EFFECTS OF WATER LEVEL FLUCTUATIONS ON PHOSPHORUS, IRON, SULFUR, AND NITROGEN CYCLING IN SHALLOW FRESHWATER ECOSYSTEMS

By

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ABSTRACT

EFFECTS OF WATER LEVEL FLUCTUATIONS ON PHOSPHORUS, IRON, SULFUR, AND NITROGEN CYCLING IN SHALLOW FRESHWATER ECOSYSTEMS

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Wetlands are often conserved, enhanced, restored, and constructed to provide ecosystem services, particularly water quality improvement and biodiversity support. Although wetlands are effective at removing some pollutants, like excess nitrogen, wetland sediments vary in their capacity to retain phosphorus (P). In addition, natural chemical stressors that tend to accumulate in wetland sediment pore waters, at times to toxic concentrations, may limit a restored wetland's ability to support biodiversity. I investigated how the variable hydrology of shallow freshwater ecosystems influences their functioning, specifically P exchange between sediments and water, and concentrations of natural stressors.

In recent decades, re-flooding historically drained areas has become common in an attempt to regain lost wetland habitat and services. In a case study in southwest Michigan, restoring wetland hydrology to historically drained land caused rapid release of large amounts of inorganic P from sediments to surface waters. Prolific growth of filamentous algae and duckweed ensued, even after available P concentrations had become lower. These observations demonstrate that when restoring wetlands by re-flooding historically drained areas, managers should consider the potential for sediment P release to jeopardize restoration goals.

Net sediment-water P exchange is controlled by several biogeochemically distinct processes, all of which are controlled to varying degrees by sediment moisture and oxygen conditions. To better understand how hydrology and sediment biogeochemistry interact to influence net sediment-water P exchange in sediments from 16 biogeochemically diverse ecosystems, we temporarily desiccated and re-flooded sediment-water microcosms and compared sediment P release to continuously flooded controls. The effects of hydrologic regime on both the direction and magnitude of P exchange depended significantly on sediment identity, and treatment effects on P release to pore and surface waters differed. Ten of the 16 temporarily desiccated sediments released more P into pore and/or surface waters than continuously inundated references of the same sediment types.

Potentially toxic levels of three naturally occurring chemical stressors (sulfide, ammonia, and iron) are prevalent in freshwater sediments, yet their roles in shaping ecosystem structure and function may be overlooked. To assess the prevalence of toxic levels of sulfide, ammonia, and iron, we sampled sediments, pore waters, and surface waters from 42 locations across 24 shallow (< 2 m deep) freshwater ecosystems in southwest Michigan and compared our measured concentrations to water quality criteria established by the U. S. Environmental Protection Agency (EPA) and to toxic thresholds in the published literature. The benthic environment of almost every freshwater ecosystem we measured was theoretically toxic or stressful to some component of aquatic life in some area or at some time. Organismal tolerances to chemical stressors vary, so the toxicant concentrations that we measured are likely shaping benthic ecological communities and influencing rates of ecosystem function.

Dedicated to

William Kinsman and Joseph Kanske,
gentlemen and scholars.

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CHAPTER 1: A REVIEW OF HYDROLOGIC REGIME EFFECTS ON SEDIMENT-WATER P EXCHANGE IN SHALLOW FRESHWATER ECOSYSTEMS

Abstract

Phosphorus (P) is both a pollutant that causes eutrophication and a globally limiting resource for food production, yet P cycling, especially in human-dominated regions, is poorly managed. Wetlands are often conserved, enhanced, restored, and constructed to mitigate excess P transport to water bodies, although wetland sediments vary in their capacity to retain P. In addition, wetlands are vulnerable to drastic natural and human-induced shifts in hydrologic regime. Due to the complex nature of sediment P binding, P exchange between wetland sediments and surface waters is the net result of many processes that are controlled to different degrees by oxygen concentrations, redox conditions, pH, temperature, and other drivers that are all strongly altered by changes in hydrology. Despite the common practice of hydrologically managing wetlands for P retention, the biogeochemical mechanisms behind sediment-water P exchange after hydrologic change remain poorly understood. Here, we review and synthesize published work on the effects of water level fluctuations on wetland sediment P retention.

Introduction

Phosphorus (P) and nitrogen (N) are the two most important nutrients contributing to unnatural eutrophication in aquatic ecosystems (Carpenter et al. 1998) because of major human-driven alterations to their global cycles (Howarth et al. 1996, Vitousek et al. 1997, MacDonald et al. 2011). In recent years, the cycling of N has received comparatively more attention from ecologists concerned with nutrient pollution than P (Seitzinger et al. 2006, Burgin and Hamilton 2007), despite the importance of P both as a limiting resource for global food production and as a eutrophication-causing pollutant (Bennett and Elser 2011).

Natural, restored, and constructed wetlands are frequently used to remove nutrients from water flowing through them (Verhoeven et al. 2006). However, the natural ability of wetlands to retain P varies (Richardson 1985), and wetlands can at times be a net source, rather than a sink, of P to downstream ecosystems (Richardson 1985, Coveney et al. 2002, Dunne et al. 2012). The goal of P removal becomes especially challenging if wetlands are managed for multiple services including N removal, biodiversity support, and flood abatement (Verhoeven et al. 2006). Sediment P cycling is complex, and the mechanisms controlling net sediment-water P exchange are variably controlled by pH, oxidation-reduction status (i.e., redox), temperature, and other factors, all of which are strongly influenced by hydrology.

Much of our knowledge of the chemical controls on sediment-water P exchange comes from studies in lakes, many of which are focused on P release from hypolimnetic sediments upon seasonal oxygen depletion in the overlying water, known as “internal loading” (Boström et al. 1988, Marsden 1989). While the same principles apply to wetland sediments, which also often experience changes in redox status over time, the influence of the additional factor of transitions between flooded and drained states is the focus of this review.

Most wetlands are subject to natural hydrologic variation in water level due to seasonal and interannual variability in temperature, precipitation, and flow regimes. For centuries, humans have imposed drastic hydrologic changes on aquatic ecosystems through major engineering projects including drainage for land use, agricultural irrigation, flood prevention, power generation, recreation, and commercial development (Vörösmarty and Sahagian 2000). The widespread drainage of wetlands in the Midwestern United States and along the Mississippi River corridor, mostly for agriculture, is particularly conspicuous (Dahl 1990). Global climate change is predicted to increase the occurrence and degree of hydrologic extremes in many parts of the world, adding additional variability and increasing the likelihood of both droughts and floods (Bates et al. 2008), with consequently increased water level fluctuations in many wetlands and other shallow water bodies.

Increasingly, environmental managers alter wetland hydrologic regimes for conservation and ecosystem restoration in an attempt to reverse the negative effects of historic hydrologic management and other human impacts. Considerable resources have been invested in re-flooding historically drained areas to restore wetlands (Zedler 2003) as well as constructing wetlands specifically for water quality improvement (Kadlec & Wallace 2009). In addition, many wetlands are actively managed with temporary draining and re-flooding to improve water quality and for other reasons including weed control (Jacoby *et al.* 1982) and crop management (e.g., rice, blueberries).

Restored and constructed wetlands are generally effective at removing excess N from inflowing waters (Carpenter et al. 1998), but their ability to retain P is much more variable (Vymazal 2007). Restoring wetland hydrology invariably involves drastic hydrologic change, often requiring flooding of historically drained areas (Zedler 2003). The mechanisms by which wetland sediments retain P are complex and controlled by multiple geochemical and biological factors. Thus, wetland response to a hydrologic regime change will depend heavily on the biogeochemical characteristics of the sediment.

Many studies have observed that flooding historically drained sediments causes release of P to surface waters (e.g., Ardón et al. 2010, Wong et al. 2011), evidently reflecting a lower P retention capacity, yet there is also some evidence that drying improves sediment P retention capacity (Mitchell and Baldwin 1998, Smolders et al. 2006). In this review, we attempt to reconcile these apparent contradictions by synthesizing published literature on the effects of drying and re-wetting on wetland sediment P retention capacity and proposing a new general hypothesis for the diversity of wetland sediments and the conditions in which they occur.

Sediment P Cycling: The Basics

Unlike N, which undergoes chemical and microbial transformations between forms with different “redox” status (e.g., nitrification transforms reduced ammonium to oxidized nitrate), P exists in ecosystems largely in one oxidation state, as either inorganic phosphate (PO_4^{3-}) or bound in organic molecules (Figure 1.1). The processes of remineralization and biotic uptake and assimilation convert phosphorus from one form to the other.

Dissolved P in water samples is usually chemically detected as “Soluble Reactive Phosphate”, defined as dissolved (i.e., filterable) P molecules (considered to be mostly

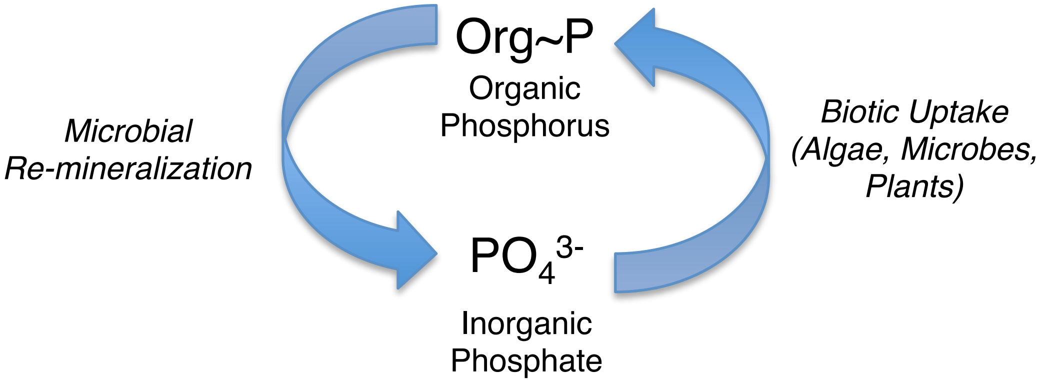

Figure 1.1 Phosphorus exists in ecosystems largely in one oxidation state, as either inorganic phosphate (PO_4^{3-}) or bound in organic molecules. The processes of remineralization and biotic uptake and assimilation convert phosphorus from one form to the other. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

PO_4^{3-}) that react with antimony and molybdenum to cause a color change that is measured spectrophotometrically (Murphy and Riley 1962). Using this method, P detected is presumed to be free phosphate (PO_4^{3-}) dissolved in the water (however see Hudson et al. 2000). Prior to SRP determination, water samples can be digested using persulfate or other means to convert organically bound P in the water to PO_4^{3-}, allowing for measurement of “non-reactive” P by calculating the difference in SRP between the digested and undigested sample. Both processes can also be completed on filtered and unfiltered samples, allowing for size fractionation that distinguishes between P in particulate (e.g., algal and microbial cells, P bound to detrital and inorganic matter) and dissolved matter. To measure P in sediments, different forms are chemically extracted and detected in the dissolved phase using similar schemes (Figure 1.2).

In sediments, P exists in inorganic and organic forms (Figure 1.3). Inorganic P in sediments is typically PO_4^{3-} in sorption-desorption equilibria with minerals, particularly iron (Fe) and aluminum (Al) oxides and oxyhydroxides (Fe~PO_4^{3-}, Al~PO_4^{3-}). While Fe oxides are subject to dissolution under reducing conditions, Al oxides are redox insensitive (Darke and Walbridge 2000, Hupfer and Lewandowski 2008). Phosphate can also coprecipitate with and sorb to calcium carbonate ($CaCO_3$) complexes that precipitate in alkaline waters when pH increases (Hamilton et al. 2009).

Organic P (Org~P) is bound in microbial, algal, animal, and plant biomass, mostly in phospholipids and nucleic acids. Most organic phosphorus, however, is found in detrital organic matter originating from settling algal cells, allochthonous organic inputs,

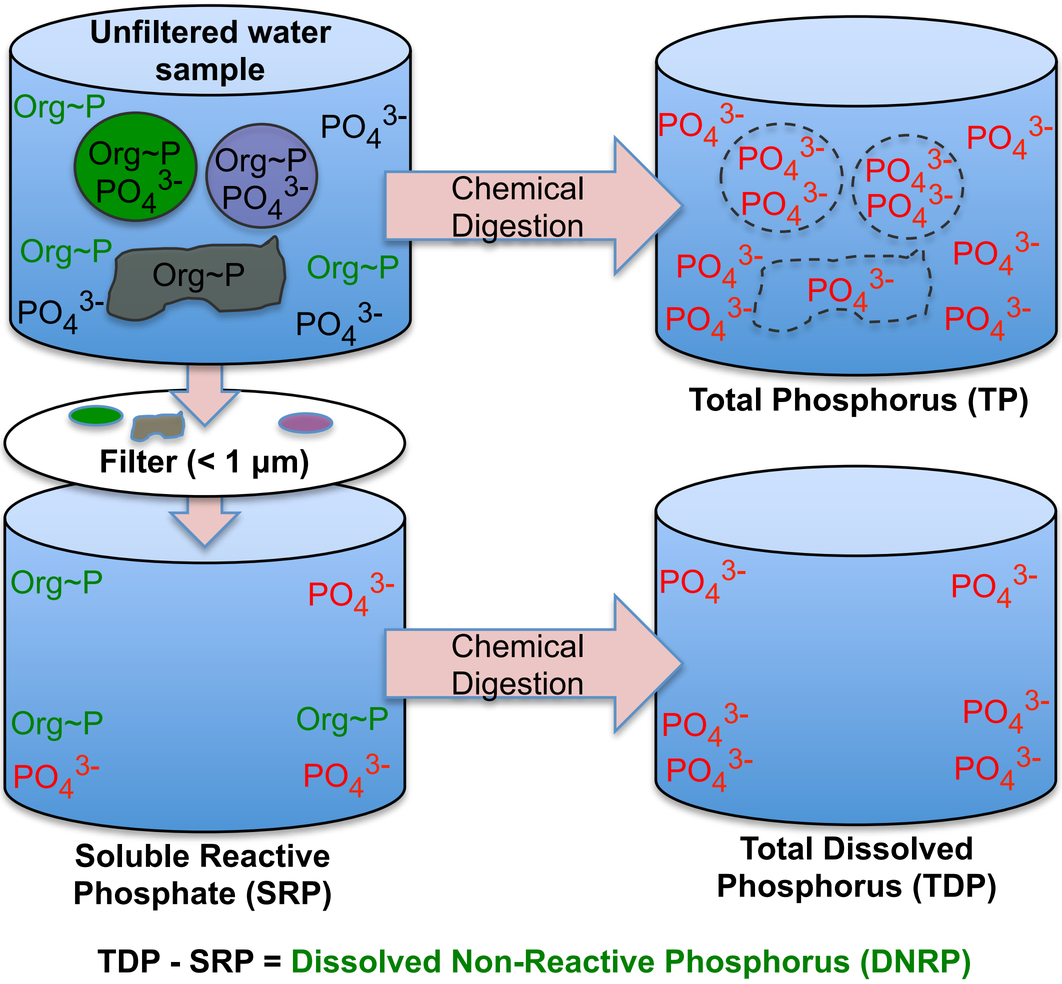

Figure 1.2 Schematic depicting typical detection of phosphate in environmental water samples. Phosphorus in water samples is typically detected as “soluble reactive phosphate” (SRP) using the colorimetric method of Murphy and Riley (1962). Samples are filtered prior to SRP analysis to measure dissolved phosphate, chemically digested to detect total phosphorus, and filtered and chemically digested to measure total dissolved phosphorus.

