4^{3-}$ being an important essential macronutrient, little work has been conducted on its interaction with schwertmannite (Eskandarpour et al., 2007; Khamphila et al., 2017). Runoff from fertilizer occurs around the world, and mine drainage lakes are not isolated from the effects of agricultural runoff. Therefore, it is likely that the two compounds will interact. However, the mechanistic understanding of this interaction remains unknown. For example, little is known about how $\text{PO}_4^{3-}$ will influence the Fe(III) oxide reducibility or the secondary mineralization pathways of the resulting minerals. Additionally, little is known about how the transformations of schwertmannite will impact $\text{PO}_4^{3-}$ mobility.

### 2.3.3 Ferrihydrite and $\text{PO}_4^{3-}$ dynamics- an analogue to schwertmannite

Synthetic schwertmannite is known to sorb large amounts of $\text{PO}_4^{3-}$ (Eskandarpour et al., 2007; Khamphila et al., 2017), and schwertmannite-rich environments can also be rich in $\text{PO}_4^{3-}$ (Collins et al., 2010; Fitzpatrick et al., 2017a). Phosphate may be important in regards to schwertmannite tunnel substitution, as the atomic radius of $\text{PO}_4^{3-}$ is similar to that of arsenate and $\text{SO}_4^{2-}$ (Regenspurg, 2003). Sulfate is an integral part of the schwertmannite tunnel structure and has been shown to exchange with arsenate (Regenspurg, 2003). But unlike arsenate, little work has investigated the influence of $\text{PO}_4^{3-}$ on schwertmannite stability, reduction, or transformation to secondary minerals.

Several studies have investigated the interactions between $\text{PO}_4^{3-}$ and the minerals ferrihydrite and lepidocrocite, however (Borch et al., 2007; Borch and Fendorf, 2008; Galvez et al., 1999; O’Loughlin et al., 2013; Paige et al., 1997; Willett, 1985). Ferrihydrite, in particular, is similar to schwertmannite in that they are both oxidized Fe(III) minerals with a large surface area and a high capacity for anion sorption (Antelo et al., 2012; Eynard, 1993; Paikaray et al., 2011; Regenspurg and Peiffer, 2005). Therefore, comparisons can be tentatively made between the two minerals.

However, there is no consensus on the influence of $\text{PO}_4^{3-}$ on ferrihydrite. Inhibited reduction and transformation of $\text{PO}_4^{3-}$ dosed ferrihydrite has been reported in the literature (Borch et al., 2007; Borch and Fendorf, 2008; Galvez
et al., 1999; Paige et al., 1997; Willett, 1985). In particular, Borch and Fendorf (2008) conducted a study on the interaction between ferrihydrite reduction and PO$_4^{3-}$ at variable PO$_4^{3-}$ loadings and circumneutral pH. They found with increased PO$_4^{3-}$ loading, the extent of microbial ferrihydrite reduction decreased (Borch and Fendorf, 2008). In addition, ferrihydrite reduction decreased PO$_4^{3-}$ mobility through Fe- PO$_4^{3-}$ mineral formation (Borch and Fendorf, 2008). They conclude that PO$_4^{3-}$ loading to ferrihydrite promotes PO$_4^{3-}$ sequestration and ferrihydrite retention instead of PO$_4^{3-}$ desorption and ferrihydrite reduction (Borch and Fendorf, 2008). However, enhanced ferrihydrite reduction in the presence of low levels of PO$_4^{3-}$ has also been reported (Kukkadapu et al., 2004; Zachara et al., 2001). Therefore, conflicting trends are described for the relation between PO$_4^{3-}$ loading and ferrihydrite reduction.

In addition to the discrepancies reported between ferrihydrite studies, schwertmannite is a different mineral. There are similarities between the two Fe(III) oxides, but also many differences, including the presence of structural SO$_4^{2-}$ in schwertmannite. This leads to a potential additional microbial pathway when the PO$_4^{3-}$-dosed schwertmannite is exposed to reducing conditions. No work has investigated schwertmannite- SO$_4^{2-}$ reduction following PO$_4^{3-}$ addition to schwertmannite or the consequent potential mineralogical formation. Therefore, more detailed studies are needed to understand the influence of PO$_4^{3-}$ on schwertmannite transformations under reducing conditions, as this interaction is likely in and around AMD and ASS environments.

2.4 Knowledge Gap

Schwertmannite is a dynamic and metastable mineral, readily transforming in both biotic and abiotic environments through reductive dissolution (Burton et al., 2007; Peine et al., 2000) and the Fe(II)-induced transformation (Burton et al., 2008b; Paikaray et al., 2017a). Although up to 5.9 g/kg PO$_4^{3-}$ has been found in acidic environments (Collins et al., 2010; Fitzpatrick et al., 2017a), little work has investigated the influence of PO$_4^{3-}$ on schwertmannite stability, anaerobic biogeochemistry or secondary Fe mineralogy. The schwertmannite structure may become stabilized, as is the case under arsenate-laden conditions (Regenspurg, 2003; Regenspurg and Peiffer, 2005), and schwertmannite may become less susceptible to reductive dissolution. Other studies of PO$_4^{3-}$-bound
ferricydrite suggest the enhancement of reduction (Kukkadapu et al., 2004; Zachara et al., 2001) and the formation of various secondary Fe minerals (Borch and Fendorf, 2008). These conflicting conclusions lead to the need for more research into the stability or transformation of schwertmannite when exposed to PO$_4^{3-}$.

Several studies suggest introducing PO$_4^{3-}$ to acidic systems to induce a controlled eutrophication event to mitigate ASW (Fyson et al., 1998; Kalin et al., 2006b). As inducing eutrophication has major consequences for both water chemistry and biota, it is very important to fully understand the potential processes involved at a mineralogical and biogeochemical level. If schwertmannite is able to retain the nutrient through surface stability, the sequestration of PO$_4^{3-}$ may inhibit the nutrient release needed for controlled eutrophication. Conversely, if schwertmannite bound PO$_4^{3-}$ is released, aqueous PO$_4^{3-}$ could induce and intensify a controlled eutrophication event in acidic lands. However, the exact interactions between PO$_4^{3-}$ and schwertmannite are unknown. This study aims to clarify both the impact of PO$_4^{3-}$ on schwertmannite stability and transformation, and elucidate the resulting fate and mobility of PO$_4^{3-}$.

Three main topics will be investigated as part of this thesis. The first study will observe the potential stability of schwertmannite under PO$_4^{3-}$ laden, acidic conditions with flow through nitrogen. This study is a batch study, observing the aqueous and mineral phases present as a result of schwertmannite stability or transformation over 41 days after exposure to PO$_4^{3-}$. Hydrogen gas will not build in the reactors, thereby limiting reduction and maintaining a low pH.

Modified study conditions for the second study will include closed batch reactors. This enables the buildup of hydrogen, and in addition to a longer incubation (82 days), allows for the potential switch from Fe(III) to SO$_4^{2-}$-reducing conditions. Here, mineralogical and aqueous conditions will be assessed to determine the evolution of schwertmannite under PO$_4^{3-}$-rich conditions.

The third study will investigate the Fe(II) induced transformation of schwertmannite. Schwertmannite, pre-sorbed with PO$_4^{3-}$, will be exposed to abundant Fe(II) and mineralogy will be observed over ten days.

In addition to the solid phase characteristics of each system, the fate of PO$_4^{3-}$ will be assessed. This determination will aid in making conclusions
about the potential sequestration or aqueous release of PO$_4^{3-}$ in each simulated environment.
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Literature Review


Phosphate as a control on schwertmannite stability, iron mineralogy and anaerobic biogeochemistry in acid-sulfate systems


Phosphate as a control on schwertmannite stability, iron mineralogy and anaerobic biogeochemistry in acid-sulfate systems


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Phosphate as a control on schwertmannite stability, iron mineralogy and anaerobic biogeochemistry in acid-sulfate systems


Chapter 3 - Phosphate-imposed constraints on schwertmannite stability under reducing conditions

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Objective: To assess the stability of schwertmannite under acidic reducing conditions before extensive Fe(III) or SO\textsubscript{4}\textsuperscript{2-} reduction occur. Schwertmannite will be held under moderately reducing conditions (facilitated by flow-through nitrogen, which suppresses the accumulation of hydrogen in the batch reactors). This is projected to limit Fe(III) reduction and alkalinity generation. Furthermore, varied phosphate loadings are projected to alter the progression and extent of schwertmannite stability in comparison to its transformation and reduction. This research component is suspected to simulate frequently disturbed natural systems, or systems which cannot fully and continuously reduce.

Author Contributions:

Concept and Design: Schoepfer and Burton
Laboratory analysis: Schoepfer
Data interpretation: Schoepfer and Burton
Original draft writing: Schoepfer
Proofreading and editing: Burton, Johnston, and Kraal

See Appendix 1 for signed author contribution
3.1 Abstract

Schwertmannite is a metastable ferric oxyhydroxysulfate mineral, which is common in acid sulfate systems. Such systems contain varying concentrations of dissolved phosphate (PO$_4^{3-}$) - an essential nutrient whose availability may be coupled to schwertmannite formation and fate. This study examines, for the first time, the effect of phosphate on schwertmannite stability under reducing conditions. Phosphate was added at 0, 80, 400 and 800 µmoles g$^{-1}$ (i.e. zero, low, medium and high loading) to schwertmannite suspensions which were inoculated with wetland sediment and suspended in N$_2$-purged artificial groundwater. pH remained between 2.7 and 4.3 over the 41 day experiment duration. Fe(II) accumulated in solution due to dissimilatory Fe(III)-reduction, which was most pronounced at intermediate PO$_4^{3-}$ loadings (i.e. in the low PO$_4^{3-}$ treatment). Partial transformation of schwertmannite to goethite occurred in the zero and low PO$_4^{3-}$ treatments, with negligible transformation in higher PO$_4^{3-}$ treatments. Overall, the results suggest that intermediate PO$_4^{3-}$ loadings enhance the reduction and transformation of schwertmannite. At zero PO$_4^{3-}$ loading, reductive dissolution appears to be constrained by the rapid transformation of schwertmannite to goethite, which thereby decreases the bioavailability of solid-phase Fe(III). Conversely, at high loadings, PO$_4^{3-}$ appears to stabilize the schwertmannite surface against dissolution; probably via the formation of strong surface complexes. These findings have important implications for understanding the contrasting environmental fate of schwertmannite in P-rich versus P-poor systems.

Keywords: Iron reduction, phosphate, schwertmannite
Graphical Abstract:
3.2 Introduction

Acid sulfate systems are found around the world in relation to mining operations, rock weathering and acid sulfate soils (Bigham and Nordstrom, 2000a). These systems are characterized by low pH due to the oxidation of iron sulfide minerals, such as pyrite (Bigham and Nordstrom, 2000a). One of the most common secondary minerals in acid sulfate systems is schwertmannite; a poorly crystalline iron(III) oxyhydroxysulfate (Bigham et al., 1996; Bigham and Nordstrom, 2000a; Blodau, 2004; Jönsson et al., 2005). Schwertmannite is metastable with regard to goethite, with previous research showing that schwertmannite is stabilized by low pH (Bigham et al., 1996; Collins et al., 2010; Jönsson et al., 2005; Regenspurg et al., 2004), high $\text{SO}_4^{2-}$ concentrations (Jönsson et al., 2005), and the presence of strongly sorbed species (e.g. Si and humic/fulvic acids) (Burton and Johnston, 2012; Collins et al., 2010; Jones et al., 2009).

The formation and stability of schwertmannite is of significant interest in acid-mine drainage (AMD) environments and in acid sulfate soils. This is because schwertmannite plays a major role in controlling water quality by influencing both the generation and consumption of acidity and by acting as a sorbent for contaminants and nutrients (Antelo et al., 2013a; Fukushi et al., 2003a; Regenspurg and Peiffer, 2005). Although schwertmannite forms under oxidizing conditions, it may be subjected to prolonged reducing conditions in re-flooded acid sulfate soils, mine pit-lake sediments or AMD-treatment wetlands (Collins et al., 2010; Küsel et al., 1999). Under reducing conditions, schwertmannite may strongly influence both Fe and S biogeochemistry and microbial electron flow through microbially-mediated dissimilatory Fe(III)-reduction (Küsel and Dorsch, 2000; Regenspurg et al., 2002) or abiotic reduction by $\text{H}_2\text{S}$ produced via dissimilatory $\text{SO}_4^{2-}$ reduction (Burton et al., 2007).

Schwertmannite is capable of sorbing large amounts of $\text{PO}_4^{3-}$, an essential nutrient which can also act as a pollutant depending on the levels of P in the system (P.C.M. Boers et al., 1998; Eskandarpour et al., 2006). Collins et al. (2010) also observed significant P-enrichment in natural schwertmannite collected from an acid sulfate soil environment. Although very little research has examined interactions between $\text{PO}_4^{3-}$ and schwertmannite, $\text{PO}_4^{3-}$ interactions have been extensively documented with respect to other iron(III)
oxides, such as ferrihydrite, goethite and hematite (Biber et al., 1994; Borch et al., 2007; Hinkle and Catalano, 2015; Latta et al., 2012; Voegelin et al., 2013). Borch et al. (2007) found that PO$_4^{3-}$ sorption stabilizes ferrihydrite against reductive dissolution, and that it also alters secondary mineral transformation pathways. As schwertmannite and ferrihydrite are similar with respect to surface area, structural disorder and metastability (Antelo et al., 2012; Eynard, 1993; Paikaray et al., 2011; Regenspurg and Peiffer, 2005), it can be hypothesized that schwertmannite stability may also be enhanced by the sorption of PO$_4^{3-}$ to the mineral surface.

The objective of this study was to examine the effect of PO$_4^{3-}$ sorption on schwertmannite stability under reducing conditions. To achieve this objective, we subjected schwertmannite, with varying loadings of added PO$_4^{3-}$, to microbially-mediated reducing conditions for 41 days and observed the aqueous and mineralogical species over time.

3.3 Methods

3.3.1 Schwertmannite synthesis

Schwertmannite was synthesized via the peroxide method (Regenspurg et al., 2004). The precipitated mineral was rinsed five times with deionized water, dried at 50° C and then ground to a fine powder using a ring mill.

3.3.2 Phosphate sorption isotherm analysis

PO$_4^{3-}$ sorption to schwertmannite was characterized at pH 2.6 using a batch sorption isotherm approach, involving 0.5 g of schwertmannite suspended in 40 mL of artificial groundwater (Table 3.7.1) with initial aqueous PO$_4^{3-}$ concentrations of 0, 0.5, 1, 2, 3, 4, 5, 6, 6.5, 7, 7.5, 8, 9, and 10 mM, added as KH$_2$PO$_4$. After a 24-hr equilibration period, the aqueous phase was separated by filtration to <0.45 µm and retained for analysis of PO$_4^{3-}$.

3.3.3 Reductive incubation experiment

The microbial reduction experiments involved 18.75 g of synthetic schwertmannite suspended in 1.5 L of artificial groundwater (as defined above) within gas-tight 2 L pyrex reaction chambers. Aqueous PO$_4^{3-}$ was added to the initial artificial groundwater on the basis of the sorption isotherm results to achieve 4 differing levels of surface site coverage corresponding to 0, 80, 400,
and 800 µmole PO$_4^{3-}$ g$^{-1}$ schwertmannite, which are hereafter denoted as zero, low, medium and high PO$_4^{3-}$ treatments.

To provide a natural consortium of microorganisms, the artificial groundwater was inoculated with ~2% (w/w) benthic sediment collected from a drainage canal in the Tuckean Swamp near Lismore, Eastern Australia (Burton et al., 2013). Determination of the presence and approximate numbers of iron and sulfur reducers in the initial inoculum using the most probable number (MPN) methods described by Benner et al. (2000) and using the calculations of Garthright and Blodgett (2003) indicated an iron reducing bacterial cell density of $\sim$10$^{7.0}$ MPN g$^{-1}$ and a sulfur reducing bacterial cell density of $\sim$10$^{4.7}$ MPN g$^{-1}$. The primary carbon substrate for microbial processes was glucose, which was present in the artificial groundwater (Table 3.7.1).

Anoxic conditions were maintained by continuously passing high-purity N$_2$(g) through the reaction chambers. The temperature was kept at 30°C, to simulate summer temperatures, using a thermostated water-bath. Four replicate schwertmannite suspensions were manually stirred for 30 seconds daily over the full experimental duration of 41 days for each treatment.

At sampling times of 2, 5, 9, 13, 17, 21, 25, 29, 33, 37 and 41 days (selected on the basis of previous studies (Burton et al., 2013, 2007; Burton and Johnston, 2012), reaction chambers were removed from the water-bath and placed on a stir plate for one minute to re-homogenize the suspension. A syringe was used to withdraw a 50 mL sample of homogeneous suspension, via a gas-tight sampling port, for characterization. The solid-phase was separated by centrifugation and dried in an anoxic chamber at room temperature (after rinsing with methanol to speed the drying process) prior to XRD analysis. The supernatant was filtered to <0.45 µm and retained for analysis. All processing of solid- and aqueous-phase samples was conducted under strictly O$_2$-free conditions within an anoxic chamber.

3.3.4 Analytical methods

The filtrate Eh and pH were measured with probes, freshly calibrated against Zobell’s solution (Zobell, 1946) and pH 4 and 7 buffers, respectively. One aliquot of filtrate was added to a color reagent to measure alkalinity (Sarazin et al., 1999), another to a 20% zinc acetate/2M NaOH solution to
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preserve the sample for H₂S analysis, followed by analysis via the methylene blue method (Cline, 1969). Samples for Fe, SO₄²⁻ and PO₄³⁻ determination were acidified and refrigerated until analysis. Fe(II) concentrations were determined through the 1,10-phenanthroline method (APHA, 2005). For total aqueous Fe, a sample was pre-reduced using hydroxylamine hydrochloride, following the same method, and Fe(III) was determined via subtraction. Phosphate was determined by the molybdenum blue method of Hansen and Koroleff in Grasshoff et al. (1999). Sulfate was quantified by total S using a Perkin Elmer Optima 8300DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) after subtracting aqueous sulfide. Quantification of Fe and S content in the initial schwertmannite was performed after solid-phase digestion (1:3 HNO₃/HCl) using a Perkin Elmer NexION 300D Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

X-ray diffraction was used to determine the mineral phases present over time using a Phillips PW 1050/70 diffractometer with a Co x-ray tube. Samples were scanned from 10 °2θ to 80 °2θ using a 0.1 °2θ step and a 1 second count time.