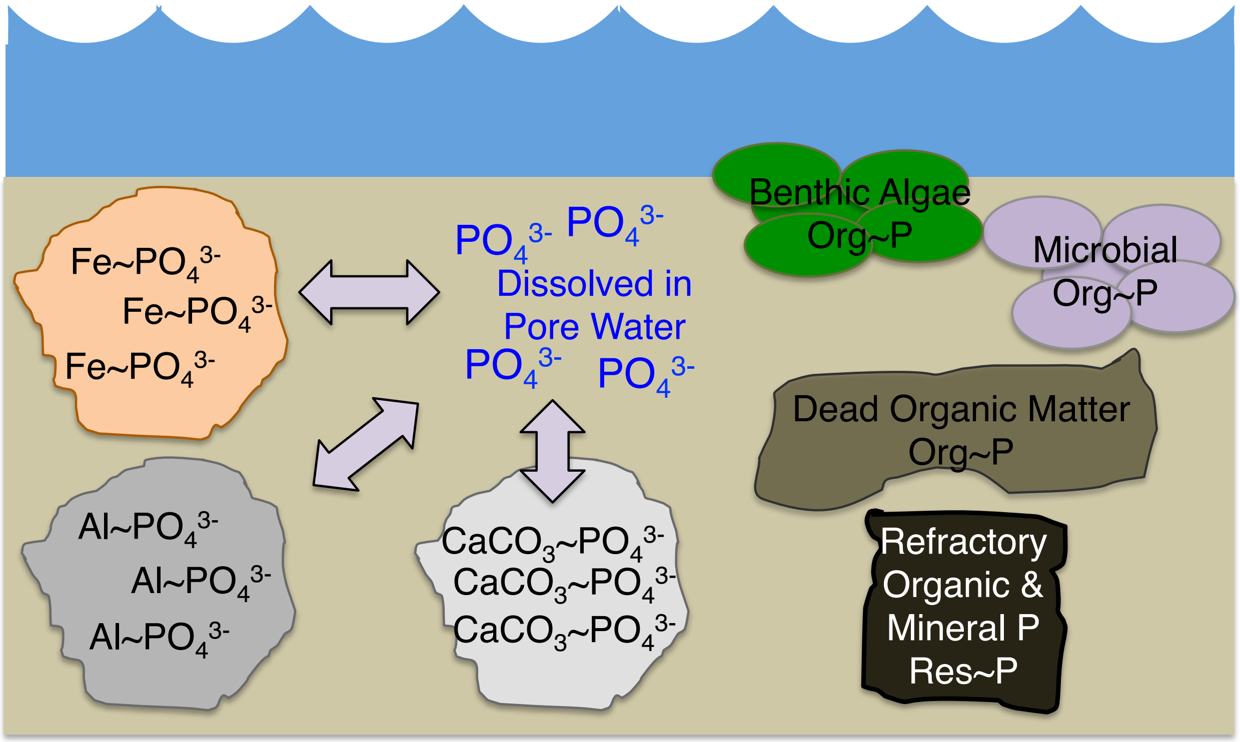

Figure 1.3 Organic and inorganic forms of phosphorus in sediments. Inorganic phosphate (PO_4^{3-}) ions dissolved in pore waters are typically in sorption equilibria with mineral solids, particularly iron and aluminum oxides and oxyhydroxides (Fe~PO_4^{3-}, Al~PO_4^{3-}). Phosphate can also sorb to or co-precipitate with calcium carbonate minerals ($CaCO_3$ ~PO_4^{3-}). Organic phosphorus (Org~P) is bound in living and dead benthic algal and microbial cells and detrital plant material. Finally, many sediments contain large amounts of residual phosphorus (Res~P), or phosphorus tightly bound in refractory organic and mineral solids that are difficult to characterize.

and vascular macrophyte remains. Organic P molecules are difficult to characterize and range in microbial availability from highly labile to recalcitrant (Reddy and DeLaune 2008). Thus, both the quality and quantity of P-containing organic matter present in an ecosystem determine the reactivity of this fraction.

Due to the multiple chemical forms of P in sediments, multiple processes control P exchange between sediments and surface waters. In this review, we use “retention” to refer to any processes by which P is associated with solid sediment particles, including covalent bonding (e.g., in organic matter), sorption, and coprecipitation, and we use “release” to refer to any process by which P becomes mobile and dissolved as PO_4^{3-} or organic molecules in interstitial sediment pore waters and/or surface water columns. Although P associated with solids can be biologically available, as long as those solids are within sediments, solid-associated P is less available to support algal growth in the water column and is less likely to be transported downstream.

How Does Sediment Drying or Draining Influence P Release Upon Re-wetting or Re-flooding?

Studies investigating the effect of drying and rewetting on sediment P flux have been conducted on quite different temporal and spatial scales, ranging from short-term sediment-water slurry incubations in microcosms up to ecosystem monitoring over many years. Most of our understanding of sediment-water P exchange after re-flooding comes from three approaches: assessing the capacity of field-sampled and/or experimentally manipulated sediments and soils to sorb inorganic PO_4^{3-} in lab-scale assays, experimental water level manipulations in mesocosms, and monitoring responses to changing hydrology in whole ecosystems (usually when historically drained areas are re-flooded). Studies across and within these three approaches utilize quite different designs and methodologies, making comparison difficult at times. For example, some studies take sediments from ecosystems that are inundated in the field and experimentally dry and re-flood them in a laboratory setting, whereas others sample sediments from ecosystems that are dry or historically drained in the field and re-flood them in the lab. The degree of desiccation varies across studies, and sometimes is not reported. Few studies have examined ecosystems spanning a wide variety of sediment characteristics, which makes it harder to compare results across the entire scope of wetland sediments that a manager may encounter. Here, we attempt to summarize results and their implications from studies using these three approaches.

Sediment Drying & Rewetting: Implications for Sediment Sorption Capacity

A dominant factor controlling a sediment's ability to sequester P is its capacity to sorb inorganic PO_4^{3-} molecules (Froelich 1988). Froelich (1988) describes the nature of PO_4^{3-} sorption well: "In solution, phosphate reacts quickly with a wide variety of surfaces, being taken up and released from particles through a complex series of 'sorption' reactions, playing hide-and-seek with both plankton and experimentalist." The sorption equilibria between dissolved PO_4^{3-} and P sorbed to benthic and suspended particle surfaces is often referred to as the "phosphate buffer mechanism," as it tends to maintain stable PO_4^{3-} concentrations in aquatic ecosystems. Many studies show that desiccated soils and sediments have lower PO_4^{3-} sorption capacity than those that remain moist or flooded, both in short-term experimental desiccation experiments (Twinch 1987, Schoenberg and Oliver 1988, Qiu and McComb 1994, Qui and McComb 2002, Song et al. 2007, Kerr et al. 2010) and in sediments collected from naturally desiccated areas in the field (Baldwin 1996, Axt and Walbridge 1999, Darke and Walbridge 2000, Kerr et al. 2010, 2011, de Vicente et al. 2010).

Researchers commonly study sediment or soil PO_4^{3-} sorption using sorption assays (Froelich 1988), in which soil or sediment samples of known mass are added to solutions of known PO_4^{3-} concentration(s), which are then incubated (usually shaken for 24 hours), after which the solid is separated from the liquid, and dissolved PO_4^{3-} is measured. The difference between the initial and final dissolved PO_4^{3-} concentration, corrected for sediment mass, (P_{sorbed}) is presumed to be the amount of PO_4^{3-} sorbed by sediments (if initial > final) or released from sediments (if final > initial). It is assumed that after 24 hr incubation, exchange processes between solid and dissolved P have reached an equilibrium, although in reality this is seldom the case, as sediment P sorption consists of a rapid, surface sorption step as well as a slow, solid state diffusion step (Froelich 1988, Pierzynski 2000).

In a multi-point sorption isotherm, researchers incubate soil with multiple solutions spanning a range of P concentrations and plot P_{sorbed} against the final concentration of PO_4^{3-} in solution to graphically represent P sorption in an adsorption isotherm or buffer diagram (Froelich 1988, Figure 1.4). The point at which the line plotted through this graph crosses the x-axis is the equilibrium phosphate concentration

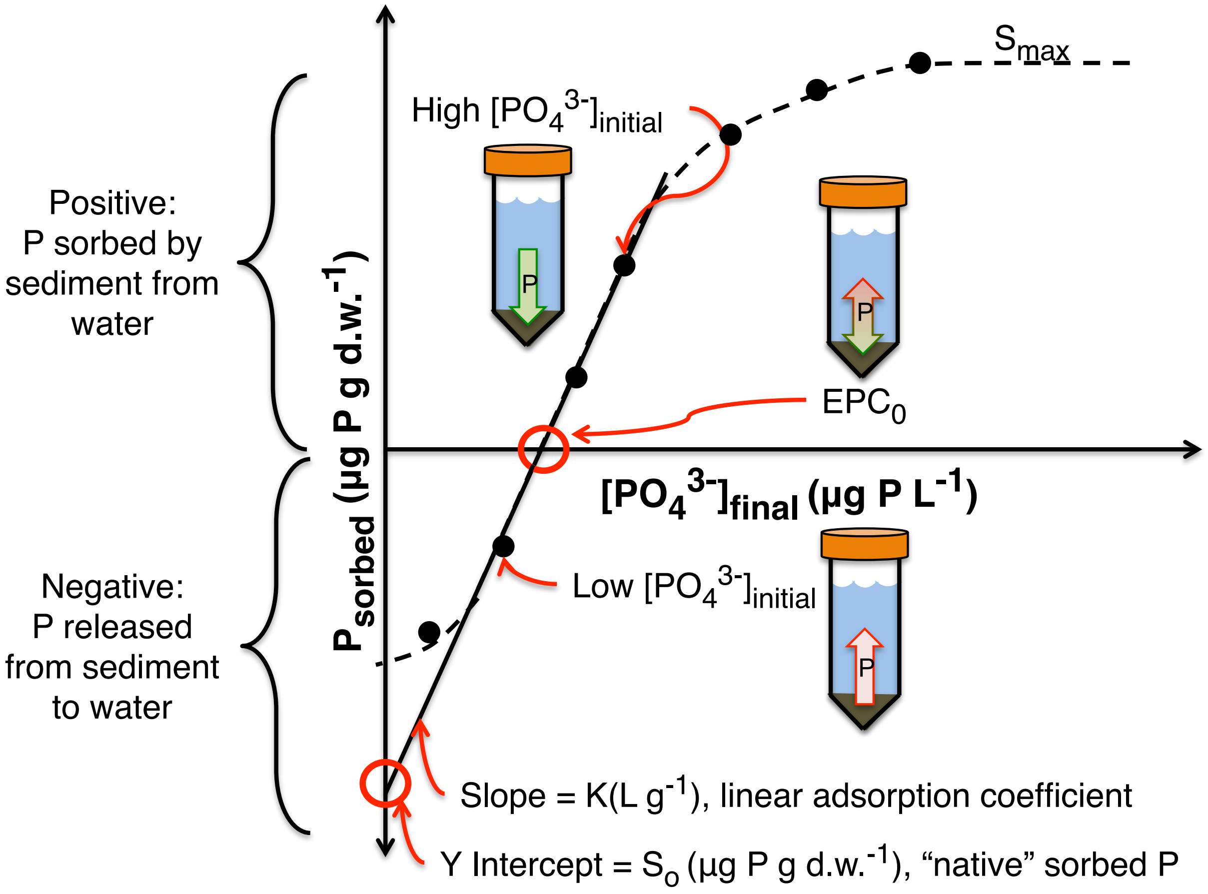

Figure 1.4 Theoretical sediment phosphorus adsorption isotherm or buffer diagram, adapted from Froelich (1988). Points represent incubations in which soil or sediment samples of known mass are added to solutions of known PO_4^{3-} concentration(s) The amount of PO_4^{3-} sorbed to or released from sediments (P_{sorbed}) is calculated as the difference between the initial and final dissolved PO_4^{3-} mass, calculated by multiplying the initial and final dissolved PO_4^{3-} concentrations by the volume of assay water. In a graph of P_{sorbed} against the final PO_4^{3-} concentration, the point at which the line crosses the x-axis is the equilibrium phosphate concentration (EPC_0), or the concentration of PO_4^{3-} at which the sorption and release rates are equal. The slope and y-intercept of the linear portion, estimated as empirical constants with least squares regression, are the linear adsorption coefficient (K) and the theoretical amount "native" P sorbed to sediments prior to the isotherm assay (S_0), respectively. Fitting sorption isotherm data to a Langmuir isotherm equation allows calculation of a sediment's theoretical sorption maximum, or the maximum amount of P a given sediment can sorb (S_{max}).

(EPC_o), or the concentration of dissolved PO_4^{3-} at which the sorption and release rates are equal. The slope and y-intercept of the linear portion of this relationship (estimated as empirical constants using least squares regression) represent the linear adsorption coefficient (K) and the estimated amount of "native" P sorbed to sediments prior to the isotherm assay (S_o), respectively. Other parameters describing sediment P sorption characteristics can be estimated using adsorption isotherm equations that were originally developed to characterize the sorption of gases onto particles, including the Langmuir, Freundlich, and Tempkin isotherms. For example, fitting data to a Langmuir isotherm allows calculation of a sediment's theoretical sorption maximum, or the maximum amount of P a given sediment can sorb (S_{max}). To provide insight into mechanisms behind observed P sorption, parameters measured with and statistically estimated from sorption assay data are statistically related to independently measured characteristics like soil texture, mineral composition, and organic matter content.