X-ray absorption spectroscopy measurements of the final (Day 41) solid-phase were made at the National Synchrotron Radiation Research Centre (NSRRC) in Hsinchu, Taiwan. Sulfur K-edge XANES data were collected under a high purity helium atmosphere on beamline 16A. Wet samples were contained between Kapton tape and Mylar film. Samples for Fe K-edge EXAFS were wet-mounted between Kapton tape and collected under normal atmosphere on beamline 17C. Standards were prepared as described by Burton and Johnston (2012). To subtract the background, normalize the data, and perform linear combination fitting (Burton et al., 2009), we used the ATHENA program (Ravel and Newville, 2005). Linear combination fits did not allow for energy float.

3.3.5 Modelling

Visual Minteq was used to calculate the equilibrium speciation and solubility state in solution. All species present in the initial groundwater as well as species determined analytically at each time point were included in the modelling.
3.4 Results

3.4.1 Initial schwertmannite properties

X-ray diffractometry confirmed that the initial mineral used in this study was schwertmannite (Figure 3.7.1a). The initial schwertmannite contained 7.4 ± 0.1 mmol g⁻¹ Fe and 1.5 ± 0.03 mmol g⁻¹ S. This Fe and S content indicate a composition of Fe₈O₈(OH)₄.96(SO₄)₁.65, which is within the compositional range reported for schwertmannite (Acero et al., 2006; Burton et al., 2010; Burton and Johnston, 2012; Regenspurg et al., 2002).

3.4.2 Phosphate sorption isotherm analysis

PO₄³⁻ sorption to schwertmannite most closely followed a Freundlich isotherm when linearly transformed (Figure 3.7.2). Here, the parameter Kᵢ is equal to 0.63 L mmole⁻¹ schwertmannite with a unitless exponent (n) of 11.6 (Figure 3.7.2). The maximum measured q value (~650 µmoles P g⁻¹ schwertmannite) was comparable to Eskandarpour et al. (2006), who suggest a sorption maximum of 865 µmoles P g⁻¹ schwertmannite.

3.4.3 Aqueous-phase trends

The pH varied as a function of time and PO₄³⁻ coverage, yet remained close to pH 3 – 4 (Figure 3.73a-d). The pH varied insignificantly with respect to initial PO₄³⁻ addition (between 3.3 at zero PO₄³⁻, and 3.0 at high PO₄³⁻). The highest final pH (4.3) occurred in the low PO₄³⁻ treatment, with the lowest (pH 2.7) occurring in the high PO₄³⁻ treatment.

Aqueous PO₄³⁻ concentrations were very low for the full experiment duration in the zero and low PO₄³⁻ treatments (Figure 3.7.3e-f). The medium PO₄³⁻ and high PO₄³⁻ treatments had much higher initial aqueous PO₄³⁻ concentrations (Figure 3.7.3g-h). These concentrations decreased over time, reaching steady state aqueous PO₄³⁻ concentrations of 6.8 µM at day 5 in the medium PO₄³⁻ treatment and 33.1 µM by day 30 in the high PO₄³⁻ treatment.

Substantial amounts of aqueous Fe(III) and Fe(II) (Figure 3.7.3i-l) developed over time in the zero, low and medium PO₄³⁻ treatments, with very little aqueous Fe being released into solution in the high PO₄³⁻ treatment. Most aqueous Fe in all treatments existed in the 2+ oxidation state. The low PO₄³⁻ treatment had the greatest level of Fe(II) production, with an aqueous Fe(II) concentration that was close to 15 mM at day 41. There were slightly lower Fe(II) concentrations in the zero PO₄³⁻ treatment (~10 mM at day 41), and
substantially less in the medium and high PO₄³⁻ treatments. Fe(II) concentrations indicate that 10%, 15%, 4.5% and 1% of the total Fe(III) content underwent reductive dissolution in the zero, low, medium and high PO₄³⁻ treatments, respectively (Figure 3.7.4).

Aqueous sulfide was below detection (< 2 μM) in all treatments throughout the experiment. All treatments displayed an initially rapid (within 2 days) release of SO₄²⁻ into solution (Figure 3.7.3m-p). This initial release of presumably surface-bound SO₄²⁻ was dependent on the level of PO₄³⁻ addition, with higher SO₄²⁻ concentrations at day 2 (i.e. our first sample time) occurring in the higher PO₄³⁻ treatments. These aqueous concentrations correspond to rapid release of 20%, 20%, 31% and 38% of the total solid-phase SO₄²⁻ content in the zero, low, medium and high PO₄³⁻ treatments, respectively. This is comparable to work by Bigham et al. (1994) and Regensburg et al. (2004) who found that ~30% of SO₄²⁻ in schwertmannite is surface bound.

Following the initial rapid SO₄²⁻ release, aqueous SO₄²⁻ then increased steadily over the full experiment duration in all treatments. However, the rate of SO₄²⁻ release and hence the final extent of aqueous SO₄²⁻ production was dependent on the level of PO₄³⁻ addition. In particular, the highest final SO₄²⁻ concentrations (~15 mM) occurred in the low PO₄³⁻ treatment, with lower concentrations occurring in the zero PO₄³⁻ treatment and even lower concentrations occurring in the medium and high PO₄³⁻ treatments. After accounting for rapid release of surface-bound SO₄²⁻ (evident at day 2), the final aqueous SO₄²⁻ concentrations equate to an additional slower release of 44%, 59%, 23% and 21% of the total solid-phase SO₄²⁻ content (Figure 3.7.7).

3.4.4 X-ray diffractrometry

There was negligible change in mineralogy over 41 days in the medium and high PO₄³⁻ treatments (Figure 3.7.1b-c, Figure 3.7.8). In contrast, XRD revealed substantial goethite formation in the zero PO₄³⁻ and low PO₄³⁻ treatments, with slightly faster transformation in the zero PO₄³⁻ treatment (Figure 3.7.5). However, both the zero and low PO₄³⁻ treatments exhibited similar XRD patterns at day 41, which reveal a mixture of crystalline goethite and poorly-ordered schwertmannite.
3.4.5 X-ray absorption spectroscopy

The Fe K-edge EXAFS spectra for the experimental samples from day 41 are shown in Figure 3.7.9. In accordance with the XRD results, the Fe K-edge EXAFS spectra of the experimental samples were well fit by a combination of schwertmannite and goethite (Table 3.7.2). The linear combination fit (LCF) results revealed that, at day 41, the zero and low PO$_4^{3-}$ treatments both contained close to 25% goethite, with relatively little goethite formation occurring in the medium and high PO$_4^{3-}$ treatments (8% and 2% goethite, respectively) (Figure 3.7.6).

Sulfur K-edge XANES spectra and linear combination fitting for samples collected at day 41 are shown in Figure 3.7.10. These spectra show a distinct peak at ~2481 eV, which corresponds to solid-phase SO$_4^{2-}$. The S K-edge XANES spectra also have a small peak at 2470.6 eV, which indicates the presence of elemental S. At day 41, 18% of the total S in the low PO$_4^{3-}$ sample was elemental S, whereas only 6% of S was elemental sulfur in the highest PO$_4^{3-}$ treatment (Figure 3.7.6).

3.4.6 Geochemical modelling

Geochemical modeling using Visual Minteq showed that the aqueous phase remained undersaturated with respect to vivianite throughout the experiment. However, supersaturated conditions developed with respect to strengite, a ferric phosphate mineral. It should be noted, however, that even if all PO$_4^{3-}$ had been removed from solution via strengite formation, this would account for only about 10%, 5%, and 1% of total Fe in the high, medium and low PO$_4^{3-}$ treatments, respectively.

3.5 Discussion

3.5.1 Fe(III)-reduction and schwertmannite dissolution

The production of Fe(II) in this study can be largely attributed to the reductive dissolution of schwertmannite via the activity of dissimilatory Fe(III)-reducing microorganisms (Eq. 3.1):

$$3\text{Fe}_8\text{O}_8(\text{OH})_6\text{SO}_4 + \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} \rightarrow 24\text{Fe}^{2+} + 3\text{SO}_4^{2-} + 6\text{CO}_2 + 42\text{OH}^-$$

Eq. 3.1

The presence of some elemental S at day 41 suggests that Fe(III)-reduction also occurred as a result of dissimilatory SO$_4^{2-}$ reduction (Eq. 3.2), with Peiffer
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and Gade (2007) showing that abiotic reduction of schwertmannite-Fe(III) by H_2S at low pH leads to the generation of Fe(II) and S^0 (Eq. 3.3):

\[ 6\text{H}^+ + 3\text{SO}_4^{2-} + \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{H}_2\text{S} + 6\text{CO}_2 + 6\text{H}_2\text{O} \]  
Eq. 3.2

\[ 3\text{Fe}_8\text{O}_8(\text{OH})_6\text{SO}_4 + 4\text{H}_2\text{S} + 14\text{H}^+ \rightarrow 8\text{Fe}^{2+} + 4\text{S}^0 + \text{SO}_4^{2-} + 14\text{H}_2\text{O} \]  
Eq. 3.3

Although dissimilatory Fe(III)- and SO_4^{2-} reduction both produce alkalinity, the pH remained acidic for all treatments. The persistence of acidic conditions is therefore consistent with the relatively small extent of Fe(III)-reduction that occurred during the 41-day experiment. For example, the greatest degree of Fe(III) reduction (which occurred in the low PO_4^{3-} treatment) amounted to dissolution of only ~16% of the total solid-phase Fe(III). However, the mass balance between H^+ production via goethite formation and OH^- production via reduction are consistent with a slowly increasing pH over the course of the 41 days (0.039, 0.066, 0.031, 0.012 mmoles OH^- g^-1 day^-1 in excess of H^+ produced).

The low pH that persisted in the experiment described here is characteristic of sediment-water interfaces in acid sulfate soils and lignite mining lakes (Blodau, 2006a, 2004; Regenspurg et al., 2002). In these type of schwertmannite-rich systems, Fe(III) reduction is typically the dominant terminal electron accepting process (Peine et al., 2000). Sulfate reduction is often assumed to require a pH of above ~4.5 (Peine et al., 2000), while Fe(III) reduction can occur readily at low pH (Küsel et al., 1999). In this experiment, the pH remained low in all treatments, and therefore, Fe(III) reduction was probably dominant. Limited sulfate reduction is also consistent with the relatively low levels of elemental S formation (4.5, 4.5, 6.9, and 2.8% of the initial S in the system as S^0, based on the S K-edge XAS data).

### 3.5.2 Transformation of schwertmannite to goethite

In addition to releasing Fe(II), the reductive dissolution of schwertmannite also liberates SO_4^{2-} (Eq. 3.1). On the basis of the Fe(II)-derived percentages of reductive dissolution mentioned above, the corresponding aqueous SO_4^{2-} concentrations (based on congruent dissolution) would be expected to be no greater than 1.9, 2.9, 0.9 and 0.2 mM for the zero, low, medium, and high PO_4^{3-} treatments, respectively. However, these expected concentrations of SO_4^{2-} equate to only between 2% and 19% of the actual aqueous SO_4^{2-} concentrations that developed over the duration of this experiment.
This discrepancy reflects the presence of an additional $\text{SO}_4^{2-}$ source, which can be largely explained by the mineralogical transformation of schwertmannite to goethite and subsequent release of structural $\text{SO}_4^{2-}$ (Eq. 3.4):

$$\text{Fe}_8\text{O}_8(\text{OH})_6\text{SO}_4 + 2\text{H}_2\text{O} \rightarrow 8\text{FeOOH} + \text{SO}_4^{2-} + 2\text{H}^+$$  \hspace{1cm} \text{Eq. 3.4}

XRD showed clear evidence of goethite formation in the two lowest $\text{PO}_4^{3-}$ treatments (Figure 3.7.5a and b), which was corroborated by the Fe K-edge EXAFS spectroscopy results. Goethite transformation was fastest in the zero $\text{PO}_4^{3-}$ treatment due to the lack of sorbing ions (Burton et al., 2013, 2008b, 2007; Burton and Johnston, 2012). The Fe EXAFS data also indicate some minor schwertmannite transformation in the two higher $\text{PO}_4^{3-}$ treatments. This concurs with the small increase in aqueous $\text{SO}_4^{2-}$ that occurred in the medium and high $\text{PO}_4^{3-}$ treatments between day 2 and 41.

The Fe K-edge EXAFS data indicate that ~25% of the initial schwertmannite transformed to goethite over the 41 day experiment in the low $\text{PO}_4^{3-}$ treatment, with slightly less transformation occurring in the zero $\text{PO}_4^{3-}$ treatment. This extent of schwertmannite transformation is comparable to that observed by Knorr and Blodau (2007), who found between 25% and 34% transformation in the absence of $\text{PO}_4^{3-}$ over 100 days at pH 3 and 20°C.

Burton et al. (2007, 2008b, 2013; Burton and Johnston, 2012) have previously documented much faster rates of schwertmannite transformation under Fe(III) reducing conditions than those observed in the zero $\text{PO}_4^{3-}$ treatment. For example, Burton et al. (2008b) found complete transformation of schwertmannite to goethite over just a few hours at near-neutral pH as a result of an abiotic Fe(II)-catalyzed transformation pathway. Burton and Johnston (2012) found that, in the absence of elevated $\text{PO}_4^{3-}$ concentrations, ~60 % to ~80 % of schwertmannite-derived Fe(III) was microbially reduced to Fe(II) within 20 days, causing pH to increase from pH ~3.5 to ~6.5 and thereby triggering almost complete transformation of schwertmannite to goethite. The present work differs from these earlier studies in that the earlier microbially-mediated experiments quickly developed near-neutral pH, which is required for the surface complexation of Fe$^{2+}$ that ultimately drives rapid Fe$^{2+}$-catalyzed transformation of schwertmannite to goethite.

Relatively slow initiation of Fe(III) reduction in the present study was possibly influenced by the absence of additional humic acid in the artificial groundwater, while glucose acted as the primary electron donor for microbial
Phosphate-imposed constraints on schwertmannite stability under reducing conditions

activity. Humic acid can act as an electron shuttle in microbial Fe(III) reduction (Amstetter et al., 2012; Kappler et al., 2004; Nevin and Lovley, 2000). Consequently, it is hypothesized that without humic acid, as in this study, Fe(III) reduction may have been slowed, thereby inhibiting alkalinity production via Fe(III) reduction and helping to retain acidic conditions.

3.5.3 Phosphate imposed constraints: An optimal zone

Our study shows a nonlinear response of schwertmannite reduction to PO₄³⁻ surface coverage over a 41-day period. Indicators of microbially-mediated reduction in this study include both Fe(II) and elemental S. Both reaction products display a similar and distinct optimal range with respect to PO₄³⁻ coverage on schwertmannite (Figure 3.7.4 and Figure 3.7.6). More specifically, conditions for schwertmannite reduction are optimal at partial coverage by PO₄³⁻. Previous research documented inhibition of ferrihydrite reduction with increasing levels of PO₄³⁻ addition (Borch et al., 2007). The current study expands on this prior work examining ferrihydrite by demonstrating a more complex, non-linear effect on schwertmannite stability in response to PO₄³⁻ loading.

Small amounts of PO₄³⁻ are known to enhance Fe(III) reduction in Fe(III) oxide species (Kukkadapu et al., 2004; Zachara et al., 2001), which is consistent with the elevated reduction of schwertmannite in the low PO₄³⁻ treatment, however caution must be taken when extrapolating to pH 3 conditions. These studies suggest that Fe-P compounds may enhance reduction by scavenging Fe(II) from the mineral surface. Microbial PO₄³⁻ enhancement is not suspected to be the dominant mechanism here. Rather, other mechanisms overshadow the potential nutrient enhancement in this system especially at high PO₄³⁻ loading. Phosphate has been found to have an inhibitory effect on Fe(III) oxide reduction. This inhibition has previously been shown with PO₄³⁻ sorption to ferrihydrite (Borch et al., 2007; Borch and Fendorf, 2008; Galvez et al., 1999; Willett, 1985). PO₄³⁻ can bind to Fe(III) oxides by exchanging two hydroxyl groups and bridging the corners of the mineral polyhedral (Bondietti et al., 1993; Stumm, 1997). Dissolution and reduction are inhibited due to the high activation energy required to break this Fe(III)-O-P bond (Stumm, 1997). Therefore, this may be an important mechanism attenuating Fe(III) reduction at higher PO₄³⁻ sorption levels.
3.5.4 Environmental Implications

This study demonstrates that PO$_4^{3-}$ can act to enhance schwertmannite-Fe(III) reduction at low PO$_4^{3-}$ coverage, and act as a barrier to schwertmannite-Fe(III) reduction at high PO$_4^{3-}$ surface coverage. At zero PO$_4^{3-}$ coverage, rapid transformation to goethite appears to partially inhibit dissimilatory Fe(III) reduction. There is an optimal zone of PO$_4^{3-}$ coverage at which reduction of Fe(III) is enhanced. The PO$_4^{3-}$ loadings used here were environmentally relevant in acid sulfate systems, as many of these environments are lowlands and receive significant agricultural input or experience dynamic redox characteristics and therefore local PO$_4^{3-}$ hotspots. Small amounts of PO$_4^{3-}$ (~45 μmoles P g$^{-1}$ schwertmannite) have been previously documented in schwertmannite rich environments (Collins et al., 2010) and the results of this study may help in understanding the persistence of schwertmannite under these and higher PO$_4^{3-}$ loadings. This study provides new insight into how interactions between PO$_4^{3-}$ and schwertmannite may affect Fe(III) reduction and Fe mineral evolution at low pH.

ACKNOWLEDGEMENTS

Funding was provided by the Australian Research Council (grant LP LP120200723). We also thank the NSRRC in Taiwan for facilitating the collection of XAS data.
3.6 References


Phosphate-imposed constraints on schwertmannite stability under reducing conditions


Regenspurg, S., Gößner, A., Peiffer, S., Küsel, K., Gobner, A., Peiffer, S., Küsel, K., 2002. Potential remobilization of toxic anions during reduction of arsenated and...
3.7 Figures

Figure 3.7.1. X-ray diffraction

X-ray diffraction pattern for a) the initial schwertmannite, b) Day 41 medium PO₄³⁻ treatment, and c) Day 41 high PO₄³⁻ treatment.
Figure 3.7.2. Phosphate sorption isotherm
Sorption isotherm of PO$_4^{3-}$ on schwertmannite at pH 2.6. The solid line shows the Freundlich isotherm modelled values, while the points indicate experimental values. The value q is a variable related to sorption capacity at equilibrium, while $C_{\text{eq}}$ is the concentration of PO$_4^{3-}$ at equilibrium. The $R^2$ value indicates the fit of the data to the model as plotted when linearly transformed.
Phosphate-imposed constraints on schwertmannite stability under reducing conditions

Figure 3.7.3. Aqueous Parameters
Changes in the aqueous-phase composition over time for zero, low, medium and high PO$_4^{3-}$ treatments. Points are mean values of four replicates with standard errors.
Figure 3.7.4. Iron reduction by phosphate treatment

Relationship between the extent of Fe(III)-reduction and the level of PO$_4^{3-}$ loading on the initial schwertmannite.
Phosphate-imposed constraints on schwertmannite stability under reducing conditions

Figure 3.7.5. Temporal x-ray diffraction patterns
Temporal evolution of XRD patterns in the zero and low PO$_4^{3-}$ treatments. The bottom pattern shows the initial schwertmannite pattern, and annotations above show goethite peaks.
Figure 3.7.6. Elemental sulfur and goethite

Percent elemental sulfur (solid line) and percent goethite (dashed line) expressed as a percentage of total S and total Fe, respectively, in the solid-phase at the end of the 41 day incubation in relation to the level of $\text{PO}_4^{3-}$ loading.
3.8 Supporting Information - Chapter 3

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Supporting Information for manuscript submitted to \textit{Environmental Science & Technology}
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\textsuperscript{B}Department of Earth Sciences - Geochemistry
Utrecht University,
Utrecht, The Netherlands

Phosphate-imposed constraints on schwertmannite stability under reducing conditions
Table 3.8.1. Artificial groundwater composition

<table>
<thead>
<tr>
<th>Wolfe’s mineral solution (in 1 L)</th>
<th>Artificial Groundwater Composition</th>
</tr>
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<tr>
<td>1.5 g nitrilotriacetic acid</td>
<td>5 mM CaCl₂</td>
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<tr>
<td>3.0 g MgSO₄ * 7H₂O</td>
<td>10 mM KCl</td>
</tr>
<tr>
<td>0.5 g MnSO₄ * H₂O</td>
<td>10 mM MgCl₂</td>
</tr>
<tr>
<td>1.0 g NaCl</td>
<td>1 mL L⁻¹ Wolfe’s mineral solution</td>
</tr>
<tr>
<td>0.1 g FeSO₄ * 7H₂O</td>
<td>1 g L⁻¹ yeast extract</td>
</tr>
<tr>
<td>0.1 g CoCl₂ * 6H₂O</td>
<td>2 g L⁻¹ glucose</td>
</tr>
<tr>
<td>0.1 g CaCl₂</td>
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<tr>
<td>0.1 g ZnSO₄ * 7H₂O</td>
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</tr>
<tr>
<td>0.01 g CuSO₄ * 5H₂O</td>
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</tr>
<tr>
<td>0.01 g AlK(SO₄)₂ * 5H₂O</td>
<td></td>
</tr>
<tr>
<td>0.01 g Na₂MnO₄ * 2H₂O</td>
<td></td>
</tr>
</tbody>
</table>

Composition of the artificial groundwater and included Wolfe’s Mineral Solution.
Phosphate-imposed constraints on schwertmannite stability under reducing conditions

Figure 3.8.1. Slow release sulfur
Percent slow release S (Day 41 minus Day 2 S concentration) as a function of the PO$_4^{3-}$ loading to schwertmannite.
Figure 3.8.2. Temporal x-ray diffraction- medium and high phosphate

Temporal evolution of XRD patterns in the medium and high PO₄³⁻ treatments. The bottom pattern shows the initial schwertmannite pattern while above patterns are samples taken at each time point.
Phosphate-imposed constraints on schwertmannite stability under reducing conditions

Figure 3.8.3. Iron EXAFS spectra
Iron EXAFS spectra, with data represented by dotted points and linear combination fits by solid lines. Standards are located in the bottom panel and linear combination fits did not allow for energy float.
### Table 3.8.2. Sulfur XANES and iron EXAFS linear combination fits

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<tr>
<th>Trt</th>
<th>Rep</th>
<th>Schwertmannite Sulfur</th>
<th>Dissolved Elemental Sulfur</th>
<th>Solid Elemental Sulfur</th>
<th>R factor</th>
<th>Chi-squared</th>
<th>Schwertmannite</th>
<th>Goethite</th>
<th>R factor</th>
<th>Chi-squared</th>
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<td>Zero 1</td>
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<td>Zero 2</td>
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<td>0.059</td>
<td>0.009</td>
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<tr>
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<td>0.963</td>
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<td>0.013</td>
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<tr>
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<td>Excess 3</td>
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<td>0.011</td>
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<td>Excess 4</td>
<td>0.997</td>
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<td>0</td>
<td>0.002</td>
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<td>0.963</td>
<td>0.017</td>
<td>0.013</td>
<td>26.19</td>
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Associated R factors and linear fit combinations for sulfur and iron XAS.
Figure 3.8.4. Sulfur XANES spectra

Sulfur XANES data, with dotted data points and solid linear fit lines. Standards are located in the bottom panel. Linear combination fitting did not allow for energy float.
Chapter 4 - Phosphate loading alters schwertmannite transformation rates and pathways during microbial reduction

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Objective: To assess the progression of Fe(III) and SO\textsubscript{4}\textsuperscript{2–} reduction given ideal schwertmannite reducing conditions (i.e. the retention of hydrogen in the batch reactors). This is projected to enhance the reactions that were observed in Chapter 3, as well as produce several reduced secondary minerals. Phosphate loading is further suspected to influence the reduced minerals that may form through the formation of vivianite. This research component is likely to simulate those environmental systems that are continuously undisturbed and allowed to reduce to their fullest extent, compared to more frequently disturbed systems.

Author Contributions:
Concept and Design: Schoepfer and Burton
Laboratory analysis: Schoepfer
EXAFS data collection: Burton
Data interpretation: Schoepfer and Burton
Original draft writing: Schoepfer
Proofreading and editing: Burton, Johnston, and Kraal

See Appendix 1 for signed author contribution
4.1 Abstract

Acid sulfate systems commonly contain the metastable ferric oxyhydroxysulfate mineral schwertmannite, as well as phosphate (PO$_4^{3-}$) - a nutrient that causes eutrophication when present in excess. However, acid sulfate systems often experience reducing conditions that destabilize schwertmannite. Under such conditions, the long-term fate of both schwertmannite and PO$_4^{3-}$ may be influenced by interactions during microbially-mediated Fe(III) and SO$_4^{2-}$ reduction. This study investigates the influence of PO$_4^{3-}$ on Fe(III) and SO$_4^{2-}$ reduction and the subsequent mineralogical transformation(s) in schwertmannite-rich systems exposed to reducing conditions. To accomplish this, varied PO$_4^{3-}$ loadings were established in microbially-inoculated schwertmannite suspensions that were incubated under anoxic conditions for 82 days. Increased PO$_4^{3-}$ attenuated the onset of microbial Fe(III) reduction. This delayed consequent pH increases, which in turn had cascading effects on the initiation of SO$_4^{2-}$ reduction and subsequent mineral species formed. Under zero PO$_4^{3-}$ loading, goethite ($\alpha$FeOOH) formed first, followed by mackinawite (FeS) and siderite (FeCO$_3$). In contrast, in higher PO$_4^{3-}$ treatments, vivianite ($\text{Fe}_3(\text{PO}_4)_2$) and/or sulfate green rust ($\text{Fe}^{II}_{14}\text{Fe}^{III}_{2}(\text{OH})_{12}\text{SO}_4$) became increasingly important over time at the expense of goethite and mackinawite compared to PO$_4^{3-}$-free conditions. The findings imply that PO$_4^{3-}$ loading alters the rates and onset of microbial Fe(III)- and SO$_4^{2-}$- reduction and the subsequent formation of secondary Fe-bearing phases. In addition, schwertmannite reduction and the associated mineralogical evolution under anoxic conditions appears to sequester large quantities of PO$_4^{3-}$ in the form of green rusts and vivianite.

Keywords: acid sulfate systems; green rust; stability; microbial reduction; X-ray diffractometry; X-ray spectroscopy
Phosphate loading alters schwertmannite transformation rates and pathways during microbial reduction

Graphical Abstract:
4.2 Introduction

Acid sulfate systems have low pH as a result of reduced inorganic sulfur (S) oxidation (Fitzpatrick, 2003). These systems, which include acid mine drainage (AMD) and acid-sulfate soils (ASS), often contain the iron (Fe) mineral schwertmannite (Bigham and Nordstrom, 2000b; Regenspurg et al., 2004). Schwertmannite is an Fe(III) oxyhydroxysulfate with the idealized formula $\text{Fe}_8\text{O}_8(\text{OH})_8\cdot x\text{SO}_4\cdot n\text{H}_2\text{O}$ where $1 < x < 1.75$ (Bigham et al., 1996; Bigham and Nordstrom, 2000b; Blodau, 2004; Jönsson et al., 2005).

A variety of Fe(III) minerals, including schwertmannite, are important terminal electron acceptors in waterlogged soils and sediments (Fredrickson and Gorby, 1996; Jones et al., 2006). The reduction of these minerals is facilitated by dissimilatory Fe(III)- and sulfate (SO$_4^{2-}$)-reducing bacteria. The reduction of Fe(III) and SO$_4^{2-}$ in schwertmannite plays a large role in mediating the pH of acid-sulfate systems and the subsequent pH-dependent release of trace elements (Antelo et al., 2013b; Fukushi et al., 2003a; Johnston et al., 2012; Regenspurg and Peiffer, 2005).

Schartmannite is metastable with respect to goethite ($\alpha\text{FeOOH}$) (Bigham et al., 1996; Burton et al., 2008b) and is unstable under reducing conditions and circum-neutral pH (Bigham et al., 1996; Collins et al., 2010; Jönsson et al., 2005; Knorr and Blodau, 2007b; Regenspurg et al., 2004). Reductive dissolution of schwertmannite via Fe(III)-reduction both raises the pH and liberates SO$_4^{2-}$ ions, which can be subject to dissimilatory SO$_4^{2-}$ reduction (Blodau, 2006b; Knorr and Blodau, 2007b). These processes drive the formation of various secondary Fe and S mineral products. For example, in experiments examining the reductive transformation of schwertmannite-rich sediment, Fe(III) and SO$_4^{2-}$ reduction led to the formation of elemental S, siderite (FeCO$_3$), goethite, and mackinawite (FeS) (Burton et al., 2007; Burton and Johnston, 2012). Schertmannite dissolution and the formation of secondary minerals have the potential to liberate and/or consume trace metals or excess nutrients (Antelo et al., 2013b; Fukushi et al., 2003a; Johnston et al., 2012, 2011a; Regenspurg and Peiffer, 2005).

Phosphate (PO$_4^{3-}$) is an abundant nutrient in acid sulfate systems (Collins et al., 2010) that can contribute to eutrophication when present in high concentrations (P C M Boers et al., 1998). Schwertmannite has been shown to be capable of sorbing large amounts of PO$_4^{3-}$ (Eskandarpour et al., 2006;
Phosphate loading alters schwertmannite transformation rates and pathways during microbial reduction

Schoepfer et al., 2017), with (Collins et al., 2010) showing the presence of large quantities of PO$_4^{3-}$ in natural schwertmannite from ASS environments. However, the impact of PO$_4^{3-}$ on schwertmannite reduction is largely unknown. In particular, the influence of PO$_4^{3-}$ on the reduction of Fe(III) and SO$_4^{2-}$ in schwertmannite-rich systems, as well as the secondary minerals that form in the presence of PO$_4^{3-}$ have not been fully investigated.

Some previous studies suggest that PO$_4^{3-}$ sorption inhibits Fe(III) reduction in ferrihydrite- and lepidocrocite-bearing systems (Borch et al., 2007; Borch and Fendorf, 2008; Galvez et al., 1999; O’Loughlin et al., 2013; Paige et al., 1997; Willett, 1985). However, other studies have demonstrated that PO$_4^{3-}$ can enhance Fe(III) reduction by scavenging the produced Fe(II) (Kukkadapu et al., 2004; Zachara et al., 2001). In a recent study, Schoepfer et al. (2017) showed that PO$_4^{3-}$ acted to stabilize schwertmannite against dissolution via Fe(III) reduction under oxygen-depleted conditions with the degree of stabilization being dependent on PO$_4^{3-}$ loading.

Although these studies provide some insights into likely schwertmannite-PO$_4^{3-}$ interactions under reducing conditions, to our knowledge, there are no long-term investigations exploring the effects of PO$_4^{3-}$ on schwertmannite stability and mineralogical transformation during prolonged exposure to reducing conditions. Particularly, very little information is available on the overall fate of schwertmannite-derived Fe and S, in addition to the secondary minerals formed during Fe(III) and SO$_4^{2-}$ reduction. Furthermore, no studies have described the effect of the reduction of Fe(III) and SO$_4^{2-}$ in schwertmannite on PO$_4^{3-}$ behaviour itself. Information on this interaction is crucial, especially in schwertmannite-rich environments vulnerable to eutrophication that have received substantial internal or external PO$_4^{3-}$ loadings.

The objective of this study is to examine how PO$_4^{3-}$ loading in an anoxic schwertmannite-rich system affects Fe(III) and SO$_4^{2-}$ reduction, and how it influences subsequent formation of secondary minerals and the resulting behaviour of PO$_4^{3-}$. To achieve this, schwertmannite suspended in artificial groundwater was dosed with varying quantities of PO$_4^{3-}$ and subjected to microbially-mediated reduction for 82 days.
4.3 Methods

4.3.1 Schwertmannite synthesis

Schwertmannite was precipitated from a ferrous sulfate solution using the H$_2$O$_2$ method as described previously (Regenspurg et al., 2004; Schoepfer et al., 2017).

4.3.2 Reductive incubation experiment

Experiments used gas-tight, 2-L Pyrex reaction vessels with 1.5-L of deoxygenated artificial groundwater (Schoepfer et al., 2017) and 18.75 g synthetic schwertmannite. The PO$_4^{3-}$ was added to the schwertmannite suspensions as KH$_2$PO$_4$ at 0, 80, 400, and 800 µmoles PO$_4^{3-}$ g$^{-1}$ schwertmannite (hereby termed zero, low, medium, and high PO$_4^{3-}$ loadings, (Schoepfer et al., 2017). Four replicate reaction vessels were assessed per PO$_4^{3-}$ treatment. The artificial groundwater was inoculated with ~2% (w/w) anoxic, benthic sediment collected from a drainage canal in the Tuckean Swamp in eastern Australia to introduce reducing microorganisms to the batch reactors (Burton et al., 2013). A most probable number (MPN) count determined the approximate number of Fe(III) and SO$_4^{2-}$ reducers in the initial sediment using the procedure described by Benner et al. (2000) and the calculations of Garthright and Blodgett (2003). This method indicated a density of Fe(III)-reducing bacteria of ~10$^{2.96}$ MPN and a cell density of SO$_4^{2-}$ reducers of ~10$^{3.64}$ MPN.

Strictly O$_2$-free conditions were maintained by placing each capped reaction vessel within a Coy anaerobic chamber (95% N$_2$ and 5% H$_2$) kept at room temperature. Schwertmannite suspensions were homogenized every two to three days for the course of the experiment. At sampling times of 2, 5, 9, 13, 19, 26, 33, 40, 47, 54, 61, 68, and 82 days (Burton et al., 2013, 2007; Burton and Johnston, 2012), reaction vessels were opened within the anoxic chamber and syringes were used to remove a 50 mL sample of homogenized suspension. These samples were centrifuged and the aqueous samples were filtered under O$_2$-free conditions to <0.45 µm and retained for analysis. The solid phase was allowed to dry under O$_2$-free conditions, with the use of methanol to speed drying for X-ray diffractometry and X-ray absorption spectroscopy (Schoepfer et al., 2017).
4.3.3 Analytical methods

$E_h$ and pH were determined with probes as calibrated against Zobell’s solution (Zobell, 1946) and pH 4 and 7 buffers, respectively. Filtrate aliquots were added to a mixed reagent to quantify alkalinity (Sarazin et al., 1999) or stored at 4 degrees Celsius with a 20% zinc acetate/2M NaOH solution to preserve the samples for subsequent sulfide analysis via the methylene blue method of Cline (1969). Remaining aqueous samples were acidified (with ultrapure HCl) and stored at 4 degrees Celsius for analysis of Fe(II), Fe(III), PO$_4^{3-}$, and SO$_4^{2-}$. Fe(II) concentrations were determined using the 1, 10-phenanthroline method of APHA (2005). Following the same method, samples were pre-reduced using hydroxylamine to determine total Fe with Fe(III) determined by subtraction. Phosphate was measured by the molybdenum blue method of Hansen and Koroleff in Grasshoff et al. (1999). Total aqueous sulfur was determined using a Perkin Elmer Optima 4300 DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) and SO$_4^{2-}$ was estimated through the subtraction of aqueous sulfide from total aqueous sulfur.

4.3.4 Solid phase X-ray diffraction

X-ray diffraction (XRD) was used to determine changes in Fe mineralogy over time. Solid-phase samples (dried under O$_2$-free conditions) were scanned from 10.00 °2θ to 80.73 °2θ using a 0.48 °2θ step and a one second count time on a Bruker D4-Endeavor diffractometer with a Co X-ray tube. Samples were exposed to atmospheric oxygen for 25 minutes during the scan.

4.3.5 Acid Volatile Sulfide

Frozen 20 mL suspension samples were thawed, centrifuged and the excess liquid decanted, and immediately analyzed for acid volatile sulfide (AVS) following the diffusion method (Hsieh et al., 2002) as modified by Burton et al. (2007). Briefly, ten milliliters of a 6M HCl/0.1M ascorbic acid solution was added to the sample in a 55 cm$^3$ reactor. While slowly shaking upright on an orbital shaker for 18 hours, the evolved H$_2$S gas was trapped in a secondary 10 cm$^3$ reactor, which contained a 2M NaOH and 4% zinc acetate solution. The trapped H$_2$S was subsequently quantified using iodometric titration.
4.3.6 Scanning Electron Microscopy

Initial schwertmannite and final samples (day 82) from each treatment were examined by scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) using a Leica Leo 440 Stereoscan with an ISIS energy dispersion microanalysis system. Dried samples were mounted on aluminum stubs and coated with carbon prior to analysis for elemental composition and mineral morphology.