The redox status of sediment Fe is commonly thought to be the main factor controlling rates of sediment-water P exchange because Fe(III) oxides sorb PO_4^{3-} more strongly than dissolved (i.e., filterable), reduced iron (Fe(II)) (Mortimer 1941, 1942, Boström et al. 1988). However, the role of mineral crystallinity in controlling PO_4^{3-} sorption may supersede sediment redox status under some conditions. For example, Baldwin (1996) found that anoxic sediment that was oxidized in solution, but not desiccated, had lower P sorption capacity than when the same sediment was desiccated, and that both the wet oxidized and desiccated sediments had lower sorption capacity than the original, anoxic reduced sediment. Patrick and Khalid (1974) posit that while Fe(III) oxides may sorb PO_4^{3-} more strongly than their reduced counterparts, poorly crystalline Fe(II) oxides in reduced sediments may have more surface area and thus have the capacity to sorb a greater quantity of PO_4^{3-}, conferring greater sorption capacity to reduced than oxidized sediments.

Changes in sorption capacity associated with drying are often thought to be due to changes in the crystallinity of Fe and/or Al oxides and oxyhydroxides. Across soils and sediments from diverse ecosystems and hydrologic regimes, one of the strongest predictors of sediment P sorption is consistently the amount of poorly crystalline Fe and/or Al oxides and oxyhydroxides (e.g., Shukla et al. 1971, Williams et al. 1971, Richardson 1985, Darke and Walbridge 2000), measured as Fe and Al extracted with an acid ammonium oxalate solution, referred to as oxalate extractable Fe and Al (McKeague and Day 1966). The irregular molecular structures of these hydrated oxides and oxyhydroxides, described as “poorly crystalline,” “amorphous”, “gel complexes”, and/or “short-range order” (Schwertmann 1966, Shukla et al. 1971, Williams et al. 1971), have higher surface areas and more hydroxyl (–OH) groups for PO_4^{3-} ions to exchange with, while minerals with more regular crystal structures have lower surface areas and fewer –OH groups (McLaughlin et al. 1981). Thus, a given amount of crystalline oxide will have lower sorption capacity for PO_4^{3-} than the same amount of a poorly crystalline oxide (Williams et al. 1971).

Flooded sediments usually contain more oxalate extractable Fe and/or Al than adjacent upland soils (Axt and Walbridge 1999, de Vicente et al. 2010, Kerr et al. 2011). In addition, flooding dry soils usually causes an increase in oxalate extractable Fe and

Al (Sah et al. 1989, Darke and Walbridge 2000, Zhang et al. 2003), and drying flooded sediments usually decreases oxalate extractable Fe and Al (Twinch 1987, Qui and McComb 2002). The mechanisms behind these patterns remain unclear, but several have been suggested. Oscillating redox conditions in continuously flooded sediments may maintain redox-sensitive Fe in the “amorphous” form via repeated oxidation and reduction (Baldwin 1996), although recent work contradicts this (Thompson et al. 2006). Aluminum oxides and oxyhydroxides are not sensitive to redox conditions, although flooding and drying may still influence their crystallinity, presumably because crystallization of both Al and Fe minerals is inhibited by organic matter that builds up in flooded areas (Schwertmann 1966, Kodama and Schnitzer 1977, 1980, Darke and Walbridge 2000). Wetland soil P sorption parameters are often equally well or more highly correlated with oxalate extractable Al than oxalate extractable Fe (Richardson 1985, Reddy et al. 1995, Axt and Walbridge 1999, Darke and Walbridge 2000). The loss of P sorption and oxalate extractable Fe and Al associated with drying may occur either through shifts in particle size distribution from finer to coarser particles (Twinch 1987, de Vicente et al. 2010) and/or irreversible “aging” of the poorly crystalline minerals to more structured crystalline forms (Lijklema 1980, Baldwin 1996, Darke and Walbridge 2000, Qui and McComb 2002), both of which lead to a loss of mineral surface area for PO_4^{3-} sorption.

Sediment Drying & Re-wetting Causes P Release: Lab Scale Experiments

Sediment P sorption capacity, as measured using lab-based sorption assays, is a static sediment characteristic that provides an indication of whether sediments will release or retain P under certain environmental conditions. Theoretically, a sediment’s lab-measured sorption capacity may not correspond with actual P release rates under field conditions because of the many natural processes affecting sediment-water P exchange that are not incorporated into lab-based sorption assays. For example, lab-based sorption assays are seldom conducted under controlled redox conditions, while field redox conditions may oscillate between oxidizing and reducing on diel and seasonal time scales. Experiments in which intact sediment cores or constructed wetland mesocosms are experimentally dried and re-flooded incorporate more realistic processes, while still allowing for some degree of experimental control.

Flooding intact sediment cores or other types of sediment-water mesocosms that have been dried in the field or experimentally in a lab usually causes sediment P release to surface and/or pore waters (Olila et al. 1997, Young and Ross 2001, Lucassen et al. 2005, Aldous et al. 2007, Loeb et al. 2008a, Schönbrunner et al. 2012). The sources of released P vary, depending on both the hydrologic history and the biogeochemical characteristics of the sediments. Because of the complex nature of P binding, and considering that the measurements of P binding fractions are limited to operationally defined components measured using sequential chemical extractions, it is often difficult to determine proximate and ultimate sources of released P.

If sufficient soil moisture remains in drained sediments, the rate of remineralization of organic P to inorganic PO_4^{3-} can increase as previously anoxic sediments that have been accumulating organic matter are exposed to oxygen, allowing for faster, more energetically favorable, microbial metabolism (Martin et al. 1997, Fisher and Reddy 2001, Schönbrunner et al. 2012). If there is insufficient inorganic sorption capacity in the sediment, then remineralized PO_4^{3-} may be released into surface waters upon re-flooding (Olila et al. 1997, Zak et al. 2010).

When soils desiccate beyond a critical moisture level, there is some evidence that released P upon re-wetting may be at least partly from microbial biomass (Olila et al. 1997, Turner and Haygarth 2001, Blackwell et al. 2010, Schönbrunner et al. 2012). When dry soils are re-wetted, microbes experience osmotic stress and release osmolytes (Schimel et al. 2007) or lyse and die (Blackwell et al. 2010). The most common osmolytes are proteins and polyols that do not contain P (Schimel et al. 2007), so P release from microbial biomass would require cell lysis. The effect of re-wetting stress on microbial P release has been poorly studied compared to effects on C and N.

P released upon re-flooding may also come from inorganic sources. If the sorption capacity of sediment is decreased during drying, then a new equilibrium will establish after re-flooding with less PO_4^{3-} sorbed to sediment particles and a higher concentration of PO_4^{3-} in surface waters (Song et al. 2007, de Vicente et al. 2010). Once oxygen is depleted in flooded sediments, which is typically rapid in re-flooded wetland sediments, microbial reduction of Fe(III) to Fe(II) will commence, mobilizing P previously sorbed to Fe(III) minerals (Mortimer 1941, 1942, Loeb et al. 2008a). In sediments with considerable Fe sulfide minerals, draining and exposure to oxygen causes oxidation of these minerals to Fe oxyhydroxides and dissolved sulfate (Boman et al. 2008). This oxidation process may regenerate some P sorption capacity but also generates acidity, which may then dissolve any PO_4^{3-} associated with $CaCO_3$, causing release of P (Lucassen et al. 2005, Smolders et al. 2006). Much of the inorganic P released upon re-flooding of historically agricultural soils is likely a reflection of high levels of stored soil P, the legacy of high loadings of fertilizer or manure (Martin et al. 1997, Pant et al. 2002, Aldous et al. 2007, Banach et al. 2009).

Wetland Draining & Re-flooding Causes P Release: Field Studies

While mesocosm experiments allow for detailed investigations of mechanisms controlling sediment-water P exchange, monitoring wetlands during changing water levels in the field provides a more realistic picture of the magnitude, timing, and longevity of P release following re-flooding over more relevant spatial and temporal scales. Re-flooding historically drained agricultural land has become a common practice in wetland restoration (Zedler 2003), but flooding often leads to P release (Newman and Pietro 2001, Coveney et al. 2002, Zak and Gelbrecht 2007, Zak et al. 2010, Ardón et al. 2010a, Wong et al. 2011), which can lead to eutrophication, potentially inhibiting the provision of desired ecosystem services, including biodiversity support and nutrient removal (Verhoeven et al. 2006). Phosphorus release upon re-flooding of historically cultivated drained land is often attributed to legacies of high P loading, but P release occurs (albeit usually at lower rates) even in re-flooded sediments without a history of high P loads (Scholz et al. 2002, Aldous et al. 2005). Phosphorus release upon re-flooding drained soils in the absence of high P loads is likely due to enhanced remineralization of highly organic sediment, transforming P from organic pools to easily mobilized PO_4^{3-} when sediments were dry, which can be released upon re-flooding in the absence of sufficient sorption capacity (Zak et al. 2010). On top of P mineralized from extant organic matter, P added in fertilizers and manure leads to an even higher amount of easily mobilized P that may enter surface waters when wetland hydrology is restored.

The ultimate fate of sediment-released P and longevity of P release will contribute to whether or not the nutrient release risks outweigh the benefits of restoring wetland hydrology to historically drained areas. P release from sediments can either be exported to downstream ecosystems or remain in the wetland, where it may cycle internally or become buried and stored in sediments. Few studies have monitored re-flooded agricultural fields both during the initial flooding event and into the future, but available evidence suggests that sediments can continue to release P at least 5-10 years after re-flooding (Montgomery and Eames 2008, Duff et al. 2009, Ardón et al. 2010a, Steinman and Ogdahl 2011, Hamilton 2011). In the absence of continued external P loading, P release rates should decline over time as sediment pools are depleted and P sorbed to sediment comes into equilibrium with P dissolved in sediment interstitial and surface waters. If sediment-released P is not exported in outflowing waters, this new equilibrium may establish a chronically high surface water P concentration within the restored wetland.

Apparent Contradictions: Sediment Drying Improves Wetland P Retention

While many studies show a net sediment P release upon re-wetting dried sediments and diminished P sorption on dried sediments, some studies show evidence of improved P sorption (Barrow and Shaw 1980, Haynes and Swift 1985, De Groot and Fabre 1993, Peltovuori and Soinne 2005) or retention capacity after drying soils or sediments (Mitchell and Baldwin 1998, Smolders et al. 2006). Australian reservoir sediments that had experienced long term (12 months) desiccation released less P in anoxic slurries than similar sediments that had been continuously flooded, presumably due to loss of sulfate-reducing bacteria, aging of oxide minerals, and carbon limitation after drying (Mitchell and Baldwin 1998). In addition, there is some evidence that draining sediments for short periods of time may reduce rates of P release due to Fe oxidation and an associated increase in PO_4^{3-} sorption (De Groot and Fabre 1993, Martin et al. 1997, Lucassen et al. 2005, Smolders et al. 2006). However, this is effective only in some cases, as oxidation of Fe sulfides in poorly-buffered soils can lead to acidification, causing release of $CaCO_3$-associated P, as well as sulfide and metal toxicity (Lucassen et al. 2005, Smolders et al. 2006).

How Does Sediment Drying or Draining Influence P Release Upon Re-wetting or Re-flooding?: Synthesis Conclusions

Despite methodological differences and some contradictory results, most studies provide evidence that 1) drying diminishes sediment P retention capacity and that 2) re-wetting experimentally or naturally drained sediments causes sediment P release. Based on studies that have investigated effects of drying on P sorption characteristics, sediment-water P exchange in mesocosms, and/or monitoring re-wetting of whole ecosystems, instances in which sediment drying or draining causes net sediment P retention upon re-flooding seem to be the exception, rather than the rule. Given the complexity of sediment P retention (Figure 1.3), understanding the mechanisms underlying observed changes in sediment-water P exchange in response to drying and re-flooding is crucial to better predict how biogeochemically diverse sediments will respond to alterations in hydrologic regime.

Reconciling Apparent Contradictions

The apparent contradictions among published studies between the effects of drying and re-wetting on sediment P retention stem largely from variation in methodology, differences in the types of sediments being studied, and in the temporal and spatial scales at which studies are conducted. Given the diverse mechanisms by which sediments retain and release P, and the differential effects of hydrologic change on each individual mechanism, it is to be expected that a sediment's biogeochemical characteristics (for example, if the sediment is rich in Fe, sulfur, or $CaCO_3$) would influence its response to drying and re-wetting. Most studies do not investigate multiple sites spanning biogeochemically diverse sediments, but those that do confirm that the effects of hydrologic regime change on sediment-water P flux depend on sediment biogeochemistry (Chapter 3, this dissertation, Qiu and McComb 1994, Martin et al. 1997, Lucassen et al. 2005, Smolders et al. 2006, Kerr et al. 2010).

The most critical difference among studies may be the duration and degree of sediment drying, as we will discuss further below. In studies that explicitly test the effects of the degree of drying on sediment P release, more severely desiccated sediments release more P than sediments that remain moist (Olila et al. 1997, Pant and Reddy 2001, Aldous et al. 2005, Schönbrunner et al. 2012). Despite the potential importance of sediment moisture content prior to re-flooding, few studies report key information regarding the degree of sediment or soil drying, making comparison among them and attempts to reconcile apparent contradictions challenging.

A General Hypothesis for the Effects of Sediment Drying and Re-flooding on Sediment-Water P Exchange

Despite the biogeochemical complexity of sediment P cycling and the vast diversity of experimental and sampling approaches in the literature, initial sediment P release upon re-flooding is most commonly observed. There are multiple mechanisms that can explain this net P release, and it remains uncertain whether or not one of these mechanisms is the dominant explanation across different scenarios, or if they all occur to different extents in different places and at different times.

After synthesizing existing evidence, we formulate a conceptual model of the effects of sediment drying and rewetting on wetland P retention (Figure 1.5). We hypothesize that the duration and degree of drying are critical in determining the net effect on sediment-water P flux upon after re-flooding because of the effects of sediment moisture on 1) microbial processes and 2) sediment mineralogy. The net effect on sediment P release depends on processes that occur during two phases: while sediment is drying and when sediment is re-flooded.

Scenario 1: Sediments remain moist

During drying: When wetland sediments are drained or dry due to dropping water levels, oxygen increases but moisture could be present so 1) mineralization rates increase, converting organic P to inorganic PO_4^{3-} and 2) reduced Fe (and Fe sulfides, if present) is transformed to poorly crystalline Fe oxyhydroxides by dissimilatory microbial oxidation. If the sediment is poorly buffered, then oxidation processes, which generate acidity, lower sediment pH. Mineralized PO_4^{3-} can sorb to Fe and/or Al oxides and oxyhydroxides if available sorption sites are present.