4.3.7 X-ray absorption spectroscopy

Solid samples (day 82) were analyzed at the National Synchrotron Radiation Research Center (NSRRC) in Hsinchu, Taiwan on beamline 17C. Samples for Fe K-edge extended X-ray absorption fine structure (EXAFS) spectroscopy were transported under O₂-free conditions and mounted at room temperature immediately prior to analysis. Reference standards included mackinawite, schwertmannite, goethite, sulfate green rust, siderite, and vivianite as suggested by the literature and XRD analysis, and were prepared as per Burton and Johnston (2012). The ATHENA program was used for background subtraction and normalization, as well as linear combination fitting of the 3-weighted EXAFS oscillations in the 2-10 Å⁻¹ range (Ravel and Newville, 2005).

4.3.8 Modelling

Visual Minteq was used to calculate saturation indices and equilibrium speciation in solution. All species present in the initial solution as well as each analytically derived species was included in the modeling.

4.4 Results

4.4.1 Initial schwertmannite properties

The initial material used in this study was schwertmannite with a minor goethite presence, as confirmed by XRD and reported in Schoepfer et al. (2017). The initial mineral contained 7.4 ± 0.1 mmoles g⁻¹ Fe and 1.5 ± 0.03 mmoles g⁻¹ S (Schoepfer et al., 2017). This corresponds to a composition of Fe₈O₈(OH)₄.96(SO₄)₁.65, which is within the range reported for schwertmannite (Acero et al., 2006; Burton et al., 2010; Burton and Johnston, 2012; Regenspurg et al., 2002).
4.4.2 Aqueous phase reductive incubation parameters

Initial PO₄³⁻ addition did not significantly influence the pH of the starting solutions (pH ~2.7 at day 2 in all treatments). The pH continuously rose until reaching pH 6.4, 6.8, 6.9, and 6.7 at day 40, 54, 82, and 47 for the zero, low, medium, and high PO₄³⁻ treatments, respectively (Figure 4.8.1 a-d).

Aqueous PO₄³⁻ was very low in the zero and low PO₄³⁻ treatments throughout the incubation (Figure 4.8.1 e-f). PO₄³⁻ was much higher initially in the medium (2.1 mM) and high PO₄³⁻ (6.7 mM) treatments. However, these PO₄³⁻ concentrations decreased rapidly over time, falling below detection (<0.5 µM) at day 9 and 26 in the medium and high PO₄³⁻ treatments, respectively (Figure 4.8.1 g-h).

Large quantities of aqueous Fe(II) were released over time in all PO₄³⁻ treatments, with the highest Fe(II) concentration occurring in the low PO₄³⁻ treatment (Figure 4.8.1 i-l). All PO₄³⁻ treatments showed two distinct phases in Fe(II) production: an initial slow phase and a secondary rapid phase of Fe(II) production. Aqueous Fe(II) reached a maximum concentration of 27 mM at day 40 in the zero PO₄³⁻ treatment after a change in Fe(II) production rates around Day 13. In the low and high PO₄³⁻ treatments, the rate of production changed at about day 33, and achieved a peak of 34 mM and 26 mM, respectively. In the medium PO₄³⁻ treatment, the Fe(II) production rate changed at Day 40, and Fe(II) did not peak until day 68 at 18 mM Fe(II). All concentrations decreased rapidly following the peak. In contrast to Fe(II), negligible Fe(III) was found in solution throughout the experiment in the zero, low, and high PO₄³⁻ treatments, with a slight increase in Fe(III) concentrations in the low PO₄³⁻ treatment from day 60 onwards (Figure 4.8.1 i-l).

Aqueous SO₄²⁻ concentrations varied as determined by the PO₄³⁻ content and time (Figure 4.8.1 m-p). Sulfate increased rapidly in the zero PO₄³⁻ treatment, and slightly slower in the low PO₄³⁻ treatment to a maximum of 12.3 mM (33 days) and 12.1 mM (40 days) respectively. The medium PO₄³⁻ treatment had released 9.6 mM SO₄²⁻ by 47 days and the high PO₄³⁻ treatment had released 11.1 mM by day 40. Following these peaks, aqueous SO₄²⁻ decreased rapidly. There was no aqueous sulfide detected at any time.

Concentrations of acid volatile sulfide (AVS) were below detection in all PO₄³⁻ treatments for the first 26, 47, 47, and 47 days in the zero, low, medium and high PO₄³⁻ treatments (Figure 4.8.1 m-p). Following this, concentrations
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increased to approximately 9.8 mM, 8.7 mM, 3.7 mM, and 7.6 mM AVS by Day 82 in the zero through to high PO$_4^{3-}$ treatments.

HCO$_3^-$ increased slowly over the first 26, 33, 40, and 33 days in the zero, low, medium, and high PO$_4^{3-}$ treatments, respectively (Figure 4.8.1 q-t). Following this slow production of alkalinity, a rapid increase occurred for each treatment to reach a maximum of 49 mM, 51 mM, 38 mM, and 54 mM HCO$_3^-$ in the zero, low, medium, and high PO$_4^{3-}$ treatments. Concentrations then decreased to 38 mM, 40 mM, 38 mM, and 43 mM HCO$_3^-$ at Day 82.

4.4.3 X-ray diffractometry and scanning electron microscopy

There were substantial changes in the XRD patterns over the entire study period in all treatments (Figure 4.8.2 a-d). In both the zero and low PO$_4^{3-}$ treatments (Figure 4.8.2 a-b), there was clear evidence for schwertmannite transformation to goethite by Day 26. By Day 47, these XRD patterns showed signs of mackinawite. By Day 82, additional siderite was present in both lower PO$_4^{3-}$ treatments. SEM-EDX observations confirm the Day 82 XRD observations (Figure 4.8.3 a-b and Table 4.8.1).

In the medium PO$_4^{3-}$ treatment (Figure 4.8.2 c), the XRD results indicate that the initial schwertmannite remained apparently unchanged until Day 47. However, by Day 82, schwertmannite had been replaced by the more crystalline goethite, siderite, and vivianite, with some additional mackinawite also present. All mineral phases were identified in the SEM images of samples collected at Day 82 (Figure 4.8.3 c and Table 4.8.1).

In the high PO$_4^{3-}$ treatment (Figure 4.8.2 d), schwertmannite (with traces of pyrite from the inoculating soil) remained through Day 26. By Day 47, vivianite and mackinawite with small amounts of goethite were present. At Day 82, siderite had formed in addition to the previous minerals, with all minerals being present in the SEM images (Figure 4.8.3 d and Table 4.8.1).

SEM-EDX data were categorized by a combination of morphology and composition to determine any PO$_4^{3-}$ fractionation among mineral species (Figure 4.8.4 a-b). The micromorphology of the zero PO$_4^{3-}$ treatment showed particulates ranging from a morphology and composition of pyrite to mackinawite, schwertmannite and siderite/goethite. The minerals in the low PO$_4^{3-}$ treatment were consistent with several of the same morphologies as the zero PO$_4^{3-}$ treatment, including schwertmannite with slight associated P. P was
mostly associated with Fe oxide minerals in the medium PO$_4^{3-}$ treatment rather than reduced minerals. Additionally, the ferrous phosphate, vivianite, was present. The high PO$_4^{3-}$ treatment had abundant vivianite and some of this vivianite was enriched in S.

4.4.4 X-ray absorption spectroscopy

Iron EXAFS data were collected on the final Day 82 samples and linear combination fits were applied (LCF, Figure 4.8.5 and Table 4.8.2). Notably, 56% and 20% of the final solid phase Fe was still in the schwertmannite form at Day 82 in the medium and high PO$_4^{3-}$ treatments, respectively. In contrast, at day 82 in the zero and low treatments, residual schwertmannite was less than ~10%. At Day 82, 40% of solid-phase Fe in the zero PO$_4^{3-}$ treatment was present as goethite, while this percentage was below detection in the high PO$_4^{3-}$ treatment. Mackinawite made up 27, 24, 13, and 16% of the final solid phase Fe in the zero, low, medium and high PO$_4^{3-}$ treatments, respectively. Vivianite was present in appreciable quantities in the high PO$_4^{3-}$ treatment only, comprising 13% of the final solid phase Fe. Siderite was not required in the LCF of the EXAFS spectrum for the medium PO$_4^{3-}$ treatment (indicating that it was present at <5% of solid-phase Fe), but was between 25 and 34% of the solid phase Fe in the other treatments. Sulfate green rust was present in the low, medium and high PO$_4^{3-}$ treatments, but was absent from the zero PO$_4^{3-}$ treatment (Table 4.8.2).

4.5 Discussion

4.5.1 Microbial Fe(III) reduction

Schwertmannite-rich systems often exhibit a low pH which can be brought to circumneutral values through the microbially-mediated reduction of schwertmannite-derived Fe(III) (Blodau, 2006b, 2004; Burton et al., 2013; Burton and Johnston, 2012; Regenspurg et al., 2002) (Equation 4.1).

$$3\text{Fe}_8\text{O}_8(\text{OH})_6\text{SO}_4 + \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} \rightarrow 24\text{Fe}^{2+} + 3\text{SO}_4^{2-} + 6\text{CO}_2 + 42\text{OH}^-$$

Eq. 4.1

The reduction of schwertmannite-Fe(III) to Fe(II) and the consequent pH increase initially occurred slowly compared to some previous studies (Figure 4.8.1). For example, similar schwertmannite reductive incubations in the absence of PO$_4^{3-}$ observed extensive Fe(III) reduction within just a few days (Burton et al., 2007; Jones et al., 2006). Burton et al. found maximum Fe(II)
concentrations within 10 days (Burton et al., 2013; Burton and Johnston, 2012), as opposed to the maximum Fe(II) concentration at 40 days in the zero \( \text{PO}_4^{3-} \) treatment of this study. The relatively slow reaction rate observed here has implications for further processes dependent on the pH, such as sulfate reduction (Peine et al., 2000) and subsequent secondary mineral formation.

In addition to \( \text{PO}_4^{3-} \) sorption onto the schwertmannite surface, strengite precipitation is feasible, as strengite was supersaturated throughout the experiment in the medium and high \( \text{PO}_4^{3-} \) treatments (Equation 4.2):

\[
\text{Fe}^{3+} + \text{PO}_4^{3-} + 2\text{H}_2\text{O} \rightarrow \text{FePO}_4 \cdot 2\text{H}_2\text{O}
\]

Eq. 4.2

Although supersaturated, strengite was not apparent in either the XRD patterns or the linear combination fits of the Fe K-edge EXAFS spectra. However, had all \( \text{PO}_4^{3-} \) precipitated as strengite, this would only amount to roughly 0, 1%, 5%, and 10% of the total Fe, making detection and quantification difficult.

Adsorption of \( \text{PO}_4^{3-} \) and Fe(III)-P precipitate formation on schwertmannite surfaces may act as a physical barrier to further schwertmannite dissolution (Rothe et al., 2016; Willett, 1985; Willett and Cunningham, 1983). Consequently, the three higher \( \text{PO}_4^{3-} \) treatments demonstrated increased lag times before the rapid Fe(III) reduction phase began (Figure 4.8.1 i-l). In comparison, O’Loughlin et al. (2010) observed a 24 hour delay in the onset of rapid reduction in \( \text{PO}_4^{3-} \)-dosed lepidocrocite. Although the magnitude of these delays are vastly different, this may be attributed to the robustness of the reducing bacteria, \( \text{Shewanella putrefaciens} \) late log phase in O’Loughlin et al. (2010), as compared to a disturbed natural assemblage in this study.

Once rapid Fe(III) reduction began, the reduction rate followed a complex dependency on the level of \( \text{PO}_4^{3-} \) loading (0.9, 1.8, 0.5, and 1.4 mmoles Fe(II) L\(^{-1}\) day\(^{-1}\) produced in the zero, low, medium and high \( \text{PO}_4^{3-} \) treatments, respectively, Figure 4.8.2 i-l). These rates were determined through linear regression of the Fe(II) concentrations during the rapid increase in Fe(II) levels. The Fe(III) reduction rate at zero \( \text{PO}_4^{3-} \) loading may be affected by the transformation to goethite, which is likely to be less bioavailable than the initial schwertmannite (Schoepfer et al., 2017, Figure 4.8.2 a). In contrast, at low \( \text{PO}_4^{3-} \) loadings, an elevated reduction rate may occur as a result of scavenging of Fe(II) from solution by precipitation with \( \text{PO}_4^{3-} \) as discussed in Kukkadapu...
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et al., Schoepfer et al., and Zachara et al. (Kukkadapu et al., 2004; Schoepfer et al., 2017; Zachara et al., 2001).

The medium PO$_4^{3-}$ treatment shows limited and slower onset of Fe(III) reduction. This is likely due to PO$_4^{3-}$ acting to stabilize the schwertmannite surface against dissolution as PO$_4^{3-}$ loading increased (Borch et al., 2007; Schoepfer et al., 2017), as was observed with ferrihydrite (Borch and Fendorf, 2008). However, this is not a linear relationship with PO$_4^{3-}$ loading, and in comparison, excess PO$_4^{3-}$ is found in the high PO$_4^{3-}$ treatment. Precipitation of PO$_4^{3-}$ with the excess Fe(II) derived from schwertmannite reduction would enhance reduction at the higher PO$_4^{3-}$ loadings when compared to the medium PO$_4^{3-}$ treatment.

4.5.2 Goethite crystallization

The XRD and XAS data show clear evidence of goethite formation in the zero and low PO$_4^{3-}$ treatments (Equation 4.3, Figure 4.8.2).

$$\text{Fe}_8\text{O}_8(\text{OH})_6\text{SO}_4 + 2\text{H}_2\text{O} \rightarrow 8\text{FeOOH} + \text{SO}_4^{2-} + 2\text{H}^+ \quad \text{Eq. 4.3}$$

This is consistent with schwertmannite being metastable with regard to goethite (Bigham et al., 1996). A major pathway for the transformation of schwertmannite to goethite under Fe(III)-reducing conditions is the Fe(II)-induced transformation (Burton et al., 2007). Previous studies have indicated that under low pH values (as found at the beginning of this study), the transformation to goethite is slow, in the order of hundreds of days for complete transformation (Acero et al., 2006; Bigham et al., 1996; Collins et al., 2010; Jönsson et al., 2005; Knorr and Blodau, 2007b; Peine et al., 2000; Regenspurg et al., 2004). However, in the presence of high Fe(II) concentrations and near-neutral pH values (such as towards the second half of the experiment), the Fe(II)-induced pathway can catalyze the transformation to goethite within hours or days (Burton et al., 2008b, 2007; Jones et al., 2009), especially in the absence of sorbing anions (Burton et al., 2013, 2008b, 2007; Burton and Johnston, 2012).

Studies describing the occurrence of the Fe(II)-induced pathway typically do not demonstrate the increasing importance of this pathway in biotic systems over time as the aqueous-phase composition and biogeochemical conditions change. Generally, studies observe either the presence, absence, or rate of this pathway in targeted abiotic studies in relation to pH (Burton et al., 2008b;
Handler et al., 2014) or co-occurring ions (Burton et al., 2010; Hinkle et al., 2015; Jones et al., 2009; Paikaray et al., 2017b). In this study, due to the attenuated progression of the chemical reactions, our results are consistent with the increasing importance of this pathway in a biotic setting over time. For the first 13 days in the zero $\text{PO}_4^{3-}$ treatment, little goethite was present (Figure 4.8.2 a) as the Fe(II) production was low (Figure 4.8.1). From day 26 through 47, Fe(III) reduction in the zero $\text{PO}_4^{3-}$ treatment was rapid, and Fe(II) was increasingly abundant in solution (Figure 4.8.1 i). Concurrent with this sharp Fe(II) increase, goethite peak height clearly increased in the XRD patterns (Figure 4.8.2 a). This suggests the increasing importance of the Fe(II)-induced pathway in the zero $\text{PO}_4^{3-}$ treatment with the evolution of Fe(II) and the pH increase in the reactors.

The extensive formation of goethite under zero $\text{PO}_4^{3-}$ loading may additionally be linked to P-limited cell growth of Fe(III) reducers, thereby enabling the ageing to goethite rather than bacterial Fe(III) reduction (Glasauer et al., 2003). Consequently, goethite is most abundant in the zero $\text{PO}_4^{3-}$ treatment, with 40% of the solid phase Fe as goethite at Day 82 (Figure 4.8.5 and Table 4.8.2).

In the present study, the extent of goethite formation decreased with increasing $\text{PO}_4^{3-}$ loading (40.1, 29.6, 16.5, and 0% Fe as goethite in the zero, low, medium and high $\text{PO}_4^{3-}$ treatments, respectively, Table 4.8.2 and Figure 4.8.5) with schwertmannite making up an increasing fraction of the final solid Fe mineral with the exception of the high $\text{PO}_4^{3-}$ treatment. The changing balance between relative schwertmannite and goethite abundances is largely due to increasing schwertmannite stability with increasing $\text{PO}_4^{3-}$ (Schoepfer et al., 2017). Increasing $\text{PO}_4^{3-}$ is known to retard the transformation of Fe(III) oxides to goethite (Borch et al., 2007; Borch and Fendorf, 2008; Refait et al., 2007), with $\text{PO}_4^{3-}$ loading of surface sites acting as a barrier to the transformation of the initial Fe oxide mineral (Bocher et al., 2004; Refait et al., 2007). Phosphate-induced stabilization of schwertmannite also diminishes the amount of Fe(III) dissolved, as has been previously observed in phosphate-bearing ferrihydrite (Borch et al., 2007; Borch and Fendorf, 2008; Galvez et al., 1999; Willett, 1985) and arsenate-bearing schwertmannite (Regenspurg, 2003; Regenspurg et al., 2002; Regenspurg and Peiffer, 2005). As ferrihydrite and schwertmannite are somewhat similar in regards to their anion sorption
Phosphate loading alters schwertmannite transformation rates and pathways during microbial reduction (Antelo et al., 2012; Eynard, 1993; Paikaray et al., 2011; Regenspurg and Peiffer, 2005), and PO$_4^{3-}$ and arsenate are similar in structure and behaviour (Antelo et al., 2005; Burton et al., 2010, 2009; Regenspurg and Peiffer, 2005), stabilization and persistence of schwertmannite by PO$_4^{3-}$ is expected.