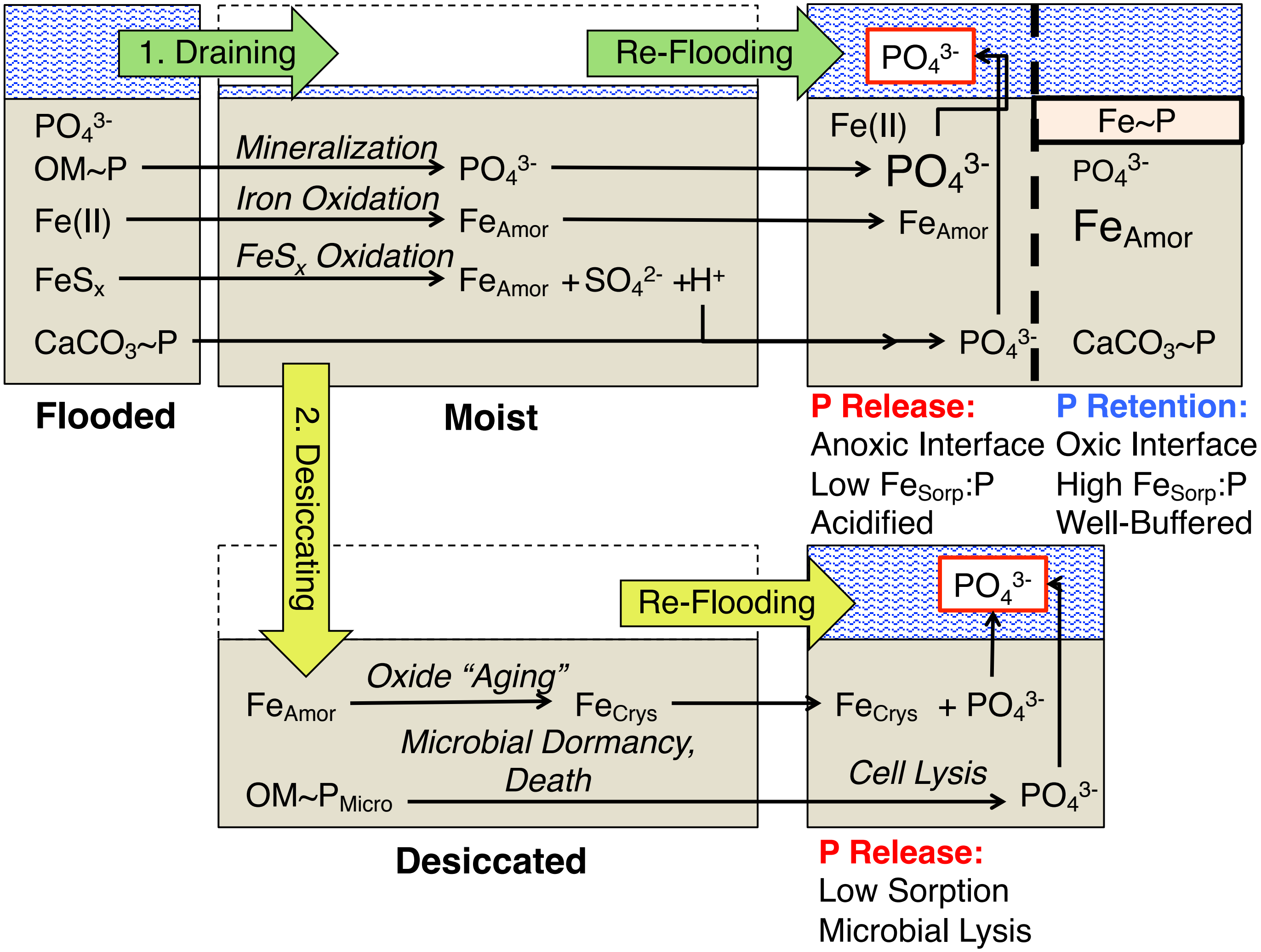

Figure 1.5 Conceptual model of sediment P processes leading to sediment retention or release after drying and rewetting. Two scenarios are depicted, one (1) in which sediments are no longer flooded, but remain moist and another (2), in which sediments desiccate. See text for explanation of abbreviations.

After re-flooding: Upon re-flooding, PO_4^{3-} will be released into the water column if 1) the sediment-water interface becomes anoxic, causing reduction of oxidized Fe and mobilization of associated PO_4^{3-}, 2) the sorption capacity of the sediments is exceeded due to a drying-associated decrease in sorption capacity and/or increases in inorganic PO_4^{3-} from mineralization**,** and/or 3) processes such as sulfide oxidation lower sediment pH, causing dissolution of $CaCO_3$ and release of associated P. Phosphate will remain in sediments as long as 1) the amount of PO_4^{3-} ions does not exceed mineral sorption sites, 2) the sediment-water interface remains oxic, and 3) the sediment is well buffered enough to prevent dissolution of $CaCO_3$-associated P (if present).

Scenario 2: Sediments desiccate

During prolonged drying/desiccation: When sediments desiccate past a certain moisture level, remineralization rates will decline as microbes die or become dormant. If microbes die, they leave behind labile biomass containing easily mineralized P. Concurrently, as discussed above, fine particles may irreversibly aggregate into larger ones and Fe and Al oxyhydroxides may “age” and become more crystalline. Both of these processes reduce mineral oxide surface area and thus PO_4^{3-} sorption sites.

After re-flooding: Upon rewetting, microbial cells may lyse due to extreme osmotic stress (although direct evidence for this is limited, see Unknowns section), mobilizing microbial biomass P, adding another P source in addition to remineralized PO_4^{3-} and desorbed PO_4^{3-}**.** The sorption capacity of the sediment will likely remain lower, causing a net release of P to surface waters, especially if sediments and/or surface waters are already high in P.

Unknowns

The hypothesized scenarios described above focus mainly on biogeochemical processes occurring in wetland sediments, particularly those influenced by oxygen and redox potential, which are linked to water saturation status. However, many unknowns remain regarding these processes, as well as the contributions of other ecosystem processes to changes in sediment-water P exchange after a wetland experiences a change in hydrologic regime, such as P assimilation by rooted plants (both aquatic and terrestrial) and benthic algae. These unknowns provide many opportunities for fruitful research with implications for wetland restoration and management. The role of plant and algal uptake in sequestering newly available P is expected to be highly variable among wetlands and over time within a particular wetland. In particular, changes in microbial processes and the structure of sediment minerals are poorly understood.

Effects of Drying on P-Sorbing Oxide Minerals

Although dried soils and sediments have often been shown to display lower P sorption capacities than their flooded or moist counterparts (Twinch 1987, Schoenberg and Oliver 1988, Qiu and McComb 1994, Baldwin 1996, Qui and McComb 2002, Song et al. 2007, de Vicente et al. 2010), the mechanism of decreased sorption is not well understood. Shifts in particle size distribution to larger particles (Twinch 1987, de Vicente et al. 2010) and aging of Fe and Al minerals from amorphous to more crystalline forms (Baldwin 1996, Qui and McComb 2002) have both been suggested. The exact process by which mineral oxides “age” to more crystalline forms, as well as the time scale over which this process occurs, has not been explicitly investigated.

Newly oxidized and precipitated Fe oxides are expected to have a more amorphous, less crystalline structure than older Fe oxides (Thompson et al. 2006). Thus, some studies suggest that in flooded ecosystems, repeated microbial Fe oxidation and reduction at redox gradients can maintain Fe oxides in their amorphous forms (Baldwin 1996), but recent work unexpectedly demonstrated that controlled redox oscillations under flooded conditions converted amorphous Fe oxides to more crystalline forms in a tropical forest soil (Thompson et al. 2006). Others have postulated that the loss of sorption capacity and increase in Fe crystallinity is simply due to a loss of fine particles in drying sediments, and that evidence for aging of minerals from amorphous to crystalline is “ambiguous” (de Vicente et al. 2010). It is well established that poorly crystalline Fe and Al oxides can sorb much higher amounts of PO_4^{3-} than more crystalline ones, at least as they are currently measured (McLaughlin et al. 1981), and thus understanding effects of hydrology on oxide mineral structure are critical to assessing a sediment’s ability to retain P and its potential response to hydrologic changes.

Effects of Drying on Microbial Processes

The direct effects of drying and re-wetting on soil microbial physiology is currently an active area of study (Schimel et al. 2007). A meta-analysis has found that microbial activity ceases at water potentials of about -14 MPa in soils due to limited solute diffusion and -36 MPa in surface litter due to dehydration (Manzoni et al. 2012). The effects of soil moisture content and re-wetting events on microbially mediated P transforming processes have not been studied nearly to the extent that re-wetting effects on C and N release have been, despite the strong circumstantial evidence for mobilization of microbial biomass P upon re-wetting dried soils (Turner and Haygarth 2001). In addition to physiological effects on microbial cells, differing hydrologic regimes select for microbial communities with different functional capacities (Mentzer et al. 2006, DeAngelis et al. 2010). Perhaps the most important functional difference among microbial taxa is differential ability to access and use inorganic and organic P sources. Because of the diversity of P-containing molecules and enzymes used to access P compounds, these processes are complex to study, and are only beginning to be understood (White 2009, Bird 2012).

Other Unknowns

Solid $CaCO_3$ can be an important P storage pool in sediments of alkaline waters (Hamilton et al. 2009), but the responses of this particular fraction to hydrologic changes have not been explicitly studied. Presumably, carbonate mineral equilibria and particularly pH are the most important drivers determining retention or release of $CaCO_3$-associated P, but hydrology may influence other factors controlling carbonate equilibria in unpredictable ways. For example, drainage of pore waters may release free carbon dioxide (increasing pH), and may result in evaporative concentration of solutes or in temperature changes that affect solubility of $CaCO_3$.

Hydrology plays a well-known role in shaping wetland plant communities (Batzer and Sharitz 2006). As wetland plant species distribution and abundance change in response to changing hydrology, the functions of plants as nutrient reservoirs, organic matter sources, and conduits for gas exchange between the atmosphere and sediments will likely change, influencing rates of many sediment P cycling processes. Hydrologic change is also frequently associated with changes in temperature regimes. Microbially mediated processes, as well as rates of chemical sorption and desorption, are all influenced by temperature, but these effects are difficult to isolate from the many other associated concurrent changes. In general, rates of sediment P release tend to be more rapid at higher temperatures (Loeb et al. 2008b).

Benthic macroinvertebrates that burrow in sediments, rework the sediment structure, and/or irrigate sediment burrows are common in aquatic ecosystems, and certain species such as tubificid worms reach especially high densities in eutrophic ecosystems (Milbrink 1983). Bioturbators alter the structure of surface sediments and increase both oxygen penetration into sediments and sediment-surface water solute exchange by pumping oxygenated surface waters into their burrows (Mermillod-Blondin and Rosenberg 2006, Meysman et al. 2006, Gallon et al. 2008, Kristensen et al. 2012). The effect of drying on the presence and activity of these and other organisms, and thus their effects on P flux upon re-flooding, will depend on their tolerance to varying degrees of desiccation and how quickly they can recolonize re-flooded sediments.

Future Research Suggestions

An improved overall understanding of wetland P retention at the ecosystem scale will require studies of the above mechanistic unknowns, as well as whole-ecosystem studies that allow us to reconcile measured effects in small mesocosms with those observed in ecosystems. In addition, we need to study ecosystems for longer time periods to better understand the long-term effects of altered hydrologic regimes. Finally, explicit study of how changes in plant, animal, and microbial communities due to hydrologic changes alter P cycling will be required to better predict outcomes of potential hydrologic regime change.

Conclusion

The net response of sediment-water P exchange to altered hydrologic regime in wetlands and other shallow water bodies is a complex function of the responses of many different P-retention processes in sediments, which are differentially controlled by temperature, redox, pH, microbial activity, and other factors. Re-flooding historically drained sediments typically causes initial sediment P release, although constructed and restored wetlands may eventually retain P over annual time scales (Coveney et al. 2002, Aldous et al. 2007, Ardón et al. 2010b). When managing wetlands for multiple ecosystem services, if P retention is desired, it is imperative to consider the biogeochemistry of the sediment in predicting the ecosystem’s initial and long term P response to altered hydrologic regimes. The effects of elevated anthropogenic P loading to soils and water bodies can take many years to diminish in part because of the high P storage capacity of soils and sediments and the potential for gradual release of this legacy P to overlying waters (Hamilton 2011). Regardless of management scheme, there is an upper limit to the amount of P a wetland can retain, and the ultimate solution to P-driven eutrophication is to reduce P inputs.

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CHAPTER 2: RE-FLOODING A HISTORICALLY DRAINED WETLAND LEADS TO RAPID SEDIMENT P RELEASE

Abstract

In recent decades, re-flooding historically drained areas has become common in an attempt to regain lost wetland habitat and services. Wetland restoration provides many benefits, but re-flooding historically drained and disturbed land can have unintended negative consequences, particularly phosphorus (P) release. To investigate the effects of re-flooding a historically drained wetland on P cycling, we monitored a restoration that entailed back-flooding old drainage ditches and re-inundating former wetland soils. Immediately after re-flooding in September 2008, previously exposed sediments released substantial amounts of P to surface waters. Soluble reactive phosphorus (SRP) concentrations in newly re-flooded areas were as high as 750 µg P L^{-1} in the days after re-flooding, constituting large increases in the total mass of P in surface water, about 20 times more SRP and 14 times more total P than prior to re-flooding. After one year, however, SRP concentrations remained lower than 50 µg P L^{-1} until the end of our sampling in January 2011 (about 15 months after re-flooding). Overall, the highest SRP concentrations occurred during the first year, and only when surface water dissolved oxygen (DO) was low (< 5.5 mg L^{-1}). Similarly low DO in Year 2 was not associated with such high P concentrations. It is likely that a combination of algal uptake during high O_2 periods and desorption from sediment iron under low O_2 conditions together led to the observed SRP-DO pattern. We provide evidence for both of these processes by (1) estimating P in filamentous algal biomass during a large

bloom that coincided with episodically low SRP concentrations throughout the wetland and (2) measuring rates of sediment P release in the absence of algal uptake in intact sediment cores with aerated and un-aerated surface waters. These observations demonstrate that when restoring wetlands by re-flooding historically drained areas, managers should consider the potential for sediment P release to jeopardize restoration goals and therefore should incorporate longer-term monitoring of water quality into restoration plans.