4.5.3 Phosphate mobility

Iron(II)-P mineral formation has previously been found to limit P mobilization upon reductive dissolution of Fe(III)-bound P (Borch and Fendorf, 2008; Rothe et al., 2016), which is consistent with the absence of detectable PO$_4^{3-}$ concentrations in the latter part of this experiment (Figure 4.8.1 e-h). Although it is assumed PO$_4^{3-}$ is retained in neo-formed solid species, often no clear solid Fe-P species are observed, even in Fe-rich systems (Benner et al., 2002; Borch and Fendorf, 2008; Fredrickson et al., 1998; Glasauer et al., 2003). This was the case in the low, medium, and high PO$_4^{3-}$ treatments (Figure 4.8.5 and Table 4.8.2), although some highly crystalline vivianite was observed in the medium treatment based on the XRD patterns and SEM images (Figures 4.8.2 c and 4.8.3 c). Vivianite was only detected in substantial quantities in the high PO$_4^{3-}$ treatment via Fe K-edge EXAFS spectroscopy. Had all PO$_4^{3-}$ been sequestered into vivianite, this would amount to 0.0, 1.8, 9.7, and 18.0% of the total solid phase Fe in the zero, low, medium and high PO$_4^{3-}$ treatments. In accord with these percentages, vivianite comprised 12.6% of the solid phase Fe, Table 4.8.2. This similarity between the theoretical and observed percentage is consistent with supersaturation with respect to vivianite only in the high PO$_4^{3-}$ treatment as determined through Visual MINTEQ modelling.

Sulfate green rust was identified in the three higher PO$_4^{3-}$ treatments via EXAFS spectroscopy (Equation 4.4, Table 4.8.2 and Figure 4.8.5). It was not observed using XRD - likely due to the presence and abundance of more crystalline species such as goethite or vivianite, which would readily mask the small green rust diffraction peak (Figure 4.8.2). Green rusts are reactive mixed-valence Fe(II)/Fe(III) minerals which form as double layered minerals containing, most commonly, carbonate, sulfate or chloride (Genin et al., 2001). Carbonate and chloride green rusts incorporate planar ions, while sulfate green rusts host tetrahedral sulfate within the plate-like structure (Genin et al., 2001). Despite the observation that PO$_4^{3-}$ may facilitate sulfate green rust formation
(Kukkadapu et al., 2004), the quantity of \( \text{PO}_4^{3-} \) did not substantially impact the amount of sulfate green rust formed (Table 4.8.2). Therefore, another factor may have controlled sulfate green rust abundance, such as low aqueous sulfate abundances at the end of the experiment to which limited microbial sulfate reduction contributed.

\[
2\text{Fe}^{3+} + \text{SO}_4^{2-} + 4\text{Fe}^{2+} + 12\text{OH}^- \rightarrow \text{Fe}^{II}_{4}\text{Fe}^{III}_{2}(\text{OH})_{12}\text{SO}_4
\]

Eq. 4.4

Several studies suggest \( \text{PO}_4^{3-} \) can inhibit the partial reduction of green rust, resulting in a fully ferric green rust end member (Kukkadapu et al., 2004; O’Loughlin et al., 2013). Phosphate can also exchange with up to 50% of the sulfate in the inner layers of sulfate green rust during ferrihydrite reductive dissolution (Hansen and Poulsen, 1999; Rothe et al., 2016). Other studies suggest a phosphate green rust exists (i.e. metavivianite, \( \text{Fe}^{II}_{4}\text{Fe}^{III}_{2}(\text{PO}_4)_2(\text{OH})_{2}*6\text{H}_2\text{O} \)) (Rothe et al., 2016), but appreciable quantities are needed for its identification in mixed mineral systems (Guilbaud et al., 2013; Hansen and Poulsen, 1999).

In \( \text{PO}_4^{3-} \)-bearing systems, green rust is often considered a precursor to vivianite (Borch and Fendorf, 2008; Glasauer et al., 2003; Hansen and Poulsen, 1999; Ruby et al., 2006). This progression from sulfate green rust to vivianite possibly starts through the slowed reduction of Fe(III), induced by \( \text{PO}_4^{3-} \) coverage (Kukkadapu et al., 2004; O’Loughlin et al., 2013). This is followed by substitution of sulfate by phosphate in the green rust mineral structure (Hansen and Poulsen, 1999; Rothe et al., 2016). With continued reduction and substitution, sulfate green rusts may be replaced by vivianite (Benner et al., 2002; Borch and Fendorf, 2008; Glasauer et al., 2003).

Vivianite was not observed in the low and medium \( \text{PO}_4^{3-} \) treatments (Figure 4.8.5). In the low \( \text{PO}_4^{3-} \) treatment, Fe(II) production was rapid and substantial, but \( \text{PO}_4^{3-} \) quantities were apparently insufficient for vivianite precipitation (Borch and Fendorf, 2008; Glasauer et al., 2003; Kukkadapu et al., 2004). In the medium \( \text{PO}_4^{3-} \) treatment, where \( \text{PO}_4^{3-} \) likely stabilizes the Fe(III) (as evident in the large quantity of schwertmannite remaining at Day 82, Figure 4.8.5), strong interactions between Fe(III) and \( \text{PO}_4^{3-} \) likely limited vivianite precipitation and thus allowed for the slower green rust formation pathway. In this medium \( \text{PO}_4^{3-} \) treatment, some highly crystalline vivianite was apparent in the XRD patterns, but not in large enough quantities for detection by EXAFS.
In the high PO$_4^{3-}$ treatment, large quantities of PO$_4^{3-}$ were present in solution for several weeks. It is possible that vivianite precipitation occurred as a result of continued complexation between Fe(III) and PO$_4^{3-}$ and the subsequent Fe(III) reduction, rather than through the intermediate green rust pathway (Borch and Fendorf, 2008; Fredrickson et al., 1998; Glasauer et al., 2003; Kukkadapu et al., 2004; Willett, 1985), Equation 4.5).

$$2\text{PO}_4^{3-} + 3\text{Fe}^{2+} + 8\text{H}_2\text{O} \rightarrow \text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$$  Eq. 4.5

### 4.5.4 Siderite precipitation

Biogenic siderite formation can be a slow process, occurring over weeks or months (Burton et al., 2013, 2007; Jensen et al., 2002; Jimenez-Lopez and Romanek, 2004). However, siderite was observed in the EXAFS datasets of the zero, low, and high PO$_4^{3-}$ treatments and is likely associated with the substantial production of both Fe(II) and HCO$_3^-$ (Eq. 4.6, Figure 4.8.1 q-t and 4.8.5).

$$\text{Fe}^{2+} + \text{HCO}_3^- \rightarrow \text{FeCO}_3 + \text{H}^+$$  Eq. 4.6

Siderite was also observed in the XRD pattern of the medium PO$_4^{3-}$ treatment, however this was not detected via EXAFS (Figure 4.8.5 and Table 4.8.2) or SEM (Figure 4.8.3 c and 4.8.4 c). This further supports slow formation, as the medium PO$_4^{3-}$ treatment was the last to produce considerable alkalinity (Figure 4.8.1).

### 4.5.5 SO$_4^{2-}$ reduction

Sulfate reduction occurred relatively late in the reductive incubation timeframe (Eq. 4.7).

$$6\text{H}^+ + 3\text{SO}_4^{2-} + \text{C}_6\text{H}_12\text{O}_6 \rightarrow 2\text{H}_2\text{S} + 6\text{CO}_2 + 6\text{H}_2\text{O}$$  Eq. 4.7

This was likely due to the abundance of easily accessible Fe(III) during the early stages of the experiments. In addition, the low pH during the early stages of the incubation experiment could have inhibited sulfate reduction (Peine et al., 2000).

Sulfate reduction rates were determined by finding the rate of change of aqueous SO$_4^{2-}$ from the peak concentration near the midpoint of the experiment to the end of the experiment. These estimated sulfate reduction rates did not vary significantly as a function of PO$_4^{3-}$ content (0.26, 0.29, 0.23, and 0.24 mmoles L$^{-1}$ day$^{-1}$). Due to the similarities in sulfate reduction rates between
treatments, sulfate reduction is not thought to be influenced directly by PO$_4^{3-}$ content under the experimental conditions considered here. Rather, PO$_4^{3-}$ influences Fe(III) reduction directly, which then impacts both the pH and consequent sulfate reduction.

Concurrent with sulfate reduction are the increase in acid-volatile sulfide concentrations and mackinawite formation (Figure 4.8.2 m-p, Figure 4.8.3, Eq. 4.8).

\[
\text{Fe}^{2+} + \text{H}_2\text{S} \rightarrow \text{FeS} + 2\text{H}^+ \\
\text{Eq. 4.8}
\]

The rapid precipitation of mackinawite in the presence of relatively high Fe(II) concentrations did not allow for aqueous sulfide to accumulate in solution to detectable levels. The percentage of mackinawite-Fe, as determined through EXAFS spectroscopy, decreased at elevated PO$_4^{3-}$ (27.4, 24.1, 12.5, and 16.2% of the solid phase Fe in the zero, low, medium and high PO$_4^{3-}$ treatments), with the medium PO$_4^{3-}$ treatment demonstrating the least mackinawite formation. The effect of PO$_4^{3-}$ on formation of these secondary phases is summarised in the conceptual model presented in Figure 4.8.6.

4.6 Conclusions

This study shows that the presence of high PO$_4^{3-}$ loading decreases the schwertmannite-Fe(III) reduction rate. The resulting prolonged presence of reducible Fe(III) and low pH conditions attenuates the onset of SO$_4^{2-}$ reduction. This strongly affects the timing of the formation as well as the abundance of the resulting secondary Fe minerals, such as goethite, vivianite, green-rust, siderite and mackinawite (Figure 4.8.6). With increased time under biotic conditions, the metastable schwertmannite transformed to its more stable counterpart, goethite, in the absence of PO$_4^{3-}$ (Figure 4.8.6). Microbial production of sulfide (H$_2$S) and bicarbonate (HCO$_3^-$) in the presence of abundant Fe(II) resulted in mackinawite and siderite as mineralogical products. With increased PO$_4^{3-}$ however, Fe mineralogy gradually shifted towards an abundance of sulfate green rust and vivianite at the expense of goethite and mackinawite (Figure 4.8.6).

The results show that schwertmannite reduction and associated secondary mineral formation is able to scavenge large amounts of PO$_4^{3-}$ from solution. This process has the potential to mitigate PO$_4^{3-}$-enriched environments (e.g. resulting from agricultural practices or wastewater discharge). The formation
of green rusts and vivianite early in the reductive dissolution process (prior to sulfate reduction) has the potential to prevent the release of PO$_4^{3-}$ back to the environment, thereby providing a sink for PO$_4^{3-}$ in eutrophic but non-sulfidic environments as recognized previously (Egger et al., 2015; Hsu et al., 2014; Rothe et al., 2016).

Higher PO$_4^{3-}$ concentrations result in lower mackinawite abundances (Figure 4.8.6). Mackinawite has a high affinity for many trace metal and metalloid contaminants (Hansen et al., 1996; Niazi and Burton, 2016b; Peng et al., 2009). As such, the decreased amount of mackinawite at high PO$_4^{3-}$ loadings may therefore drastically alter contaminant mobility in reduced acid sulfate environments.
ACKNOWLEDGEMENTS
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References


Phosphate loading alters schwertmannite transformation rates and pathways during microbial reduction


Phosphate loading alters schwertmannite transformation rates and pathways during microbial reduction


### Figures

#### Figure 4.8.1. Aqueous parameters

Aqueous pH (a-d), $\text{PO}_4^{3-}$ (e-h), Fe (i-l), $\text{SO}_4^{2-}$ (m-p) and $\text{HCO}_3^-$ (q-t) data and solid AVS data (m-p) as a function of time over the course of 82 days. Values are means of four replicates within each treatment with standard errors. Treatments include zero, low, medium and high $\text{PO}_4^{3-}$ loading, which correspond to 0, 80, 400, and 800 µmoles $\text{PO}_4^{3-}$ g$^{-1}$ schwertmannite.

<table>
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<tr>
<th>Zero $\text{PO}_4^{3-}$</th>
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<td><img src="image2.png" alt="Graph" /></td>
<td><img src="image3.png" alt="Graph" /></td>
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</table>

- Zero $\text{PO}_4^{3-}$
- Low $\text{PO}_4^{3-}$
- Medium $\text{PO}_4^{3-}$
- High $\text{PO}_4^{3-}$
Figure 4.8.2. X-ray diffractograms
Mineral evolution in the zero (a), low (b), medium (c), and high PO$_4^{3-}$ (d) treatments. Samples began as schwertmannite, with some pyrite from the inoculating soil in the medium and high treatments (not identified in the figure). G = goethite, V = vivianite, M = mackinawite, S = siderite.
Figure 4.8.3. SEM imagery

SEM imagery in the zero (a), low (b), medium (c), and high PO$_4^{3-}$ (d) treatments, where schwertmannite, goethite, vivianite, mackinawite, and siderite were observed. Numbers refer to EDX spot analysis and corresponding EDX elemental percentages can be found in Table 4.7.1.
Table 4.8.1. SEM-EDX spot analysis
Percent Fe, S, and P in each spot as analysed by SEM-EDX. Spectrum values correspond to numbers in Figure 3.

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<th>S %</th>
<th>P %</th>
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<td>21.13</td>
<td>2.58</td>
<td>1.65</td>
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Figure 4.8.4. SEM-EDX ternary analysis

SEM-EDX data as separated by treatment and identity (identity= combination of morphology and chemical composition), identifying the percentage of Fe, S, and P present at each spot analysed by EDX. The sum of Fe, S, and P equal 100%, although minor fractions of other varying elements were present in the zero (a), low (b), medium (c), and high PO$_4^{3-}$ (d) treatments.
Figure 4.8.5. EXAFS linear combination fits
Extended x-ray absorbance fine edge structure (EXAFS) data (solid lines) and linear combination fits (dashed lines). Samples were fit per treatment according to a combination of standards as identified using XRD identification and literature values. Numerical values for the fits are included in Table 4.7.2.
Table 4.8.2. Iron EXAFS linear combination fits

Iron EXAFS linear combination fits including fitting parameters. The sum of the weights was not forced to equal 1.

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<tr>
<th>Trt</th>
<th>Schwertmannite</th>
<th>Goethite</th>
<th>Machkinawite</th>
<th>Siderite</th>
<th>GR-Sulfate</th>
<th>Vivianite</th>
<th>R-factor</th>
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Phosphate loading alters schwertmannite transformation rates and pathways during microbial reduction and phosphate as a control on schwertmannite stability, iron mineralogy and anaerobic biogeochemistry in acid-sulfate systems.

Figure 4.8.6. Conceptual diagram

Conceptual diagram demonstrating the timeline of secondary mineral formation in a biotic schwertmannite system amended with different loadings of phosphate. Boxes refer to solid phase minerals, while aqueous phases are depicted in circles.
Chapter 5 - Contrasting effects of phosphate on the rapid transformation of schwertmannite to Fe(III) (oxy)hydroxides at near-neutral pH

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Objective: To determine the influence of phosphate on schwertmannite transformation to secondary Fe(III) oxyhydroxides, both before and after the addition of aqueous Fe(II). Schwertmannite is known to transform to several oxyhydroxides through the Fe(II) induced reaction. However, the influence of phosphate on this reaction is unknown. By using Mössbauer spectroscopy, this study aims to characterize the initial synthetic schwertmannite, schwertmannite exposed to varying phosphate loadings, and the resulting secondary minerals following Fe(II) addition. Phosphate has been suspected to stabilize schwertmannite, however this characterization is incomplete. Natural schwertmannite rich sediments are thought to transform to goethite through the Fe(II) induced reaction, however the addition of phosphate, a common nutrient and/or pollutant in many environments, has unknown consequences for the stability of schwertmannite and other Fe(III) oxyhydroxides.

Author Contributions:
Concept and Design: Schoepfer and Burton
Laboratory analysis: Schoepfer
Mössbauer spectroscopy data collection: Nigel Dawson
Data interpretation: Schoepfer and Burton
Original draft writing: Schoepfer
Proofreading and editing: Burton and Johnston

See Appendix 1 for signed author contribution
5.1 Abstract

Schwertmannite is a metastable Fe(III)-bearing mineral that can rapidly transform to more stable Fe(III) oxides upon Fe(II) sorption, via a dissolution-reprecipitation pathway. Phosphate (PO$_4^{3-}$) is a nutrient found in varying quantities in natural systems, but the influence of PO$_4^{3-}$ on the Fe(II) induced transformation of schwertmannite is not well characterized. Here, we aim to quantify the aqueous and mineralogical effects of varied PO$_4^{3-}$ loading, and subsequent Fe(II) loading, to schwertmannite at circumneutral pH. Phosphate was presorbed to schwertmannite at loadings of 0, 80, 400 and 800 µmoles g$^{-1}$ in anoxic artificial groundwater. Fe(II) was then added at a final concentration of 12.5 mM and aqueous and mineralogical products were observed over 10 days. No transformation occurred before Fe(II) addition in the zero PO$_4^{3-}$ treatment. In contrast, the three highest PO$_4^{3-}$ treatments partially transformed to a microcrystalline Fe(III) oxyhydroxide before Fe(II) addition, possibly due to exchange of sulfate with the added PO$_4^{3-}$. Within one hour of subsequent Fe(II) addition in the zero PO$_4^{3-}$ treatment, all schwertmannite had transformed to goethite via the Fe(II)-induced transformation pathway. Increases in the level of PO$_4^{3-}$ loading attenuated goethite precipitation via Fe(II)-induced transformation of the precursor schwertmannite and microcrystalline Fe(III) oxyhydroxide, with 65%, 34%, and 21% solid phase Fe as goethite at day 10 in the low (80 µmoles g$^{-1}$), medium (400 µmoles g$^{-1}$) and high (800 µmoles g$^{-1}$) PO$_4^{3-}$ treatments. Under low PO$_4^{3-}$ loading, the addition of Fe(II) induced the transformation of the microcrystalline Fe(III) oxyhydroxide and schwertmannite to a mixture of lepidocrocite and goethite. In the medium and high PO$_4^{3-}$ treatments, schwertmannite and the microcrystalline Fe(III) oxyhydroxide were instead stabilized by surface-complexation of PO$_4^{3-}$ and did not extensively transform to more crystalline phases. Overall, the results show that PO$_4^{3-}$ loadings can both drive rapid schwertmannite transformation to a new microcrystalline Fe(III) oxyhydroxide phase, and subsequently inhibit the Fe(II)-induced formation of lepidocrocite and goethite.

Keywords: Lepidocrocite; Fe(II) catalyzed; phosphate inhibition; goethite; microcrystalline Fe(III) oxyhydroxide
Contrasting effects of phosphate on the rapid transformation of schwertmannite to Fe(III) (oxy)hydroxides at near-neutral pH

Graphical Abstract
5.2 Introduction

Schwertmannite is a metastable ferric oxyhydroxysulfate mineral with the variable formula $\text{Fe}_8\text{Os}(\text{OH})_{8-2x}\text{m}(\text{SO}_4)_{x}\cdot n\text{H}_2\text{O}$, where $1 < x < 1.75$ (Bigham et al., 1996; Bigham and Nordstrom, 2000; Blodau, 2004; Jönsson et al., 2005). The x-ray diffraction (XRD) lines of schwertmannite are strongly broadened indicating that schwertmannite is poorly-ordered with reduced crystal development. Schwertmannite plays a large role in controlling the water quality of some soils and sediments through both the sorption of pollutants (Acero et al., 2006; Schoepfer et al., 2017) and the pH dynamics of dissolution (Burton et al., 2007; Burton and Johnston, 2012; Fitzpatrick et al., 2017a).

In schwertmannite containing systems, there is often a natural progression from the initial schwertmannite to secondary, more crystalline minerals such as goethite ($\alpha$-FeOOH), with a release of structural $\text{SO}_4^{2-}$ in the process (Equation 5.1) (Bigham et al., 1996; Burton et al., 2008; Choppala and Burton, 2019).

$$\text{Fe}_8\text{Os}(\text{OH})_{8-2x}\text{m}(\text{SO}_4)_{x} + 2\text{H}_2\text{O} \rightarrow 8\text{FeOOH} + \text{SO}_4^{2-} + 2\text{H}^+$$

Equation 5.1

Schwertmannite is just one of several poorly-ordered iron oxides that can transform to more crystalline iron oxide minerals with time or oscillating redox conditions (Lair et al., 2009; Liu et al., 2007; Pasakarnis et al., 2015; Thompson et al., 2006; Tomaszewski et al., 2016; Winkler et al., 2018). The transformation rates of these minerals and the resulting intermediary products often depend on environmental parameters such as pH and the presence of additional ions or compounds (Burton et al., 2019, 2010, 2008b; Jones et al., 2009; Liu et al., 2016, 2007; Paikaray et al., 2017a). The complete transformation of schwertmannite to goethite can take hundreds of days under acidic conditions, such as are commonly found in many schwertmannite containing environments (Bigham et al., 1996; Collins et al., 2010; Jönsson et al., 2005; Knorr and Blodau, 2007a; Regenspurg et al., 2004). However under less acidic conditions, like those often found in several natural environments such as in soils, especially following the reduction of schwertmannite-Fe(III) (Blodau, 2004; Burton et al., 2006b; Johnston et al., 2011b), the increased pH increases the negative surface charge of schwertmannite, which can thus attract the resulting Fe(II) (Jönsson et al., 2005; Paikaray et al., 2017a). Sorption of this Fe(II) can induce complete dissolution and transformation within hours or days at near-neutral pH, often depending on the Fe(II) surface coverage to the iron oxide (Burton et al., 2010, 2008b; Houngaloune et al., 2015; Paikaray et al., 2017a).
Previous studies have focused on the kinetics of the Fe(II) induced transformation of schwertmannite as a function of pH (Burton et al., 2008b; Paikaray et al., 2017a) or its occurrence in the presence or absence of additional aqueous compounds such as silica, arsenic, natural organic matter, chromium or copper (Burton et al., 2010; Burton and Johnston, 2012; Houngaloune et al., 2015; Jones et al., 2009; Paikaray et al., 2017a). Both of these factors play a role in determining the sorption of Fe(II) to the mineral surface through surface charge alterations or physical barriers to Fe(II) sorption (Paikaray et al., 2017a). Limited Fe(II) surface coverage, driven by either the surface charge or external compound sorption, is also thought to influence the precipitation of an intermediate lepidocrocite phase (γ-FeOOH) during the Fe(II) induced transformation of ferrihydrite and schwertmannite (Boland et al., 2014; Hansel et al., 2005; Paikaray et al., 2017a; Pedersen et al., 2005).

Schwertmannite and schwertmannite rich sediments have a high affinity for phosphate (PO$_4^{3-}$) (Boukemara et al., 2017; Eskandarpour et al., 2006), and PO$_4^{3-}$ is ubiquitous in schwertmannite rich environments (Collins et al., 2010; Fitzpatrick et al., 2017a). Sorption of PO$_4^{3-}$ has been found to stabilize both ferrihydrite (Borch et al., 2007) and schwertmannite (Schoepfer et al., 2017) during microbial Fe(III) reduction, with negligible PO$_4^{3-}$ release in the process. In addition, PO$_4^{3-}$ has also been shown to exchange with sulfate in and on the schwertmannite structure during both sorption (Schoepfer et al., 2017) and coprecipitation (Regenspurg and Peiffer, 2005). This exchange potentially influences the schwertmannite transformation pathways in both the presence and absence of Fe(II). However, the effects of PO$_4^{3-}$ on abiotic schwertmannite transformation pathways have not yet been directly investigated.

This study focuses on the ferric oxyhydroxides that form as a result of schwertmannite sorption with PO$_4^{3-}$ and Fe(II) under abiotic and circumneutral conditions. Mössbauer spectroscopy, x-ray diffraction, and aqueous spectroscopy are employed to characterize and monitor the evolution or stabilization of aqueous and mineral phases.
5.3 Methods

5.3.1 Schwertmannite synthesis

Schantzmannite was precipitated from a ferrous sulfate solution as described by Regenspurg et al. (2004) and Schoepfer et al. (2017), and contained 7.4 mmol Fe g⁻¹ and 1.5 mmol S g⁻¹. This method produces a specific surface area of 4-14 m² g⁻¹, corresponding to 405-1417 m² mol⁻¹ Fe⁻¹ (Burton et al., 2009; Regenspurg et al., 2004).

5.3.2 Schwertmannite transformation

These experiments involved three replicates within gas tight 2-L pyrex reaction vessels, which contained 18.75 g synthetic schertmannite and 1.5 L deoxygenated artificial groundwater. The artificial groundwater was made up of 5 mM CaCl₂, 10 mM KCl, 10 mM MgCl₂ and 1 mL L⁻¹ Wolfe’s mineral solution (Schoepfer et al., 2017), and was buffered to pH 6.5 with 0.05 M MES/MOPS (2-morpholinopropanesulfonic and 2-morpholinoethanesulfonic acid). Phosphate was then added to each reaction vessel in a Coy anoxic chamber (glove box) at loadings of 0, 80, 400, and 800 µmol g⁻¹ schwertmannite (corresponding to zero, low, medium, and high PO₄³⁻ loading and sorption to schwertmannite, (Schoepfer et al., 2017). The atmosphere of the anoxic chamber was maintained as a mixture of 95% N₂ and 5% H₂.

The suspensions were mixed and permitted to equilibrate for 24 hours (Burton et al., 2008b). Following this 24 hour equilibration period, a 50 mL subsample of homogenized mineral-water suspension was removed anoxically from the reaction vessels, centrifuged, and filtered to <0.45 µm within the anoxic chamber to determine initial aqueous and solid phase conditions. The solid sample was dried using methanol and stored under anoxic conditions for solid phase analysis (Schoepfer et al., 2017), while the aqueous samples were acidified and stored at 4 °C for later analysis of Fe(II), Fe(total), PO₄³⁻, and SO₄²⁻ following the methods outlined below.

After the initial 24-hr PO₄³⁻ sorption and equilibration period, deoxygenated Fe(II) was then added as an FeCl₂ solution to a final concentration of 12.5mM (1 mmole Fe(II) g⁻¹) in each vessel (Burton et al., 2008b). Subsamples (50- mL) were withdrawn anoxically at 1, 2, 3, 4, 5, 6, 7, 8, and 12 hours, as well as 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days. Samples were centrifuged, returned to the anoxic chamber, and filtered to <0.45 µm.
5.3.3 Aqueous phase analytical methods

Aqueous PO$_4^{3-}$ concentrations were determined through the molybdenum blue method of Hansen and Koroleff in (Grasshoff et al., 1999). Fe(II) concentrations were determined through the 1, 10-phenanthroline method of (APHA, 2005). Following the same method, samples were pre-reduced using hydroxylamine hydrochloride to assess total Fe. Fe(III) was determined by subtraction. Total sulphur was evaluated using a Perkin Elmer Optima 4300 DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The sulphur content from the MES/MOPS buffer was subtracted from the total sulphur content, resulting in an aqueous SO$_4^{2-}$ concentration.

5.3.4 Solid phase X-ray diffractometry

X-ray diffraction (XRD) was used to determine the mineral phases present in each sample. Dried samples were ground and loaded anoxically into slides for a Bruker D4-Endeavor diffractometer with a Co x-ray tube. Samples were scanned from 10.00 °2θ to 80.73 °2θ using a 0.48 °2θ step and a one second count time (Schoepfer et al., 2017). Samples were exposed to atmospheric oxygen for a period of 25 minutes during analysis.

5.3.5 Mössbauer spectroscopy

Transmission $^{57}$Fe Mössbauer spectroscopy was utilized to determine the changes in Fe mineral speciation as well as the quantity of each species with respect to PO$_4^{3-}$ treatment and incubation time. Sampling points for $^{57}$Fe Mössbauer spectroscopy analysis were chosen based on XRD data (at 0, 1, and 8 hours as well as 2, 5, and 10 days).

Dried and ground samples were deposited in an HDPE sample holder with an area of 1.76 cm$^2$. Spectra were collected at 77K (liquid nitrogen temperature) using an MR35A Mössbauer system with an MA-250 velocity transducer and the transmission mode data was collected on a 1024 channel MCD (Fast ComTec GmbH). A constant acceleration mode was set to ± 10 mm sec$^{-1}$ using a 1.85 GBq $^{57}$Co source (Wissell Inc.). Samples were analysed for approximately 24 hours under room atmosphere.

All Mössbauer spectra were autofolded and fit using the MossA software (Prescher et al., 2012). Sample velocity was calibrated in transmission mode against an α-Fe foil standard with a calculated velocity of 9.644 mm s$^{-1}$, a calculated center shift (CS) of 0.11716 mm s$^{-1}$, and a source calibration full
Mössbauer fits were kept as simple as possible, only adding spectra if it greatly decreased the error term. Parameters of each spectra were first allowed to vary individually. The intensity of each spectra was allowed to vary near the end of the initial fit, once the peak positions were approximately consistent with the peak positions determined visually. Parameters were constrained when the fitting process greatly distorted the spectra. Following this distortion, parameters (most commonly the a13 or full width at half maximum (FWHM)) were returned to the default (a13= 3.0) or recently estimated value (FWHM), and constrained.

Spectral area is the area fraction of the total intensity of the peaks. Each spectra was identified and displayed as a fraction of the total area. This always summed to one, or 100%.

## 5.4 Results

### 5.4.1 Aqueous PO$_4^{3-}$

Aqueous PO$_4^{3-}$ was consistently below the method detection limit (<0.25 µM) in the zero PO$_4^{3-}$ treatment (Figure 5.8.1a). In the low and medium PO$_4^{3-}$ treatments, PO$_4^{3-}$ remained below 0.8 µM PO$_4^{3-}$ throughout the ten day experiment, although PO$_4^{3-}$ slightly increased with time (Figure 5.8.1b-c). Before Fe(II) addition, the high PO$_4^{3-}$ treatment contained roughly all of the PO$_4^{3-}$ as aqueous PO$_4^{3-}$, but within 24 hours of Fe(II) addition, this aqueous PO$_4^{3-}$ dropped to <0.1% of the initial added PO$_4^{3-}$ in solution and remained very low throughout the rest of the experiment (Figure 5.8.1d).

### 5.4.2 Aqueous Fe(II)

Before Fe(II) addition, no aqueous Fe(II) was detectable in any treatment (Figure 5.8.1e-h). Previous experimental work confirms that Fe(II) is not removed from solution in the absence of schwertmannite (Burton et al., 2008b). A decrease in Fe(II) concentration from 12.5mM to <30 µM occurred in all treatments between the moment of Fe(II) addition and the first sampling point at one hour following Fe(II) addition. Aqueous Fe(II) in the zero PO$_4^{3-}$ treatment then increased to ~70 µM by 8 hours, and with further time, decreased to around
20 µM. This is in contrast to the low PO$_4^{3-}$ treatment, where Fe(II) increased from 11 µM to 107 µM over the initial 4 days, before slowly decreasing to ~70µM. The two higher PO$_4^{3-}$ treatments exhibited minimal changes throughout the experiment after Fe(II) sorption with 35-45 µM remaining in the aqueous phase. Despite apparent differences, however, the total amount of aqueous Fe(II) for all treatments was less than 1% of the added Fe(II).

5.4.3 Aqueous SO$_4^{2-}$ trends

The initial SO$_4^{2-}$ released into the aqueous phase during the pre-equilibration period (i.e. prior to Fe(II) addition) was proportional to PO$_4^{3-}$ loading, with 0.32, 0.35, 0.41, 0.67 mmol g$^{-1}$ SO$_4^{2-}$ released in the zero, low, medium and high PO$_4^{3-}$ treatments, respectively (Figure 5.8.2, red squares). This equates to dissolution of 22% to 47% of the initial solid phase SO$_4^{2-}$. Following Fe(II) addition, the aqueous SO$_4^{2-}$ concentrations generally increased over the first two days. Following the 2 day mark, SO$_4^{2-}$ concentrations then remained relatively steady. This same pattern occurred in all PO$_4^{3-}$ treatments, with an aqueous SO$_4^{2-}$ average at 67%, 53%, 53%, 49% of the total schwertmannite-SO$_4^{2-}$ in the zero, low, medium and high PO$_4^{3-}$ treatments, respectively between days 2 and 10.

The difference in the amount of aqueous SO$_4^{2-}$ that was present before Fe(II) addition compared to that at day 10 was inversely proportional to the level of PO$_4^{3-}$ loading (Figure 5.8.2, black circles). After accounting for the SO$_4^{2-}$ released in the pre-equilibration period, 0.58, 0.62, 0.38, 0.15 mmol g$^{-1}$ additional SO$_4^{2-}$ was released in the zero, low, medium and high PO$_4^{3-}$ treatments, respectively, at day 10.

5.4.4 X-ray diffractometry

The initial material was identified as schwertmannite with some minor goethite (Figure 5.8.3a). Before Fe(II) addition in the zero PO$_4^{3-}$ treatment (Figure 5.8.3b), slightly more crystalline goethite was present than in the initial mineral as evident by the more intense peak at ~24.6 °2θ. This goethite peak decreased in intensity with increased PO$_4^{3-}$ loading (Figure 5.8.3 e, h, k).

Mineral evolution over the ten day period varied according to PO$_4^{3-}$ treatment. The zero PO$_4^{3-}$ treatment contained abundant goethite by day 10 (Figure 5.8.3d). In addition to goethite, lepidocrocite formed in the low PO$_4^{3-}$ treatment following the addition of Fe(II), (Figure 5.8.3e-g). In contrast, the XRD patterns suggest that schwertmannite persisted in the medium (Figure
Phosphate as a control on schwertmannite stability, iron mineralogy and anaerobic biogeochemistry in acid-sulfate systems

5.8.3h-j) and high (Figure 5.8.3k-m) PO$_4^{3-}$ treatments, with only slight goethite presence at these higher levels of PO$_4^{3-}$ sorption. No Fe-PO$_4^{3-}$ phases were observed in the XRD patterns.

5.4.5 Mössbauer spectroscopy- identification of components

Mössbauer spectra were obtained at 77K for the initial schwertmannite sample, and for samples before Fe(II) addition and at 1 hr and 10 days following Fe(II) addition (Figure 5.8.4). Spectral identification was guided by x-ray diffraction phase identification as well as through literature values (Supplemental information Table 5.9.1). Spectral components were identified as schwertmannite, goethite and lepidocrocite, as well as a microcrystalline Fe(III) oxyhydroxide, based on Mössbauer parameters such as the quadrupole split and the full width at half maximum (Chen et al., 2018; Murad, 1998; Thompson et al., 2011). No Fe-PO$_4^{3-}$ minerals were observed in any treatments (Supplemental information Table 5.9.1). If present vivianite would have been apparent through its large CS and strengite through the appearance of two overlapping doublets (Dyar et al., 2014).

All phases generally displayed similar center shifts (CS) between ~0.45 and 0.48 and therefore, this parameter was not useful for identification. Schwertmannite had the largest quadrupole splitting (QS, ~0.7) and was visually a broad asymmetrical doublet at 77K (Murad, 1998). Lepidocrocite had a QS of ~0.56 and was a distinct, sharp symmetrical doublet (Murad, 1998). Goethite, a sextet at 77K, was easily defined as the only full sextet, however it appeared as two sextets, which probably reflects goethite with two distinct particle size characteristics (Gabbasov et al., 2014). In some samples, a collapsed sextet was also present, which had a near zero QS and low BHF (magnetic hyperfine field).

5.4.6 Trends in Mössbauer spectroscopy before Fe(II) addition

The initial synthetic material was well fit by a schwertmannite doublet (88% spectral area) and a goethite sextet (Figure 5.8.4a). This initial material was pre-equilibrated with the four PO$_4^{3-}$ loadings for 24 hours before the addition of Fe(II). Prior to Fe(II) addition a schwertmannite doublet (68% spectral area) was identified in the zero PO$_4^{3-}$ treatment, with the remaining spectral area comprising a goethite sextet (Figure 5.8.4b).

Before Fe(II) addition in the low PO$_4^{3-}$ treatment (Figure 5.8.4e), the Mössbauer spectrum was composed of a schwertmannite doublet (50% spectral
area), a goethite sextet (26% spectral area) and a collapsed sextet (identified as a microcrystalline Fe(III) oxyhydroxide, 25% spectral area) (Chen et al., 2018; Thompson et al., 2011). With increasing PO$_4^{3-}$, but before Fe(II) addition (Figure 5.8.4 e, h, k), the collapsed sexted increased to 36% and 58% of the spectral area in the medium and high PO$_4^{3-}$ treatments, respectively, at the expense of goethite (32 and 20% spectral area). Schwertmannite occupied the remainder at 32% and 33% of the spectral area in the medium and high PO$_4^{3-}$ treatments, respectively.