Introduction

During the 19th and early 20th centuries in the United States, wetlands were systematically drained and “reclaimed” for agricultural use (Dahl 1990). In recent decades, managers have begun restoring wetland hydrology to some of these historically drained areas in an attempt to regain lost habitat and ecosystem services (Zedler 2003). This practice is incentivized in the United States through the Wetland Reserve Program (http://www.nrcs.usda.gov/programs/wrp/), a voluntary program in which the US Department of Agriculture’s Natural Resources Conservation Service provides landowners financial support in exchange for wetland protection, restoration, and enhancement.

Wetland restoration provides a number of benefits, but re-flooding historically drained and cultivated land can have unintended negative consequences. Often, drained wetland sediments release nutrients upon re-flooding, as has been shown both in lab-scale re-flooding experiments (Olila et al. 1997, Young and Ross 2001, Lucassen et al. 2005, Aldous et al. 2007, Loeb et al. 2008, Schönbrunner et al. 2012) and in monitoring of whole ecosystem re-flooding (Newman and Pietro 2001, Coveney et al. 2002, Ardón et al. 2010, Wong et al. 2011). However, some dried and re-flooded sediments do not release nutrients at higher rates than continuously flooded ones, which may reflect biogeochemical differences that are not well understood (Mitchell and Baldwin 1998, Baldwin et al. 2000, Smolders et al. 2006). Nutrient release due to re-flooding can inhibit the restored wetland's ability to support biodiversity and improve water quality, which are two common goals of wetland restoration (Hansson et al. 2005, Verhoeven et al. 2006). Thus, managers need to understand the circumstances that make sediment nutrient release likely.

Although studies show that sediments often initially release phosphorus (P) when re-flooded after long term drainage (particularly when they were fertilized for cultivation), the sources and mechanisms of P release remain uncertain. High rates of P release upon re-flooding are often attributed to the legacy of fertilizer application, but P release has been shown to occur even in dried and re-flooded sediments without a history of high P loads (Aldous et al. 2005). It seems that there are complex natural processes which lead to sediment P release upon re-flooding that can be exacerbated by elevated P loading associated with agricultural use.

The persistence of sediment P release after initial re-flooding and the ultimate fate of the released P are also difficult to predict. Released P can either be exported to downstream ecosystems or remain in the wetland, where it may cycle internally and eventually become buried in sediments or assimilated into biomass. Few studies have monitored re-flooded agricultural soils both during the initial flooding event and into the future, but studies of wetlands that were re-flooded in the past have shown that sediments can continue to release P at least 5-10 years after re-flooding (Montgomery and Eames 2008, Duff et al. 2009, Steinman and Ogdahl 2011). Theoretically, P release rates should decline over time as sediment P pools are depleted and the sediment comes into equilibrium with its overlying water, assuming the absence of new P loading, but in some cases this can take a very long time (Hamilton 2011).

To investigate the immediate and longer term (2 years) effects of re-flooding a historically drained wetland on P cycling, we monitored a wetland restoration project that entailed construction of a control structure to back-flood old drainage ditches and the surrounding wetland soils. We studied the aqueous and sediment biogeochemistry during the summer prior to re-flooding and for two years after the re-flooding event. We sought to address the following questions: (1) Will the sediments release P upon re-flooding, and if so, how much?, **(**2) What are the mechanisms of P release?**, (**3) What is the longevity of P release?, and (4) What is the ultimate fate of released P?

Methods

Site Description

We studied a 9 ha (when flooded to the sill level of the dam) wetland located in the Fort Custer Training Center (FCTC), a military training area in southwestern Michigan, USA. The historically drained “Area 2 Wetland” was re-flooded in September 2008 as part of a restoration effort (Langerveld 2009). Ditches draining the wetland appear in aerial photographs from at least the late 1930’s (Figure 2.1), indicating that the area had been drained for at least 75 years, and perhaps as many as 100 (Langerveld 2009). During this time however, the wetland flooded periodically

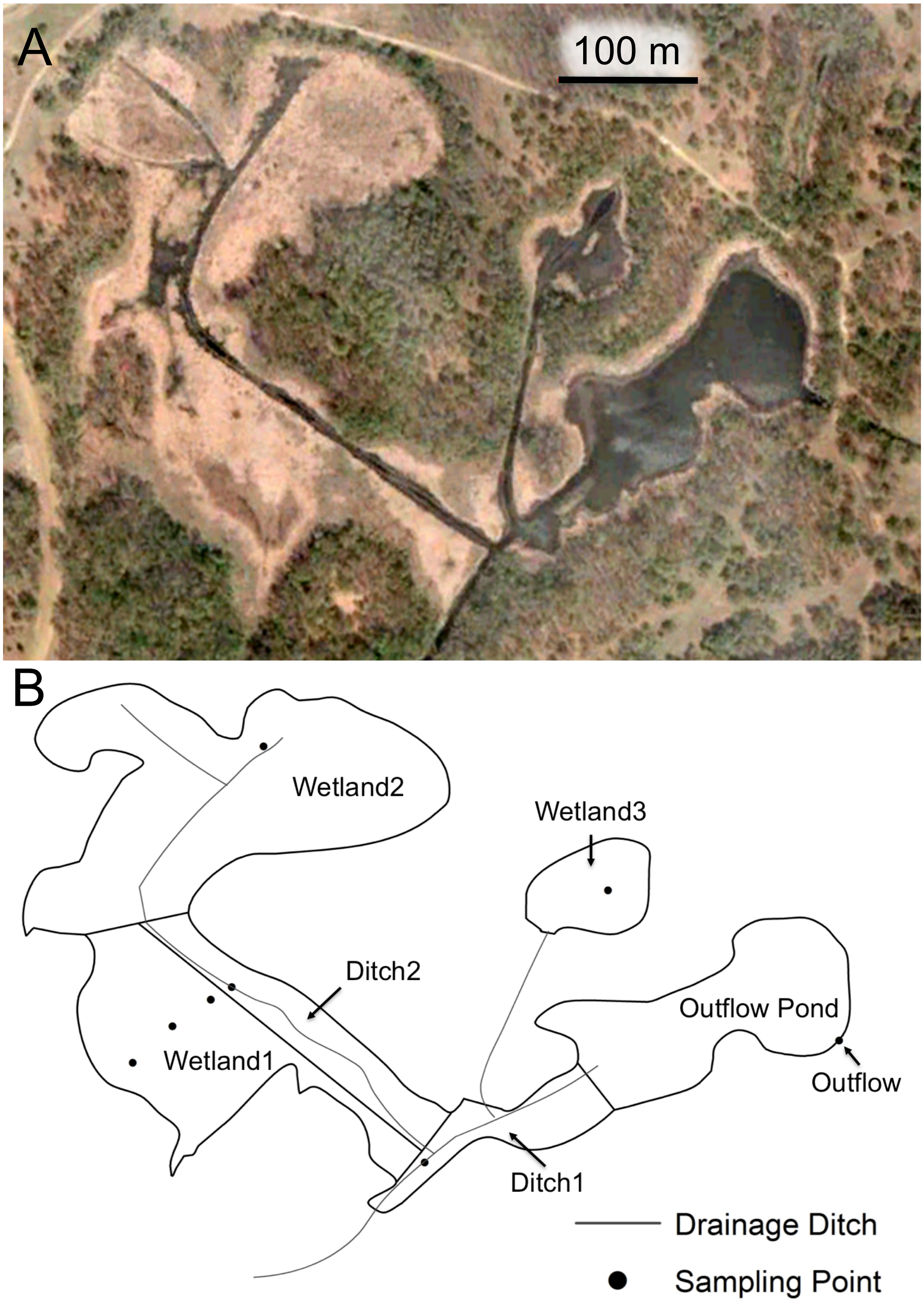

Figure 2.1 Aerial photograph of the Fort Custer Training Center “Area 2 Wetland” taken on 25 April 2005 (Google Earth) with drainage ditches visible (A). Drainage ditches appear in aerial photographs from the 1930’s. Outline of Fort Custer Training Center “Area 2 Wetland” divided into sampling zones, with point locations of repeated sampling for water chemistry monitoring (B).

for shorter but unknown periods of time due to beaver activity, most recently in the late 1990s (Langerveld 2009). Prior to flooding, curlytop knotweed (*Polygonum lapathifolium*), stinging nettle (*Urtica diocia*), and reed canarygrass (*Phalaris arundinaceae*) were the dominant plant species, and water tables remained high enough to prevent shrub and woody encroachment. Soils in the wetland are >90% Houghton muck, a euic, mesic Typic Haplosaprist (Soil Survey Staff 2012).

Prior to the extreme precipitation event in September 2008 that flooded the wetland to its full capacity, only the drainage ditches and a portion of the outflow pond were fully inundated. The sediment surface in the Wetland zones was exposed, but the water table was not far below the surface. The summer of 2008 was relatively hot and dry, so even areas that were usually inundated were exposed by late summer (e.g., the sediment surface in Ditch2 was exposed in mid-September)

A partnership between FCTC, Ducks Unlimited, the US Fish & Wildlife Service, and other organizations restored this wetland area to improve biodiversity, provide opportunities for scientific research and education, and provide floodwater protection for the FCTC roadways and downstream property. In July 2008, a water control structure (Agri-Drain, Adair, IA) and emergency spillway were installed at the wetland outflow (Figure 2.1), establishing the maximum water level of the water body at 874 ft above sea level. Water levels remained close to their original level (~870.7 ft) until September 2008, when an extreme precipitation event (239 mm rain in 3 days, KBS LTER 2012, www.lter.kbs.msu.edu) associated with the remnants of Hurricane Ike flooded the wetland to its full capacity. After this re-flooding event, the wetland remained at or near its full flooded capacity throughout our sampling period.

The wetland drains a relatively small area (~100 ha), most of which is within the FCTC. Even when fully flooded, there is no single, channelized inflow that feeds the wetland. Drainage Ditch1 drains a larger area prior to entering the study wetland, but was rarely, if ever, flowing measurably. Based on dissolved Mg^{2+} concentrations measured in surface waters, which is a reliable indicator of groundwater in this particular landscape (Whitmire and Hamilton 2005), it is likely that groundwater represents more than half of hydrologic inputs to Ditch1 and the Outflow Pond. The newly re-flooded wetland areas, however, contained much lower Mg^{2+} concentrations, indicating a lower influence of groundwater and a low rate of mixing between newly re-flooded areas and the "inflowing" Ditch1. It seems that Ditch1 is fed partially by groundwater upstream but short-circuits most of the wetland as it flows into the Outflow Pond. During the study period, one storm water drain directed run off from an unpaved parking area into the wetland.

Wetland Morphology

To understand relationships between wetted area and volume, we measured depths at marked waypoints with high spatial resolution throughout the wetland on August 10 and 12, 2009 and October 20, 2010. Depths measured repeatedly at the same locations revealed that the water level difference between these dates was ~10 cm. In a geographic information system (ArcGIS 10), we adjusted the existing National Wetlands Inventory polygons for the Area 2 Wetland to more accurately represent the wetland's shoreline and location of drainage ditches based on aerial photographs and marked waypoints with known depths. We used depth measurements to estimate depth contours at 0.2 m intervals, with which we estimated the volume of the fully flooded wetland using the truncated cone method (Wetzel and Likens 2000). Subsequently, we divided the wetland into zones corresponding to repeated water sample points (Figure 2.1) and estimated the volume within each zone using the same method.

Water Chemistry Dynamics

To test the effects of ecosystem re-flooding on nutrient cycling, we monitored surface and pore water chemistry during the summer prior to and for over two years after re-flooding. We established locations for repeated samplings in the inflow ditch (Ditch 1), the main drainage ditch (Ditch 2), three areas that were initially upland but later flooded (Wetlands 1-3), and at the wetland's outflow (Figure 2.1). Prior to the re-flooding event, we sampled surface waters in inundated drainage ditches and the outflow at least twice each, and also obtained one sample from Wetland 3, in which there was a small amount of water (< 20 cm deep and a very small proportion of the zone's area).

After re-flooding in September 2008, we monitored water chemistry approximately monthly until January 2011. One of the zones, Wetland 1, contained three repeatedly sampled points along a transect running perpendicular to Ditch 2 (Figure 2.1). At each point for each sampling event, we measured surface water temperature, dissolved oxygen, pH, and specific conductivity (corrected to 25°C) using a Hydrolab multisonde. We collected surface water samples for measurement of major ions and nutrients. For analyses of dissolved ions and nutrients, surface waters were filtered (0.45 μm, Pall Supor). We sampled sediment pore water from a known depth (~7-12 cm) using a plastic syringe and tubing connected to a screened filter at the end of a drive-point sampler. We filtered pore water samples through a 0.45 μm cellulose-acetate filter with a glass fiber pre-filter (Steriltech) and added reagents in the field for analysis of dissolved (filterable) reduced iron (Fe(II)) and free hydrogen sulfide (H_2S). At least monthly, and approximately every other week during 2009, we measured discharge at the outflow of the wetland using a Marsh-McBirney flow meter, recorded water depth at a standard location, and sampled surface water at the outflow for soluble reactive phosphate (SRP), total dissolved P (TDP), and total P (TP).

We measured SRP concentrations in surface and pore water samples using the molybdate blue colorimetric method (Murphy and Riley 1962), and nitrate (NO_3^- -N), sulfate (SO_4^{2-}), calcium (Ca^{2+}), and magnesium (Mg^{2+}) ions using membrane-suppression ion chromatography. Total ammonium nitrogen (NH_4^+ -N) was measured using the indophenol blue method (Grasshoff et al. 1983, Aminot et al. 1997) and long-pathlength spectrophotometry. We measured TDP and TP after persulfate digestion of membrane filtered (0.45 μm) and unfiltered samples, respectively, followed by colorimetric SRP analysis. Dissolved organic carbon (DOC) was measured in filtered surface and pore water samples using a Shimadzu TOC 5000 analyzer. We measured Fe(II) in pore waters using reaction with ferrozine based on a method modified from Lovley and Philips (1987) and Stookey (1970), in which the pore water sample was immediately added to a solution of 50 mM HEPES buffer containing ferrozine (1 g L^{-1}). After color formation, we measured sample absorbance on a spectrophotometer at 562 nm. We measured H_2S using the methylene blue spectrophotometric method (Golterman and Clymo 1969).