5.4.7 Trends in Mössbauer spectroscopy after Fe(II) addition

One hour following Fe(II) addition, the schwertmannite doublet in the zero PO$_4^{3-}$ treatment had completely disappeared and was replaced by two goethite sextets, separated by a small CS (Figure 5.8.4c+d).

Similarly, following Fe(II) addition in the low PO$_4^{3-}$ treatment, the one goethite sextet split into two distinct sextets with slightly different CS (roughly 0.05 mm s$^{-1}$ apart, Figure 5.8.4f+g). This small shift in CS was enough to drastically lower the error term, further confirming the presence of two distinct goethite sextets. Significantly, the broad schwertmannite doublet (large QS, ~0.76 mm s$^{-1}$) was replaced by a narrower doublet with parameters consistent with lepidocrocite (QS ~0.58 mm s$^{-1}$). One hour following Fe(II) addition in the low PO$_4^{3-}$ treatment, the collapsed sextet had disappeared, leaving only lepidocrocite and goethite. The resulting lepidocrocite/goethite pattern remained throughout the ten day study with roughly 30-35% lepidocrocite and 65-70% goethite.

In both the medium and high PO$_4^{3-}$ treatments, the collapsed sextet of the micro-crystalline Fe(III) oxyhydroxide occupied 36% and 40% of the spectral area at one hour following Fe(II) addition (Figure 5.8.4i+l). By ten days, this had decreased to 25 and 35% of the spectral area in the medium and high PO$_4^{3-}$ treatments, respectively (Figure 5.8.4j+m). Schwertmannite occupied 41% and 43% of the spectral area in each medium and high PO$_4^{3-}$ treatment at 10 days, with the remainder being goethite.

No Fe(II) minerals were observed in any spectra, which would have been readily observable since Fe(II) has a much higher CS than Fe(III). This is attributed to oxidation of surface Fe(II) while samples were being analysed by Mössbauer spectroscopy (~24 hour analysis). However, given only 1 mmole g$^{-1}$
Fe(II) was added, complete Fe(II) oxidation could only account for only 12% of the total Fe. Hence, this level of Fe(II) oxidation is unlikely to substantially affect our interpretation of the Mössbauer results. Furthermore, geochemical modelling suggests that the precipitation of Fe(II)-PO$_4^{3-}$ (vivianite) was not thermodynamically favourable in any PO$_4^{3-}$ treatment at any timepoint. Strengite (Fe(III)-PO$_4^{3-}$) was supersaturated in the first 8 hours of the high PO$_4^{3-}$ treatment, following which it then became undersaturated.

5.5 Discussion

5.5.1 PO$_4^{3-}$ drives rapid schwertmannite transformation

No major mineralogical changes were observed in the zero PO$_4^{3-}$ treatment before Fe(II) addition. The amount of SO$_4^{2-}$ released before Fe(II) addition in the zero PO$_4^{3-}$ treatment (22% of total schwertmannite-SO$_4^{2-}$) was consistent with previous studies suggesting surface bound SO$_4^{2-}$ occupies roughly 20-30% of the total SO$_4^{2-}$ in schwertmannite (Bigham et al., 1990; Regenspurg et al., 2002; Schoepfer et al., 2017). The remainder of the SO$_4^{2-}$ can be found within the schwertmannite tunnel structure (Loan et al., 2004; Wang et al., 2015).

With increased PO$_4^{3-}$ loading, increased SO$_4^{2-}$ loss from the schwertmannite mineral was observed, with approximately 0.42 mmoles g$^{-1}$ SO$_4^{2-}$ released per 1 m mole g$^{-1}$ PO$_4^{3-}$ sorbed (Figure 5.8.2, red squares). This SO$_4^{2-}$ release is similar to the ratio found with the sorption of arsenic(V) to schwertmannite (0.53 at pH 5.5) (Burton et al., 2009). Increased SO$_4^{2-}$ loss is consistent with PO$_4^{3-}$ either 1) exchanging with SO$_4^{2-}$ on the schwertmannite surface through ligand exchange (Burton et al., 2009; Fukushi et al., 2003b; Schoepfer et al., 2017) or 2) PO$_4^{3-}$ alternatively exchanging with SO$_4^{2-}$ within the schwertmannite tunnel structure during a co-precipitation pathway (Regenspurg and Peiffer, 2005). The co-precipitation pathway is similar to that which is observed with SO$_4^{2-}$-exchanged selenate (Waychunas et al., 1995). Additionally, Regenspurg and Peiffer (2005) suggest that during co-precipitation, aqueous ions with a larger ionic radius than SO$_4^{2-}$ (such as PO$_4^{3-}$) will distort the schwertmannite tunnel structure, decreasing the schwertmannite crystallinity and structure. Although the present study did not employ co-precipitation with PO$_4^{3-}$, rather, PO$_4^{3-}$ was added to preformed schwertmannite, a similar structural deformation is hypothesized as a result of
the PO$_4^{3-}$ and SO$_4^{2-}$ exchange during the pre-equilibration period (Schoepfer et al., 2017).

The SO$_4^{2-}$ release, which was induced by PO$_4^{3-}$ addition, also corresponded to a significant mineralogical transformation of schwertmannite to a “microcrystalline Fe(III) oxyhydroxide” (Chen et al., 2018; Thompson et al., 2011). According to Mössbauer spectroscopy, this new mineral phase comprised 25%, 36%, and 58% of the total Fe in the low, medium and high PO$_4^{3-}$ treatments before Fe(II) addition, respectively. Although this proposed new mineral phase was not identified in our x-ray diffraction patterns (presumably due to its small crystallite size and lack of long range order) a microcrystalline Fe(III) oxyhydroxide has been observed to display a collapsed sextet using Mössbauer spectroscopy (Chen et al., 2018; Coward et al., 2018; Thompson et al., 2011). A collapsed sextet has previously been identified as ferrihydrite (Kukkadapu et al., 2004, 2003) or nano-goethite (Hansel et al., 2003; Pasakarnis et al., 2015). However, the same collapsed sextet was observed in the medium and high PO$_4^{3-}$ treatments in the absence of sharp XRD goethite peaks, ruling out the assignment of goethite to the collapsed sextet. As such, no specific mineral was assigned to this spectra, rather the available information points to a microcrystalline Fe(III) oxyhydroxide with a potential high degree of structural defects that decrease crystallinity (Thompson et al., 2011).

The PO$_4^{3-}$ - SO$_4^{2-}$ exchange and hypothesized structural deformation is supported in this study by both the increase in aqueous SO$_4^{2-}$ with increased PO$_4^{3-}$ loading, as well as the precipitation of an x-ray amorphous microcrystalline Fe(III) oxyhydroxide (Chen et al., 2018; Coward et al., 2018; Thompson et al., 2011). Thompson et al. (2011) also suggest that this microcrystalline Fe(III) oxyhydroxide can have a high degree of ion substitution which further decreases its crystallinity and structure. In the current study, the amount of microcrystalline Fe(III) oxyhydroxide formed is directly proportional to the amount of SO$_4^{2-}$ released ($r^2 = 0.999$) (with the exception of the zero PO$_4^{3-}$ treatment where we did not see any microcrystalline Fe(III) oxyhydroxide precipitation).
5.5.2 The Fe(II) induced transformation of schwertmannite in the absence of PO$_4^{3-}$

Boland et al. (2014) have described the Fe(II) induced ferrihydrite transformation, which is similar to the Fe(II) induced schwertmannite transformation. Within this process, an Fe(II) atom interacts with the Fe(III) oxide surface and is oxidized. This newly formed Fe(III) acts as a nucleus for the precipitation of a more crystalline mineral and simultaneously produces new surface sites for Fe(II) sorption. The electron produced is transferred to an existing Fe(III) atom, reducing it and forming aqueous Fe(II) to continue the cycle (Boland et al., 2014). The Fe(II) induced pathway is known to occur readily with regard to schwertmannite (Burton et al., 2010, 2008b; Jones et al., 2009; Paikaray et al., 2017a).

According to both XRD and Mössbauer spectroscopy, goethite formation occurred within one hour in the absence of PO$_4^{3-}$ (Figures 5.8.3 and 5.8.4). Compared to similar Fe(II) induced schwertmannite transformation studies, complete transformation to goethite within one hour is rapid (Burton et al., 2008b; Paikaray et al., 2017a). This is likely due to the high sorbed Fe(II) : solid Fe(III) ratio employed here, which was up to 10x greater than these similar studies (0.135). Although the initial quantity of aqueous Fe(II) added was comparable to (Burton et al., 2008b), near complete Fe(II) sorption to schwertmannite occurred here in all PO$_4^{3-}$ treatments, inducing a rapid reaction to goethite.

Within this goethite mineral fraction, there were two suspected particle sizes present, as evident from two distinct, yet similar, sextets in the Mössbauer spectra (Figure 5.8.4). The center shifts (CS) of some iron oxides are known to increase with an increase in particle size (Gabbasov et al., 2014), and we suspect this occurred here with goethite. Newly precipitated goethite is hypothesised to have a small particle size, while some of the original nano-goethite is hypothesized to aggregate over the ten day study, obtaining a larger size and demonstrating a larger CS with time. These two particle sizes lead to the determination of two pathways for goethite formation in this study: 1) goethite formation via schwertmannite dissolution in the absence of Fe(II), and 2) the Fe(II) induced transformation of schwertmannite.
Increased PO$_4^{3-}$ loading progressively inhibits the Fe(II) induced transformation of schwertmannite

This study indicates that PO$_4^{3-}$ can interfere with the Fe(II)-induced transformation of schwertmannite. In the absence of PO$_4^{3-}$, schwertmannite quickly dissolves and transforms to goethite, as confirmed by XRD and Mössbauer data from the zero PO$_4^{3-}$ treatment. Complete dissolution of schwertmannite and microcrystalline Fe(III) oxyhydroxide occurred within one hour of Fe(II) addition in the low PO$_4^{3-}$ treatment as well (Figure 5.8.4 and 5.8.5), with lepidocrocite and goethite precipitating at ~40 and ~60% of the solid phase Fe mineral abundance, respectively. Lepidocrocite is often an intermediary phase, preceding the precipitation of goethite in the pH range of 5.5-6.5 and under slow oxidation of Fe(II) species (Boland et al., 2014; Hansel et al., 2005; Paikaray et al., 2017a; Pedersen et al., 2005). However in the low PO$_4^{3-}$ treatment, lepidocrocite remained abundant throughout the ten day study, suggesting that PO$_4^{3-}$ induced the stabilization of lepidocrocite rather than the usual pathway towards complete goethite precipitation (Boland et al., 2014; Liu et al., 2007).

Before Fe(II) addition in the medium and high PO$_4^{3-}$ treatments, both solid phase schwertmannite and the microcrystalline Fe(III) oxyhydroxide were present, much like in the low PO$_4^{3-}$ treatment (Chen et al., 2018; Coward et al., 2018; Murad, 1998; Thompson et al., 2011). Following Fe(II) addition, however, large quantities of schwertmannite and microcrystalline Fe(III) oxyhydroxide remained, as well as some additional goethite. The large PO$_4^{3-}$ loadings (~5 and 10% mole fraction PO$_4^{3-}$) therefore acted to stabilize the schwertmannite and the microcrystalline Fe(III) oxyhydroxide from transformation to lepidocrocite or goethite (Zachara et al., 2011). This inhibition may be similar to the inhibition imposed by arsenate during schwertmannite transformation to goethite (Burton et al., 2010; Paikaray et al., 2017a; Pedersen, 2006).

Much like in the zero PO$_4^{3-}$ treatment, over 99% of the aqueous Fe(II) was immediately removed from solution in the low, medium and high PO$_4^{3-}$ treatments, however no vivianite was observed in the XRD patterns or Mössbauer spectra due to the thermodynamic unfavourability for formation of these phases (based on Visual MINTEQ modelling) in addition to its slow formation kinetics in areas of possible local supersaturation (Walpersdorf et al.,...
Together these data suggest that Fe(II) was not removed from solution by discrete precipitation with aqueous PO\textsubscript{4}\textsuperscript{3-}.

Exponentially less goethite precipitation occurred in these highest PO\textsubscript{4}\textsuperscript{3-} treatments (100%, 51%, 36%, and 20% of the Fe mineral phase was identified as goethite in the zero, low, medium and high PO\textsubscript{4}\textsuperscript{3-} treatments at day 10, Figure 5.8.5), which is hypothesized to be a result of lowered Fe(II) – Fe(III) electron exchange induced by PO\textsubscript{4}\textsuperscript{3-} pre-sorption (Jones et al., 2009; Paikaray et al., 2017a; Williams and Scherer, 2004b). In support, all PO\textsubscript{4}\textsuperscript{3-} in all treatments was removed from the aqueous phase by day 10.

### 5.6 Conclusions

Phosphate is common in many schwertmannite-rich natural environments (Collins et al., 2010; Fitzpatrick et al., 2017b). However in this laboratory study, the addition of PO\textsubscript{4}\textsuperscript{3-} to schwertmannite at circumneutral pH (pH 6.5) was shown to induce a rapid transformation of schwertmannite to a new micro-crystalline Fe(III) oxyhydroxide mineral. In natural sediments, this PO\textsubscript{4}\textsuperscript{3-} -induced transformation could change the sediment makeup from a schwertmannite-rich sediment to a more amorphous composition that is not easily identifiable by powder x-ray diffraction. PO\textsubscript{4}\textsuperscript{3-} also induced inhibition of the Fe(II) – Fe(III) electron exchange process, substantially retarding Fe(II) oxyhydroxide transformation and subsequent goethite precipitation. This is supported by the attenuated goethite precipitation and retained Fe(II) oxyhydroxide under increased PO\textsubscript{4}\textsuperscript{3-} loadings in this study.

Consequently, PO\textsubscript{4}\textsuperscript{3-} has demonstrated the following dual role: (i) in the absence of Fe(II), PO\textsubscript{4}\textsuperscript{3-} induces rapid schwertmannite transformation to a micro-crystalline Fe(III) oxyhydroxide phase and (ii) in the presence of Fe(II), PO\textsubscript{4}\textsuperscript{3-} stabilizes the iron oxides (schwertmannite and the new microcrystalline Fe(III) oxyhydroxide) from transformation to goethite or lepidocrocite. The degree of stabilization is dependent on the PO\textsubscript{4}\textsuperscript{3-} loading level (Schoepfer et al., 2017). Sorption of ions or compounds to schwertmannite have been shown to stabilize schwertmannite from transformation to goethite in the presence of Fe(II) (Burton et al., 2010; Jones et al., 2009). However, the rapid PO\textsubscript{4}\textsuperscript{3-}-induced transformation of schwertmannite to a micro-crystalline Fe(III) oxyhydroxide is a novel and significant discovery, with this new mineral being expected to scavenge PO\textsubscript{4}\textsuperscript{3-}. 

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Phosphate-stabilized schwertmannite will likely have different properties than PO$_4^{3-}$-stabilized microcrystalline Fe(III) oxyhydroxides, goethite or lepidocrocite in regards to contaminant sorption, pH dynamics, or the response of these minerals under reducing conditions. Additionally, the rapid removal of aqueous PO$_4^{3-}$ and Fe(II) via sorption to schwertmannite may help to stabilize water quality through: 1) limiting PO$_4^{3-}$-induced eutrophication or 2) limiting aqueous Fe(II) oxidation and subsequent potential acidity generation. Therefore, although large quantities of PO$_4^{3-}$ are often thought to be a pollutant in the environment in terms of its eutrophication potential, this study indicates the potential for stability and containment of both PO$_4^{3-}$ and iron oxide minerals in both PO$_4^{3-}$- and Fe(II)-loaded schwertmannite systems.

Future work into the characterization and of the newly precipitated microcrystalline Fe(III) oxyhydroxide phase which forms via PO$_4^{3-}$-induced transformation of schwertmannite is needed. This could include the use of synchrotron source x-ray diffraction, which may produce clearer and more distinct x-ray diffraction lines than the standard laboratory XRD instruments, aiding in mineral identification. Furthermore, scanning electron microscopy and transmission electron microscopy may also provide helpful information to define the mineral morphology and structure. These methods may further the knowledge of schwertmannite transformation in the presence of PO$_4^{3-}$.

ACKNOWLEDGEMENTS: We would like to thank Nigel Dawson for facilitating collection of the Mössbauer spectroscopy data. Funding was provided by the Australian Research Council (grant LP LP120200723).
5.7 References


Phosphate as a control on schwertmannite stability, iron mineralogy and anaerobic biogeochemistry in acid-sulfate systems


Contrasting effects of phosphate on the rapid transformation of schwertmannite to Fe(III) (oxy)hydroxides at near-neutral pH


5.8 Figures

Figure 5.8.1. Aqueous parameters
Aqueous PO$_4^{3-}$ (a-d), aqueous Fe(II) (e-h), and aqueous SO$_4^{2-}$ (i-l) parameters as responding to time throughout the incubation period (1 hr through 10 days). Left hand y-axis documents concentrations, while the right hand y-axis shows the percentage of the total compound in the reactors. Error bars denote standard deviations.
Figure 5.8.2. Sulfate release

The amount of aqueous $\text{SO}_4^{2-}$ at each $\text{PO}_4^{3-}$ loading before Fe(II) addition (red line), and at 10 days after subtracting the $\text{SO}_4^{2-}$ released before Fe(II) addition at zero hours. Error bars denote standard deviations.
**Figure 5.8.3. X-ray diffractometry**

X-ray diffraction patterns for the initial solid sample (a), solid samples before Fe(II) addition, 1 hour after Fe(II) addition, and 10 days following addition for the zero (b-d), low (e-g), medium (h-j) and high (k-m) PO$_4^{3-}$ treatments. The grey bar notes scale on the y-axis.

<table>
<thead>
<tr>
<th>Initial mineral</th>
<th>Before Fe(II)</th>
<th>1 hour</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>e. G</td>
<td>f. L</td>
<td>g. L</td>
<td></td>
</tr>
<tr>
<td>h. G</td>
<td>i. G</td>
<td>j. G</td>
<td></td>
</tr>
<tr>
<td>k. G</td>
<td>l. G</td>
<td>m. G</td>
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Contrasting effects of phosphate on the rapid transformation of schwertmannite to Fe(III) (oxy)hydroxides at near-neutral pH

Phosphate as a control on schwertmannite stability, iron mineralogy and anaerobic biogeochemistry in acid-sulfate systems

Figure 5.8.4. Mössbauer spectra
Mössbauer spectra collected at 77K for the initial mineral (a) and solid samples before Fe(II) addition, 1 hour after Fe(II) addition, and 10 days following Fe(II) addition for the zero (b-d), low (e-g), medium (h-j) and high (k-m) PO$_4^{3-}$ treatments.
Figure 5.8.5. Mössbauer derived mineral abundances
Mössbauer-derived solid phase mineral abundances (fraction) for the zero, low, medium and high PO$_4^{3-}$ treatments before Fe(II) addition, and 1 hour and 10 days following Fe(II) addition.
5.9 Supporting Information - Chapter 5

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Supporting Information for manuscript submitted to *Environmental Science & Technology*

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Southern Cross University
Lismore, NSW 2480, Australia
Table 5.9.1. Reference Mössbauer parameters used in the determination of the phases present in each sample.

<table>
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<th>Mineral phase</th>
<th>CS (mm s$^{-1}$)</th>
<th>QS (mm s$^{-1}$)</th>
<th>BHF (T)</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>Schwertmannite</td>
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<td>-0.37</td>
<td></td>
<td>(Murad, 1998)</td>
</tr>
<tr>
<td>Goethite</td>
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<td>-0.2</td>
<td>46</td>
<td>(Murad, 1998)</td>
</tr>
<tr>
<td>Lepidocrocite</td>
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<td>0.65</td>
<td></td>
<td>(Murad, 1998)</td>
</tr>
<tr>
<td>Vivianite</td>
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<td>2.93</td>
<td></td>
<td>(Dyar et al., 2014)</td>
</tr>
<tr>
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<td>2.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.42</td>
<td>0.3</td>
<td></td>
<td>(Dyar et al., 2014)</td>
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<td>Fe(III) oxyhydroxide</td>
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<td>0-27</td>
<td>(Thompson et al., 2011)</td>
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<td>(Barrero et al., 2006)</td>
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Table 5.9.2. Mössbauer parameters for the zero PO$_4^{3-}$ treatment

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<th>FWHM (mm s$^{-1}$)</th>
<th>QS (mm s$^{-1}$)</th>
<th>BHF (T)</th>
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<th>a13</th>
<th>Phase</th>
<th>Area fraction</th>
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<td>1.558</td>
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### Table 5.9.3. Mössbauer parameters for the low $\text{PO}_4^{3-}$ treatment

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<th>QS (mm s$^{-1}$)</th>
<th>BHF (T)</th>
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<th>Area fraction</th>
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<td>1.41</td>
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<td>1.216</td>
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<td>Fe(III) oxyhydroxide</td>
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<td>-</td>
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### Table 5.9.4. Mössbauer parameters for the medium $\text{PO}_4^{3-}$ treatment

<table>
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<th>QS (mm s$^{-1}$)</th>
<th>BHF (T)</th>
<th>$a_{12}$</th>
<th>$a_{13}$</th>
<th>Phase</th>
<th>Area fraction</th>
</tr>
</thead>
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<td>Medium</td>
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<td>0.464</td>
<td>0.575</td>
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<td>0.738</td>
<td>0.777</td>
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<td>0.453</td>
<td>-</td>
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<tr>
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<td>3.034</td>
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<td>0.909</td>
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<td>0.461</td>
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<td>0.453</td>
<td>0.888</td>
<td>0.789</td>
<td>-</td>
<td>0.453</td>
<td>-</td>
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<tr>
<td>Medium</td>
<td>240</td>
<td>0.419</td>
<td>3.034</td>
<td>0</td>
<td>0.077</td>
<td>0.909</td>
<td>2.596</td>
<td>Fe(III) oxyhydroxide</td>
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Table 5.9.5. Mössbauer parameters for the high PO₄³⁻ treatment

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<tr>
<th>Treatment</th>
<th>Hour</th>
<th>CS (mm s⁻¹)</th>
<th>FWHM (mm s⁻¹)</th>
<th>QS (mm s⁻¹)</th>
<th>BHF (T)</th>
<th>a₁₂</th>
<th>a₁₃</th>
<th>Phase</th>
<th>Area fraction</th>
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<tr>
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<td>0</td>
<td>0.413</td>
<td>1.325</td>
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<td>47.55</td>
<td>1.5</td>
<td>3</td>
<td>goethite</td>
<td>0.196</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>0.457</td>
<td>1.156</td>
<td>0.729</td>
<td>-</td>
<td>0.467</td>
<td>-</td>
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<td>0.086</td>
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<td>goethite</td>
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<td>0.015</td>
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<tr>
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<td>-</td>
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<td>-</td>
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<td>-0.272</td>
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<tr>
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<td>1.257</td>
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<td>0.744</td>
<td>0.881</td>
<td>-</td>
<td>0.455</td>
<td>-</td>
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</tr>
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<td>High</td>
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<td>0.02</td>
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<td>-</td>
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<td>5.253</td>
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<td>1.354</td>
<td>2.745</td>
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<td>0.207</td>
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<td>3</td>
<td>Fe(III) oxyhydroxide</td>
<td>0.359</td>
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</tbody>
</table>
Contrasting effects of phosphate on the rapid transformation of schwertmannite to Fe(III) (oxy)hydroxides at near-neutral pH

Works Cited - Supplemental information:


Chapter 6 - Conclusions

6.1 Summary

The three studies presented as part of this thesis provide new insight into the largely unexplored field of PO$_4^{3-}$ and schwertmannite dynamics. Namely, they provide an overview into the ability of PO$_4^{3-}$ to control schwertmannite stability, anaerobic biogeochemistry, and iron mineralogy in schwertmannite rich systems under various environmental conditions. The interactions between PO$_4^{3-}$ and schwertmannite are not straightforward, rather, the interactions are highly dependent on PO$_4^{3-}$ concentration, time of incubation, and the presence or absence of microbial communities as well as aqueous Fe(II). As such, there can be no single blanket conclusion as to how PO$_4^{3-}$ affects schwertmannite stability, reduction or transformation. However, four key points are identified below.

6.2 Conclusion 1: Phosphate induces a rapid change in the initial schwertmannite mineral structure.

The third study introduced Mössbauer spectroscopy as a tool for mineral identification and quantification. Using this tool, a poorly ordered, microcrystalline Fe(III) oxyhydroxide was identified (Chen et al., 2018; Thompson et al., 2011), following the addition of aqueous PO$_4^{3-}$ to schwertmannite under short term anoxic conditions in the absence of Fe(II) or reducing bacteria. This transformation of schwertmannite to a microcrystalline Fe(III) oxyhydroxide has not been previously reported, and is highly significant. Previous work on schwertmannite stability in the presence of anions like arsenate (Paikaray et al., 2017a, 2012), or similar studies of ferrihydrite transformation in the presence of PO$_4^{3-}$ (Borch et al., 2007; Borch and Fendorf, 2008) all document stability of the iron oxide by the selected anion. Schwertmannite, therefore, has been assumed to be stable in the presence PO$_4^{3-}$, only transforming its mineralogy as a result of the biogeochemical forcing of reducing bacteria (Burton et al., 2007; Küsel, 2003) or elevated Fe(II) concentrations (Burton et al., 2008b; Paikaray et al., 2017a; Paikaray and Peiffer, 2014). The work presented here, however, supports that schwertmannite can additionally transform in the presence of PO$_4^{3-}$. This transformation was only detectable through the use of advanced techniques such as Mössbauer spectroscopy.
Therefore, what proportion of natural schwertmannite actually corresponds to this microcrystalline ferric oxyhydroxide? Do the properties associated with natural schwertmannite (Bigham and Nordstrom, 2000a; Collins et al., 2010; Fitzpatrick et al., 2017a) remain true for the minerals found in a naturally PO$_4^{3-}$-loaded system? Or can some recorded properties of natural schwertmannite instead be attributed to this microcrystalline ferric oxyhydroxide? Collins et al. (2010) support that natural schwertmannite reacts and transforms much differently than synthetic schwertmannite, likely a result of the sorption of compounds including PO$_4^{3-}$. Therefore, it is vital to understand the make-up of the initial minerals present in natural systems, as their identification and properties could drastically alter our understanding of biogeochemical processes. And furthermore, as the micro-crystalline Fe(III) oxyhydroxide mineral was only detected with Mössbauer spectroscopy, this highlights the need for studies of natural systems using advanced techniques.

6.3 Conclusion 2: Phosphate stabilizes the schwertmannite mineral structure.

Rather than continue to induce changes in the schwertmannite structure upon the introduction of reducing bacteria or upon Fe(II) addition, PO$_4^{3-}$ instead stabilized the schwertmannite through the end of each of the three experiments under medium and high PO$_4^{3-}$ loadings. For example, in the first study investigating schwertmannite stability under acidic reduction conditions, the greatest extent of schwertmannite stability was recorded in the medium and high PO$_4^{3-}$ treatments as a result of PO$_4^{3-}$-Fe(III) surface complexation. Increased stability with increased PO$_4^{3-}$ was also found in the second study even following extensive Fe and S reduction, where schwertmannite remained in the medium and high treatments after 82 days. The medium treatment was the slowest to reduce, with slightly faster reduction in the high PO$_4^{3-}$ treatment, possibly a result of Fe(III)-PO$_4^{3-}$ complexation on the schwertmannite/iron oxyhydroxide surface, enhancing dissolution under high PO$_4^{3-}$ loading (Borch and Fendorf, 2008; Fredrickson et al., 1998; Glasauer et al., 2003; Willett, 1985).

Iron oxide stabilization in the medium and high PO$_4^{3-}$ treatments was also apparent in the third study, following addition of Fe(II). X-ray diffractometry indicated retained schwertmannite throughout the 10 day study despite a large concentration of Fe(II) added. Phosphate is suspected to form surface complexes with Fe(III) on the schwertmannite surface, isolating the bulk mineral from the
effects of Fe(II). Mössbauer spectroscopy further revealed an abundance of the microcrystalline Fe(III) oxyhydroxide at day ten in the medium and high PO$_4^{3-}$ treatments. No schwertmannite/ oxyhydroxide remained in the low PO$_4^{3-}$ treatment, a result of lowered PO$_4^{3-}$ loading, however the resulting lepidocrocite and goethite minerals were also stabilized from 1 hour through 10 days by PO$_4^{3-}$.

The stability of a schwertmannite system exposed to PO$_4^{3-}$ has consequences for acidity dynamics, contaminant mobility, and PO$_4^{3-}$ itself. Low pH is one result of inhibited Fe(III) reduction (Peine et al., 2000). Many metal and metalloid contaminants are mobile at low pH values (Borch et al., 2010) and consequently, the presence of PO$_4^{3-}$ may indirectly induce metal mobility. Further supporting this mobility is the smaller abundance of mackinawite at high PO$_4^{3-}$ loadings, as mackinawite is a strong sorbent for many contaminants (Hansen et al., 1996; Niazi and Burton, 2016a; Peng et al., 2009). In contrast, PO$_4^{3-}$ sorption to schwertmannite is nearly complete, even at high PO$_4^{3-}$ loadings, causing the precipitation of Fe- PO$_4^{3-}$ minerals or the complexation of Fe and PO$_4^{3-}$, and as such, preventing PO$_4^{3-}$ release to the water column. So with the addition of PO$_4^{3-}$, microbial reduction is inhibited, the pH remains low, and contaminant mobility may increase, but PO$_4^{3-}$ mobility decreases. This has major, but contrasting implications for the health of the ecosystem- the eutrophication potential of the water body is decreased, but the mobile metals can travel far from the source waters, expanding the reach of acid sulfate systems far beyond their spatial and temporal footprint.

6.4 Conclusion 3: Goethite abundance decreases with increased phosphate loading.

All studies support a decrease in the abundance of the goethite mineral at the end of the experiment with an increase in PO$_4^{3-}$ loading. This can be explained partly by the tendency of schwertmannite to rapidly transform to goethite via the Fe(II) pathway in the absence of PO$_4^{3-}$ (Burton et al., 2008b; Paikaray et al., 2017a). The first two biotic incubation studies experienced at least some Fe(III) reduction, producing Fe(II). The third study directly and artificially added aqueous Fe(II), spurring the Fe(II) induced transformation of schwertmannite. Therefore, without the stabilizing effect of PO$_4^{3-}$ (i.e. the zero PO$_4^{3-}$ treatment) goethite precipitation was relatively rapid and extensive in all studies.
Goethite is a highly crystalline mineral and is less bioavailable than schwertmannite due to this crystallinity (Faiivre, 2003). Its presence in a natural environment brings long-term stability to the natural mineral assemblages (Larsen and Postma, 2001; Roden and Zachara, 1996). However, with increasing PO$_4$$_3^-$, the goethite stability is lost, favored instead by PO$_4$$_3^-$-stabilized schwertmannite. Phosphate stabilized schwertmannite has demonstrated mixed results under long term reduction. For example, study two saw the precipitation of mixed valence green rusts, which are more susceptible to changing redox conditions when compared to goethite (Genin et al., 2001; Hansen and Poulsen, 1999; Kukkadapu et al., 2004). Therefore, a greater range of mineral transformations are possible in the presence of PO$_4$$_3^-$ than in the more stable goethite system.

6.5 **Conclusion 4: Aqueous phosphate is not released in any study**

Many studies suggest PO$_4$$_3^-$ is released upon iron oxide reduction (e.g. Bonneville et al., 2004; Lovley, 1991; Megonigal and Neubauer, 2009), however this was not observed. Despite various study conditions, no PO$_4$$_3^-$ was released from the mineral form to the aqueous form at any time, rather, PO$_4$$_3^-$ was sequestered to the solid phase as either vivianite or was associated with the stabilized Fe(III) oxides.

The finding of PO$_4$$_3^-$ sequestration, despite high initial PO$_4$$_3^-$ loadings, is highly significant for those potentially eutrophic environments. Eutrophication has the potential to drastically alter the microbial and plant community dynamics of acid sulfate waters (Sekhon and Bhumbla, 2013) which can thus promote reducing conditions in a water body. Reducing conditions are often desired for acid sulfate water mitigation, however the lack of aqueous PO$_4$$_3^-$ by its sequestration in iron minerals may alter the anticipated dynamics of eutrophication. Several studies suggest controlled eutrophication as a method for acid sulfate water remediation through the addition of PO$_4$$_3^-$ (Fyson et al., 1998; Kalin et al., 2006a; Lessmann et al., 2003; Wei et al., 2008). However, this work supports the rapid sequestration of PO$_4$$_3^-$ in iron minerals and no aqueous PO$_4$$_3^-$ release, minimizing the effectiveness of PO$_4$$_3^-$ addition.
6.6 Future Work

These studies have highlighted the need for more research into the dynamics between schwertmannite and $PO_4^{3-}$. Specifically, the main goal of future studies should be to target and understand the dynamics of the new microcrystalline Fe(III) oxyhydroxide that forms as a result of $PO_4^{3-}$ addition to schwertmannite. This finding has the potential to revolutionize how schwertmannite-rich environments are studied, as schwertmannite may not be the dominant mineral in $PO_4^{3-}$-laden, “schwertmannite-rich” environments. Therefore, what we know about schwertmannite in natural settings may in fact be the knowledge of a mixture of several minerals. Consequently, there is a need to understand the mechanisms involved regarding schwertmannite and $PO_4^{3-}$ dynamics within a natural system, in addition to the specific behavior of this microcrystalline iron oxyhydroxide before we can make conclusions about schwertmannite stability, reducibility, or even contaminant sorption in natural systems impacted by $PO_4^{3-}$.

Furthermore, these studies also highlight the influence of varied concentrations of $PO_4^{3-}$ on schwertmannite behavior. These studies demonstrate that concentration matters, via the non-linear stability results obtained in the first study, the varied mineral formation in the second study and the dynamic stability and transformation of the iron oxides in the third study. Does this non-linear, concentration-based relationship hold for other anions and iron oxides? Or is the relationship between $PO_4^{3-}$ and schwertmannite unique? To answer many of these questions, a more process orientated approach to the natural environment is required, using a variety of advanced techniques.

Furthermore, better characterization of schwertmannite exposed to $PO_4^{3-}$ is needed, as is an investigation into the secondary minerals produced from schwertmannite transformation and/or reduction. Currently, laboratory based x-ray diffraction was used to assume x-ray amorphous behavior of the microcrystalline Fe(III) oxyhydroxide formed from the addition of $PO_4^{3-}$ to schwertmannite. However, a better method for characterization may be synchrotron-based x-ray diffraction. This advanced technique may more clearly display x-ray diffraction lines and distinguish pure schwertmannite from the microcrystalline Fe(III) oxyhydroxide that precipitated and was only identified using Mössbauer spectroscopy. Additionally, characterization of the newly precipitated goethite and lepidocrocite is desired, such as through determining
the mean crystallite dimension based on the full width at half height of the main x-ray diffraction lines. The secondary mineral formation pathways may differ between standard precipitation methods, the Fe(II) induced transformation, and the PO$_4^{3-}$ influenced transformation of schwertmannite to secondary oxyhydroxides, however this is yet uncharacterized and further work is needed to clarify these precipitation processes.
6.7 References


Appendices
Appendix 1 - Statement of contributions of others

Statement of author contribution: Edward Burton

Schoepfer (80%), Burton (10%), Johnston (5%), Kraal (5%)

Schoepfer (80%), Burton (10%), Kraal (8%), Johnston (2%)

Schoepfer (85%), Burton (10%), Johnston (5%)

I, Edward Burton, co-author on the articles listed above, agree with the stated author contributions.

Edward Burton
Statement of author contribution: Scott Johnston

Schoepfer (80%), Burton (10%), Johnston (5%), Kraal (5%)

Schoepfer (80%), Burton (10%), Kraal (8%), Johnston (2%)

Schoepfer (85%), Burton (10%), Johnston (5%)

I, Scott Johnston, co-author on the articles listed above, agree with the stated author contributions.

Scott Johnston
Statement of author contribution: Peter Kraal

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Schoepfer (80%), Burton (10%), Johnston (5%), Kraal (5%)

Schoepfer (80%), Burton (10%), Kraal (8%), Johnston (2%)

Schoepfer (85%), Burton (10%), Johnston (5%)

I, Peter Kraal, co-author on the articles listed above, agree with the stated author contributions.

Peter Kraal
Appendix 2 - Publications included in thesis

List of publications included in thesis


List of conference proceedings