Sediment Sampling

To characterize Area 2 Wetland sediments during our study period, we collected sediments at least once each year and measured a number of biogeochemical characteristics that are important in P cycling. To investigate the effects of re-flooding on sediment P binding fractions, we sampled sediments immediately prior to (September 3, 2008) and immediately after (September 17, 2008) the flooding event. On these dates, sediment samples were taken throughout the wetland (Ditches 1-2, Wetland 1-3) for sequential P extraction as well as analysis of organic matter (OM) by loss on ignition, total P (sedTP), and total iron (TFe). To gain an understanding of a broader range of characteristics, in 2009 and 2010 sediments were collected from a subset of sites (Wetland 1 and Wetland 3) and analyzed for OM, sedTP and TFe, as well as poorly crystalline, oxalate extractable iron (Ox-Fe), total solid carbonates ($CaCO_3$), total aluminum (TAl) and acid volatile sulfides (AVS).

Sediment Chemical Analyses

To prepare sediment for measurement of OM, sedTP, TFe, and $CaCO_3$, we dried a sub-sample of moist sediment to a constant weight and ground it with a mortar and pestle to homogenize it. We then combusted triplicate sub-subsamples (~1 g d.w.) of dried and ground sediment for analysis of OM as loss on ignition (550°C). We extracted combusted samples for 10 minutes in boiling 1 M hydrochloric acid (HCl) for measurement of sedTP, TFe, and TAl (Andersen 1976). To measure Ox-Fe, an indicator of poorly crystalline iron minerals, we extracted ~0.4 g moist sediment in a 0.2 M acid ammonium oxalate solution for 4 hours in darkness (Walbridge et al. 1991). We measured $CaCO_3$ in triplicate sub-samples of dried and ground sediment (0.1-1 g d.w.) by acidifying in a sealed container, measuring carbon dioxide produced using a pressure transducer, and calculating the original carbonate concentration using the Ideal Gas Law. We measured iron in Ox-Fe and TFe extracts using flame atomic absorption spectrophotometry.

We froze approximately 100 g of moist sediment for analysis of AVS, which was later measured following US EPA Method 821-R-91-100 by acidifying sediment samples with HCl to convert AVS to hydrogen sulfide, which was then trapped as S^{2-} in an alkaline solution (0.5 M NaOH) and measured colorimetrically after reaction with a mixed diamine reagent (H_2SO_4, N,N-dimethyl-p-phenylenediamine oxalate, and ferric chloride hexahydrate) (Allen et al. 1991). Analytical sulfide standards were prepared from a stock solution standardized versus thiosulfate.

Sequential P Extraction

We used a sequential extraction procedure (Paludan and Jensen 1995) to measure operationally defined P binding fractions in sediments collected immediately prior to and immediately after flooding. These measurements allowed for a more mechanistic understanding of dominant P retention and release processes in each sediment, because different chemical forms of P respond differently to changes in biological, physical, and chemical conditions. Many sequential extraction procedures have been used to extract individual P binding fractions in soils and sediments, although all provide operationally defined results that are useful indicators, but not pure samples, of chemical forms of P (Pettersson et al. 1988).

We collected sediments at sampling points in Ditch 2, Wetland 1 (3 samples, a-c), and Wetland 2 (2 samples, a-b). Sediments from Ditch 2 were separated into two samples—one from the overlying loose sediment (“floc” layer) and one representing the top 10 cm of the more consolidated sediment below. All other sediment samples were taken from the top 10 (± 3) cm of sediment and homogenized in a glove bag with an anoxic atmosphere prior to sequential P extraction to avoid alteration of P binding fractions by oxidation of field-collected anoxic sediment.

We sequentially extracted P binding fractions in triplicate samples of wet sediment (0.5-5 g d.w.). The first step used de-oxygenated de-ionized water to extract loosely bound P (H_2O~P). Next, bicarbonate-buffered dithionite (0.11 M) extracted phosphate (PO_4^{3-}) bound to redox-sensitive oxidized iron minerals (BD~P) and some non-reactive (mostly organic) P (BD~DNRP) (Reitzel et al. 2006). This step also may extract some apatite P in calcareous sediment (Reitzel 2005). The third step used sodium hydroxide (0.1 M NaOH) to extract PO_4^{3-} bound to redox-insensitive aluminum and iron oxides that undergo anionic exchange with hydroxide (NaOH~SRP) and non-reactive organic and inorganic P (pyro- and polyphosphates). Non-reactive P extracted by NaOH was acidified to separate out precipitating humic-acid associated P (HA~P) from other non-reactive P molecules (NaOH~DNRP). The remaining binding fractions, apatite and calcareous-bound P and residual P, were estimated by subtracting the sum of all other fractions from independently measured sedTP (HCl+Res~P). We expect carbonate-associated P to be of minimal importance at this site due to low total calcium carbonate concentrations in the sediments (<1 mg $CaCO_3$ g d.w.$^{-1}$). We did not detect any non-reactive H_2O~P, so results are not reported for that fraction.

We did not measure SedTP in the Ditch 2 post-flood floc layer sample due to an insufficient amount of material, so the sedTP value from flocculent Ditch 2 sediment prior to flooding was used to estimate HCl+Res~P. Two samples taken ~1m apart were collected at Wetland 2a in the pre-flood sampling, and P fraction data from these two were averaged to produce a single value for Wetland 2a prior to statistical analysis.

Algal Sampling

To estimate the amount of filamentous algal biomass produced in the water column after the re-flooding and sediment P release, as well as the potential amount of P stored in the algal biomass, we sampled algae during a bloom in April 2009 that coincided with particularly low surface water SRP concentrations throughout the wetland. We sampled algae at 31 locations within the newly re-flooded wetland: at 12 points along the existing water sampling transect spaced ~10 m apart in Wetland 1, six points along a transect extending from the bend in Ditch 2 and 4 points surrounding the water sampling point in Wetland 2, and at 6 points clustered around the water sampling point in Wetland 3 (Figure 2.1).

To sample algal biomass, we used a cylindrical sampling device of 15 cm diameter with an open top and bottom. We carefully placed the sampler on the surface of the sediment (after cutting through algal mats at the edge of the tube if they were present) to capture a known volume of surface water containing a representative amount of algae. We used a metal rod with a stopper on the end to homogenize the surface water within the sampling device and then sub-sampled the resultant mixture. This algal mixture was re-homogenized in the lab using a blender and algal biomass in a subsample was collected on a filter for chlorophyll *a* analysis and measurement of biomass as dry weight. Chlorophyll *a* was extracted from algae on filters in cold 90% ethanol and measured using fluorometry (Welschmeyer 1994). Algal biomass requires about 0.01-0.02% P by dry mass for normal growth, but algal biomass P can be as high as 0.45% if algae exhibit luxury uptake, which is common in high P environments (Reddy and DeLaune 2008). We used a mid-range value of 0.25% algal biomass P for our calculations.

Intact Core Experiment

To investigate the role of dissolved oxygen in overlying water in controlling sediment P release in the absence of photosynthesis, we sampled intact cores and compared rates of P release to surface waters in un-oxygenated surface water treatments and in continually oxygenated controls. We collected 12 intact cores from each of two locations in the Fort Custer Area 2 Wetland in June (Wetland 1) and July (Wetland 2) of 2009. Sediments were sampled at the same location where sediments were collected for chemical analysis in 2009 and 2010. Cores had a diameter of 4.5 cm, with a sediment depth of about 15 cm and an overlying water column of about 15 cm. We kept intact cores in a dark room at room temperature.

To test the effects of oxygen on sediment P release in the intact cores, we aerated six of the cores from each site with an aquarium bubbler to 90-100% equilibrium with atmospheric oxygen ("High O_2") and left the remaining six from each site un-aerated ("Low O_2": < 25% saturation, mean 9%). The surface water in un-aerated cores was carefully mixed biweekly prior to sampling to produce a uniform water column and to mimic mixing created by bubblers in the aerated treatments. Approximately twice a week for 50 days, we sampled surface water in each core for SRP concentration. The total volume of water sampled was replaced with low-P stock water (~2 µg P L^{-1}) sampled from the pond at the Area 2 Wetland outflow. Little to no algal growth was observed during the experiment.

We used surface water SRP concentrations measured through time in the experimental intact cores to estimate sediment P release rates (µg P d^{-1} m^{-2}) using the first 14 and 21 days of sampling from Wetland 1 and Wetland 3 cores, respectively. We calculated maximum P release (mg P m^{-2}) by subtracting the average SRP concentration in flood water from the maximum SRP concentration measured through time within each intact core.

Calculations & Statistics

To better understand the initial release and later fate of P within the system, we used our measurements of water-column and sediment P, as well as discharge measurements and bathymetry, to estimate P stocks in surface water, P exported from the outflow, and P in sediment binding fractions. To estimate the amount of P exported from the wetland, we used discharge and P measurements to estimate “instantaneous” export rates for sampling dates (g P d^{-1}). We used these daily export rates to estimate total export over time (g P) by calculating the area under the time series curve between consecutive sampling dates and adding these values. Although we do not have discharge measurements between September 3, 2008 (immediately before re-flooding) and February 25, 2009 (163 days after re-flooding), discharge and water levels during this time period in nearby water bodies (Fair Lake, Crooked Lake, Augusta Creek; data not shown) support interpolation under the time series as shown in Figure 2.2.

To estimate the amounts of SRP, TDP, and TP in the wetland surface water, we scaled up point measurements of water chemistry to the entire zone within which each sampling point was located, taking an average if multiple sampling points were sampled on the same day within one zone. To estimate the bulk mass of P in different sediment P binding fractions that could potentially be exchanged with surface waters, we used an estimated organic soil bulk density of 0.5 g cm^{-3} and made the assumption that the top 5 cm of sediment was most likely to interact with surface waters.

All statistical analysis were carried out in R version 2.13.2 (R Development Core Team 2011). Unless stated otherwise, variables were natural-log transformed prior to analysis to more closely meet linear model assumptions of normal distributions and equal variance. To examine the effects of re-flooding on sediment P binding fractions, we conducted pairwise comparisons between each binding fraction that we directly measured in sediment samples (n=7) collected just prior to and shortly after flooding ($\alpha=0.05$). To test if P release rates and maximum P release were significantly different among aeration treatments in intact cores, we ran linear models on untransformed data with treatment as a fixed factor for each site ($\alpha=0.05$).

Results

Geomorphology and Flooding

After the Area 2 Wetland was fully flooded, most of its area consisted of newly re-flooded sediments and most was shallow, with an area-weighted mean depth of 46 cm (Table 2.1). A decrease from the fully flooded water level of 20 cm would expose 20% of the wetland area, and a decrease of 40 cm would expose 60%. The re-flooding in September 2008 rapidly and drastically changed conditions in Wetland zones, which make up the majority of the ecosystem's area, from exposed soils dominated by grasses to inundated sediments under about 0.5 m of water.

Discharge from the outflow stream was highest each year in mid-winter and early spring, declining to zero by late summer/early fall (Figure 2.2). The largest discharge rate (32 L s^{-1}) recorded was in late February 2009 after re-flooding, which we assume to be representative of discharge rates throughout winter 2008-2009. The wetland was flushed at the highest rate at this time, with a residence time for the entire wetland of 4.7 days, but considering only the more rapidly flushed areas (Ditch1 and the Outflow Pond) the residence time would have been only 2.7 days.

Using discharge measurements and wetland volume to estimate residence times produced wide-ranging estimates because of widely variable discharge rates measured, from 4 days to 18 years. Residence time of the entire wetland's volume ranged from 4.6-6791 days (average=1322 days). The residence time of the more rapidly flushed portion of the wetland (Ditch1 + The Outflow Pond) ranged from 2.7-3986 days (average=776 days). The shortest residence times occurred in mid-winter and early

Table 2.1 Area, volume, area-weighted mean depth, and maximum depth measured within each zone of the Fort Custer Area 2 Wetland. Percent groundwater influence was calculated from dissolved magnesium (Mg^{2+}) concentrations (Whitmire and Hamilton 2005) using a mixing model assuming groundwater and precipitation represent the only inputs of dissolved Mg^{2+} to the ecosystems and assuming Mg^{2+} concentrations of 0.05 mg L^{-1} for precipitation (1979-2002 NADP/NTN 2003) and 23 mg L^{-1} for groundwater (Kalamazoo County mean).

Zone	Area (m^2)	Volume (m^3)	Mean Depth (cm)	Groundwater Influence (%)
Ditch1	5603	3597	65	73%
Ditch2	6580	3394	52	48%
Outflow Pond	16569	13623	83	67%
Wetland1	19471	3101	25	39%
Wetland2	34277	3364	36	33%
Wetland3	4940	2000	41	51%
Entire Wetland	**87440**	**29079**	**46**	

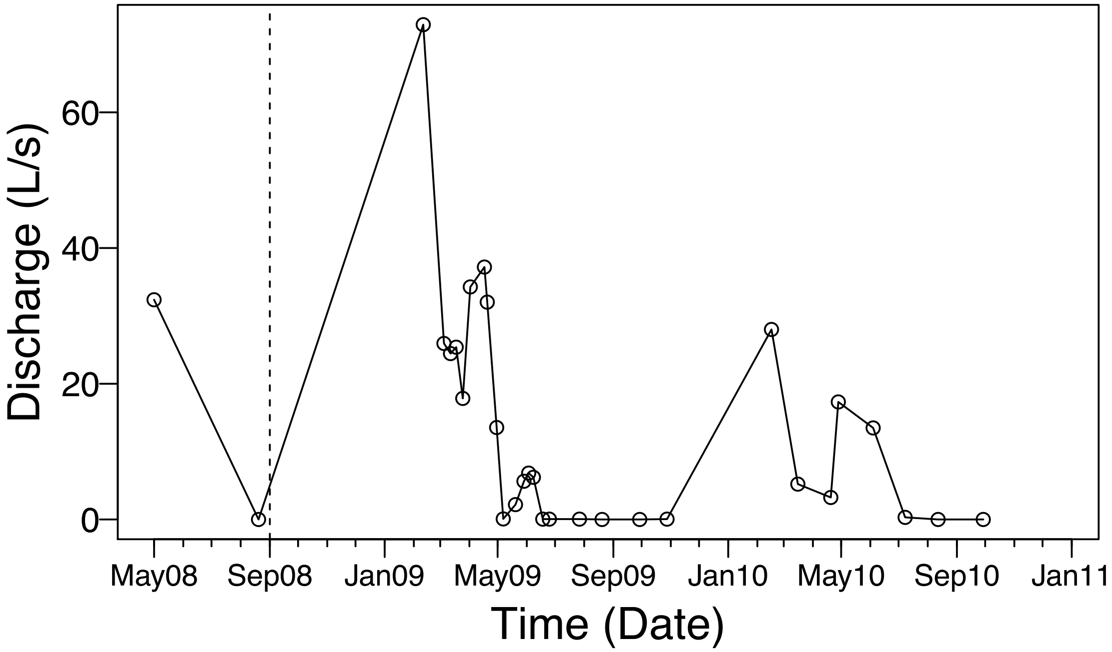

Figure 2.2 Discharge measured at the outflow of the Fort Custer “Area 2 Wetland” over time, before and after the wetland was re-flooded for restoration (re-flooding timing shown by the vertical dashed line).

spring, and the longest residence times occurred in summer and early fall, when little or no water was flowing out of the wetland.

Sediment P Release

Surface water P concentrations were high throughout the re-flooded wetland after the rapid September 2008 re-flooding event (Figure 2.3). In newly re-flooded areas, SRP concentrations in the days after re-flooding were as high as 750 µg P L^{-1}. Concentrations of SRP remained high during winter 2008-2009 (Wetland Zones mean ± S.E. 323 ± 118 µg P L^{-}). In April 2009, SRP concentrations throughout the wetland declined rapidly to less than 20 µg P L^{-1}. These lower SRP concentrations coincided with a large bloom of filamentous algae (see *Algal Biomass and P Sampling*).

Concentrations of SRP increased moderately during summer 2009, but by one year following the re-flooding event (September 2009), SRP concentrations measured throughout the wetland remained lower than 50 µg P L^{-1} until the end of our sampling period (January 2011). The highest SRP values (>45 µg P L^{-1}) were only observed during the first year following re-flooding, and only when surface water dissolved oxygen was < 5.5 mg L^{-1} (Figure 2.4). Similarly low dissolved oxygen concentrations in Year 2 were not associated with such high P concentrations.

The high P concentrations we observed reflect large post-flooding increases in mass of surface water P. Immediately after re-flooding, the wetland’s surface waters contained about 20 times more SRP and 14 times more TP than during months prior to

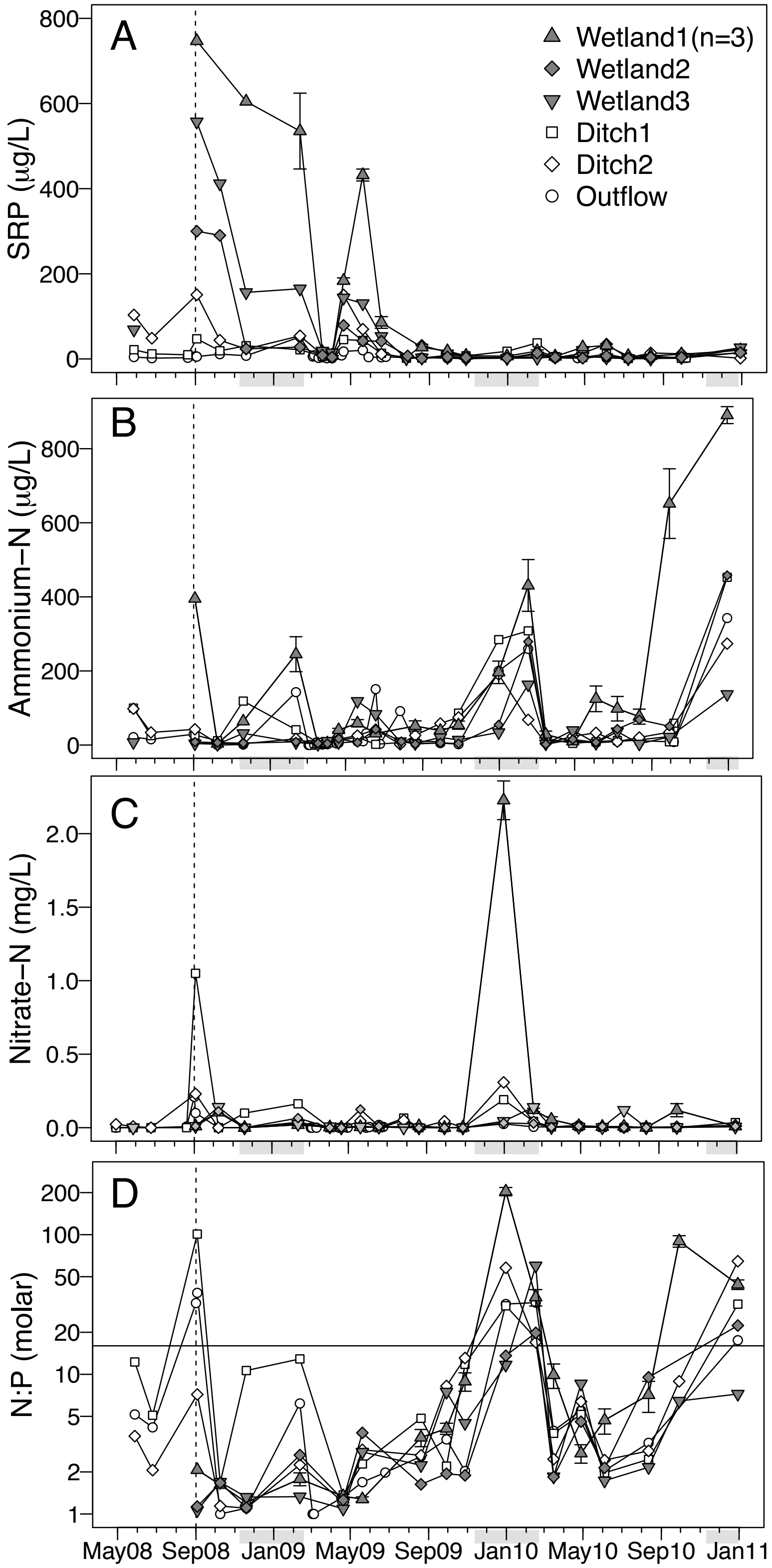
A
B
C
D
Wetland1(n=3)
Wetland2
Wetland3
Ditch1
Ditch2
Outflow
SRP (μg/L)
Ammonium−N (μg/L)
Nitrate−N (mg/L)
N:P (molar)
0
200
400
600
800
0.0
0.5
1.0
1.5
2.0
1
2
5
10
20
50
100
200
May08
Sep08
Jan09
May09
Sep09
Jan10
May10
Sep10
Jan11

Figure 2.3

Figure 2.3 (continued) Soluble reactive phosphate (SRP), ammonium-N (NH_4^+), nitrate-N (NO_3^-), and available molar N:P ratios (i.e., dissolved inorganic N to TDP) measured in surface water through time in the re-flooded Fort Custer Area 2 Wetland. Dashed vertical line represents heavy rain event (Sept 15-17, 2008) that re-flooding historically drained areas sampled at locations labeled Wetland 1-3. Grey shaded areas on the x axes represent periods when surface water was under ice. Prior to September 15, 2008, the soil surface of Wetlands 1-3 was exposed to air. After re-flooding, these areas were covered by water 0.1-1 m deep (area-weighed average depth=0.46 m) throughout the rest of the study period. See map (Figure 2.1) for water sampling locations. The horizontal line in panel D represents Redfield N:P ratio (16:1).

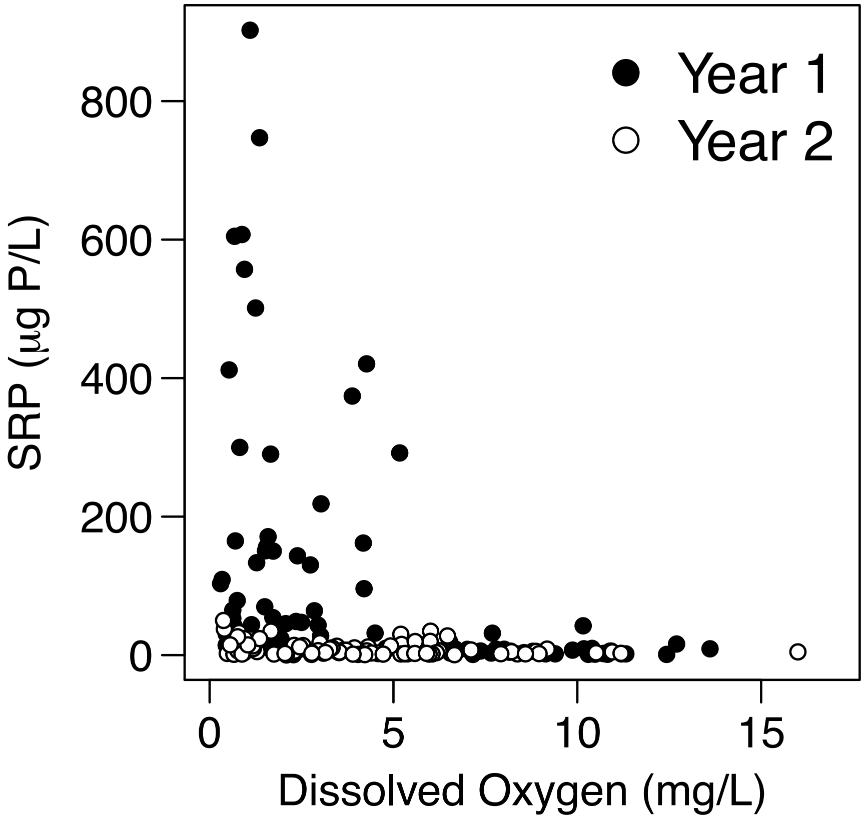

Figure 2.4 Soluble reactive phosphorus (SRP) and dissolved oxygen measured in surface waters of a re-flooded historically drained wetland in the first year following re-flooding (September 15, 2008-2009) and the second year following re-flooding (September 2009-January 2011).

re-flooding. Prior to re-flooding (summer 2008), standing water in inundated areas contained approximately 233, 438, and 503 g SRP, TDP, and TP, respectively. Immediately after flooding (September 17, 2008), the standing water of the wetland contained about 5200 g of SRP and TDP (TDP constituted nearly 100% of SRP) and 7400 g TP.

Newly flooded sediments represent the most probable source of the large P increase throughout the wetland. Immediately after flooding, SRP represented virtually 100% of TDP. The amount of SRP in the inflowing drainage ditch (Ditch 1) only increased four fold, indicating that P entering the wetland from overland flow, precipitation, and the drainage ditch inflow can only explain a minority of the observed increase in P concentrations in water at the wetland's center (Wetland 1). In response to flooding, sediments released on average 382 g SRP and 534 g TP per day, or 6.5 mg SRP m^{-2} d^{-1} and 9.1 mg TP m^{-2} d^{-1} (sum of Wetland 1-3 Area, September 3-17, 2008).

P Export

Phosphorus export via the outflow stream was highest in the months after initial flooding because of higher discharge rates and P concentrations during this time. We estimate that during our study period after re-flooding, the Area 2 Wetland exported a total of 110 kg P, most of which (84%) was dissolved and non-reactive (presumably mostly organic). The majority (72%) of P export during our study occurred in the first five months following re-flooding (September 3, 2008- February 25, 2009).

Nitrogen Dynamics

Nitrogen in surface waters exhibited a starkly different pattern than P (Figure 2.3). Nitrate concentrations ranged from 0-0.3 mg NO_3^--N L^{-1} throughout the wetland during the study period, except in one instance in January 2010 when higher (1.5 mg NO_3^--N L^{-1}) concentrations were observed in Wetland 1. Ammonium was also generally low (<200 µg NH_4^+-N L^{-1}) throughout the first year following re-flooding, except for a few episodically high (200-400 µg NH_4^+-N L^{-1}) events immediately after flooding and in April 2009. During the second year after re-flooding, however, high ammonium concentrations (>200 µg NH_4^+-N L^{-1}) were observed in spring and fall 2010, and in winter 2010-2011. Taken together as molar N:P ratios ((NO_3^- +NH_4^+)/TDP), the nutrient patterns we observed reveal that during our study period, average N:P ratios were spatially variable (Table 2.2, 2.3), but N:P ratios were lower than the Redfield ratio (16:1) in most places during most of our study, except in a few zones immediately following re-flooding (Outflow, Ditch 1), throughout the wetland during winter 2010, and in a few zones during winter 2011 (Figure 2.3).

Dissolved Organic Carbon

Dissolved organic carbon (DOC) concentrations were more variable among sites before re-flooding and during the first year after re-flooding than in Year 2 (Figure 2.5). During Year 1, DOC concentrations ranged from 8.7-77 mg L^{-1} with a mean of 33 mg L^{-1}. In Year 2, the range was smaller, from 11-37 mg L^{-1} with a mean of 18 mg L^{-1}.

Table 2.2 Surface water chemistry at sampling sites in the Fort Custer wetland restoration site. Values are means (one standard error in parentheses) of approximately monthly measurements made after a re-flooding event (average of all measurements taken between September 17, 2008 to January 13, 2010.

	SRP (µg L^{-1})	TDP (µg L^{-1})	TP (µg L^{-1})	NH_4^+ (µg N L^{-1})	NO_3^- (µg N L^{-1})	N:P*	SO_4^{2-} (mg L^{-1})	Mg^{2+} (mg L^{-1})	DOC (mg L^{-1})	Sp. Cond. (µS cm^{-1})
Ditch 1	15 (3)	30 (4)	61 (7)	64 (24)	67 (42)	6.4	2.6 (0.8)	25 (3)	22 (2)	441 (13)
Ditch 2	29 (9)	50 (11)	110 (20)	43 (13)	33 (15)	5.7	3.9 (2.3)	20 (2)	25 (3)	325 (18)
Wetland 1	108 (28)	162 (39)	299 (47)	158 (34)	147 (71)	2.4	1.2 (0.5)	10 (1)	35 (3)	280 (8)
Wetland 2	40 (18)	85 (28)	139 (35)	50 (23)	21 (71)	1.7	1.3 (0.4)	13 (1)	27 (2)	240 (20)
Wetland 3	78 (31)	113 (40)	179 (47)	35 (10)	23 (10)	2.3	4.3 (0.4)	14 (1)	21 (2)	305 (13)
Outflow Pond	8 (2)	28 (3)	49 (6)	42 (13)	15 (6)	5.4	2.0 (0.4)	15 (1)	22 (1)	375 (7)

*N:P calculated as the molar ratio of within-site averages of dissolved inorganic N (NO_3^--N + NH_4^+-N) to total dissolved P.

Table 2.3 Pore water chemistry at sampling sites in the Fort Custer Area 2 Wetlands. Values are means (standard errors in parentheses) of approximately monthly measurements for after a re-flooding event (average of all measurements taken between September 17, 2008 to January 13, 2010.

	SRP (μg L^{-1})	TDP (μg L^{-1})	NH_4^+ (μg N L^{-1})	NO_3^- (μg N L^{-1})	N:P*	SO_4^{2-} (mg L^{-1})	Mg^{2+} (mg L^{-1})	Fe(II) (mg L^{-1})	H_2S (μg S L^{-1})
Ditch 1	168 (42)	652 (393)	2304 (508)	23 (7)	7.9	2.0 (0.5)	12 (1)	26.1 (5.09)	30 (22)
Ditch 2	85 (62)	397 (170)	4297 (702)	87 (74)	24	4.1 (0.4)	14 (0)	42.8 (8.15)	30 (10)
Wetland 1	1233 (223)	1283 (281)	4860 (484)	27 (7)	8.6	1.6 (0.4)	9 (0)	36.1 (3.57)	33 (62)
Wetland 2	151 (30)	160 (18)	3874 (673)	15 (5)	54	0.9 (0.4)	7 (1)	11.7 (2.89)	44 (33)
Wetland 3	144 (37)	197 (41)	2263 (384)	16 (6)	26	1.0 (0.3)	12 (1)	7.1 (0.94)	43 (42)

***N:P** calculated as the molar ratio of within-site averages of dissolved inorganic N (NO_3^--N + NH_4^+-N) to total dissolved P.

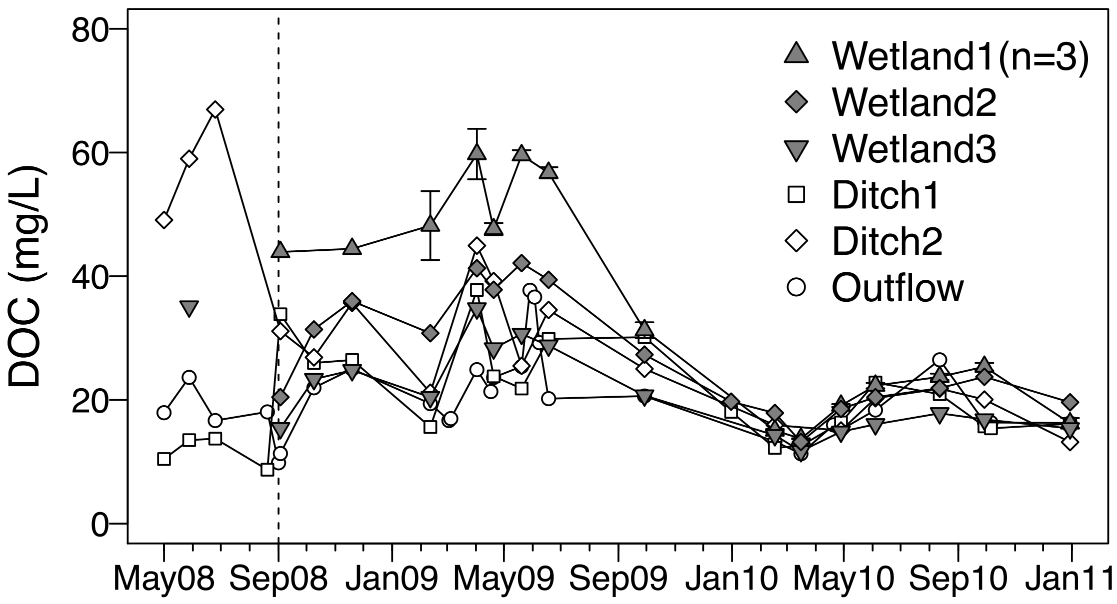

Figure 2.5 Dissolved organic carbon (DOC) concentrations measured in surface water through time in the re-flooded Fort Custer Area 2 Wetland. Dashed vertical line represents flood event (Sept 15-17, 2008) that re-flooding historically drained areas sampled at locations labeled Wetland 1-3. Prior to September 15, 2008, the soil surface of W1-3 was exposed to air. After re-flooding, these areas were 0.5-1 m deep throughout our study period. See map (Figure 2.1) for water sampling locations.

Immediately after re-flooding, DOC increased in Ditch 1, but decreased in Ditch 2 and at the outflow. Concentrations of DOC at all sampling locations peaked in early spring of 2009, and declined through the following fall and winter. DOC increased slightly again during summer of 2010 throughout the wetland, and tended to decline or remain the same in winter 2010-2011.

Pore Water Chemistry

In general, dissolved P and N in pore waters did not display seasonal patterns. Average pore water SRP and NH_4^+-N concentrations after re-flooding—630 µg P L^{-1} and 5,000 µg NH_4^+-N L^{-1}, respectively—were higher than surface water concentrations, suggesting a strong diffusion gradient from the sediment pore-water environment to surface waters. Wetland 1, the zone in which the highest surface water SRP concentrations were observed, typically contained the highest pore water SRP concentrations (Table 2.2, 2.3). Pore water SRP concentrations were high compared to pore waters of other wetlands in the region sampled in late summer (mean = 400 µg P L^{-1}, N = 54 sites: Kinsman-Costello et al., unpublished data). Pore water dissolved Fe(II) concentrations were also very high compared to regional wetlands, with Area 2 Wetland pore waters averaging 27 mg Fe L^{-1} and southwest Michigan wetlands containing on average 8.3 mg Fe L^{-1} (N = 54 sites: Kinsman-Costello et al., unpublished data). Concentrations of H_2S were generally low or undetectable, although higher values were sometimes measured in spring or summer (maximum = 245 µg H_2-S L^{-1} in June 2009).

Algal Biomass and P Content

In the spring and summer months of both years studied, large amounts of filamentous algae and duckweed (*Lemna* spp. and *Wolffia* spp.) dominated the surface of the re-flooded wetland. On April 16, 2009, water-column integrated samples of algae, which was mostly filamentous and floating in clumps on the water surface and in the water column (i.e., metaphyton, Goldsborough and Robinson 1996), revealed high algal biomass and chlorophyll *a* in the newly re-flooded wetland (Table 2.4). Assuming that algal biomass contained 0.25% P by dry weight, we estimate that on this day, algal biomass in the wetland contained 21.5 ± 1.4 kg P. Despite the lower surface water P concentrations in the Year 2 (2010), duckweed and filamentous algae still dominated the water surface in late spring and early summer.

Sediment Characteristics

The wetland sediments were generally characterized by high OM, iron, and P, and low $CaCO_3$ and AVS, as compared to other wetland sediments from the region (Chapter 3, this dissertation). Sediments throughout the wetland were high in OM, ranging from about 10-80%, with an overall average of 60% (Table 2.5). The wetland sediments were also quite high in TP (area-weighted average = 1229 µg P g^{-1}) and TFe (area-weighted average = 11.56 mg Fe g^{-1}), but with moderate Fe:P molar ratios (area-weighted average = 6.78). On the other hand, sediments contained relatively low amounts of $CaCO_3$ and AVS, constituents typically characteristic of sediments in water bodies with high groundwater influence (Table 2.5).

Table 2.4 Algal biomass (by dry mass), chlorophyll *a* (Chla), and estimated total algal P (assuming 0.25% P) of depth-integrated surface water algal samples taken on April 16, 2009 in the Fort Custer restored wetland. Most of the algae growing at this time was filamentous and floating on the water surface and in the water column. Values are mean ± standard error of multiple sampling points (N) within each of the three zones.

Zone	N	Biomass ($g\ m^{-2}$)	Chla ($\mu g\ L^{-1}$)	Chla ($g\ m^{-2}$)	Algal P Concentration ($\mu g\ P\ L^{-1}$)	Algal P in Zone (g P)
Wetland 1	13	204 ± 26	664 ± 67	25 ± 2	2318 ± 299	9013 ± 1164
Wetland 2	12	96 ± 12	400 ± 63	20 ± 2.92	1090 ± 136	4238 ± 529
Wetland 3	6	186 ± 11	764 ± 46	48 ± 2.61	2117 ± 124	8229 ± 483

Table 2.5 Sediment characteristics measured in the top 10 cm of cores sampled from two drainage ditches (Ditches 1-2) and three newly re-flooded wetland areas (Wetlands 1-3) of the Fort Custer Wetland. Sediment characteristics for the entire wetland are averages weighted by the areas of the zones in which they were measured. Except for organic matter, all values are expressed as per gram dry weight. All sites were sampled in 2008 for organic matter (OM), total phosphorus (TP) and total iron (TFe) measurements. In addition, Wetland 1 and Wetland 3 were sampled in 2009 and 2010 for OM, TP, and TFe, and in 2010 only for oxalate-extractable iron (Ox-Fe), total aluminum (Total Al), total calcium carbonate ($CaCO_3$), and acid volatile sulfides (AVS).

Site	Organic Matter (%)	Total Phosphorus (µg P g^{-1})	Total Iron (mg Fe g^{-1})	TFe:P Molar Ratio	Ox-Fe (mg Fe g^{-1})	Total Al (mg Al g^{-1})	$CaCO_3$ (mg g^{-1})	AVS (µmol S g^{-1})
Ditch1	13	334	10.75	17.88				
Ditch2	45 ± 4	904 ± 163	12.47 ± 2.18	7.99 ± 1.55				
Wetland1	65 ± 7	1502 ± 286	18.63 ± 3.83	7.57 ± 1.71	11.31	4.79	0.68	1.31
Wetland2	68 ± 3	1245 ± 246	7.98 ± 0.7	4.81 ± 1.59				
Wetland3	63 ± 3	1489 ±198	8.24 ± 0.46	3.17 ± 0.43	5.62	10.51	0.58	0.94
Entire Wetland	60	1229	11.56	6.78	10.16	5.95	0.66	1.24

The relative distributions of sequentially extracted P binding fractions reflect the high organic matter and iron content of these sediments (Figure 2.6). Redox-sensitive iron-bound P (BD~SRP) represented 19% of total P on average, and P associated with organic matter (NaOH~DNRP and HA~P) represented about 25% of total P. While initial flooding did not greatly change sediment total P content, relative amounts of some chemical forms of sediment phosphorus changed significantly. Phosphorus extracted by the BD solution, both non-reactive and reactive, significantly decreased after flooding, while non-reactive P extracted with NaOH and P associated with humic acids increased significantly after flooding (Table 2.6).

The "loosely sorbed" (H_2O~P) and redox-sensitive iron-bound (BD~SRP) P binding fractions are theoretically more reactive and most readily released. The total mass of H_2O~SRP and BD~SRP in sediments before flooding far exceeded the observed increase in surface water P. For example, the top 5 cm in Wetland 1 contained an average of about 2 kg H_2O~P and 82 kg BD~SRP prior to flooding, compared to an observed P increase in surface waters in that zone of about 2 kg SRP. The average declines we observed in BD~SRP and BD~DNRP, extrapolated to the entire wetland area, each represent approximately 100-300 kg P. The average increases in NaOH~DNRP and HA~P correspond to about 72-180 and 60-140 kg P, respectively. Overall, it appears that as much as 600 kg P were released from the BD~SRP and BD~DNRP fractions combined, and all or part of this was transformed to NaOH~DNRP and HA~P. Thus, release of all of the H_2O~P and/or a small fraction of

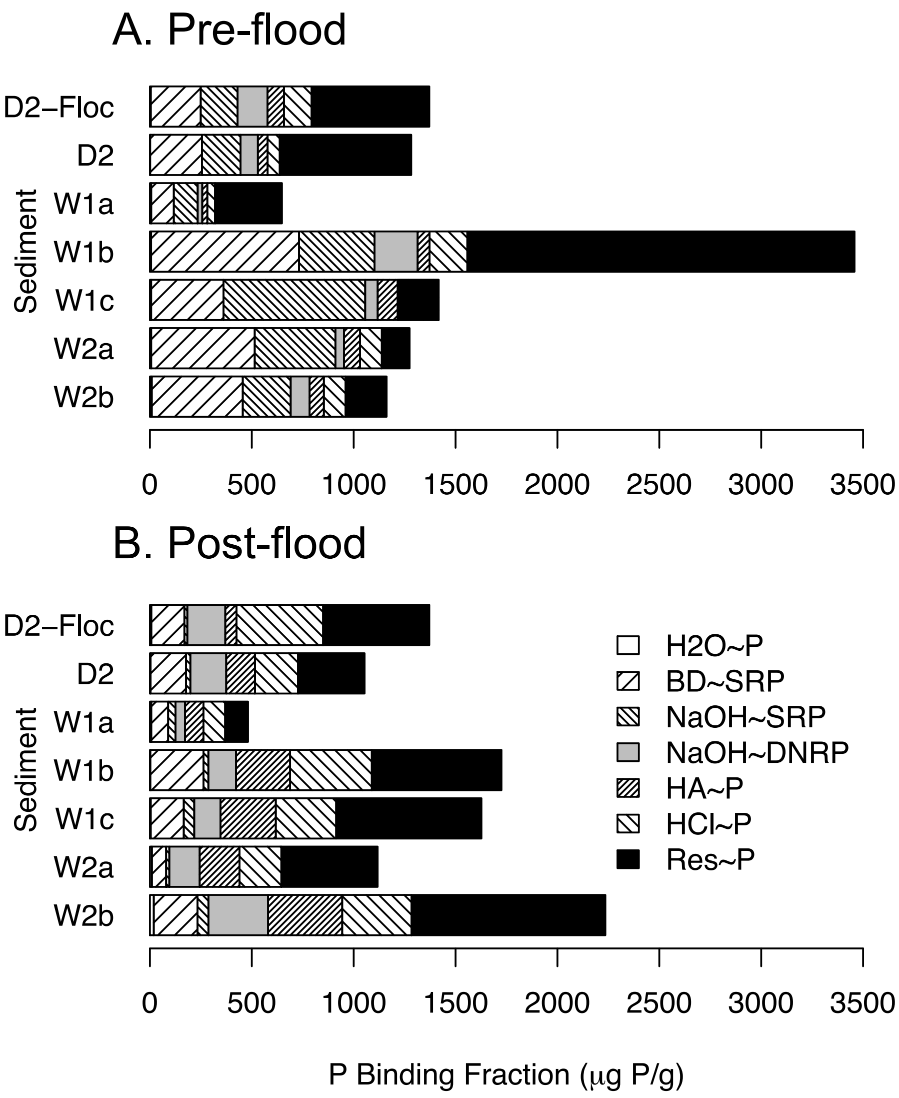

Figure 2.6 Comparison of sequentially extracted P binding fractions in sediments collected immediately prior to (10 days) and immediately after (2 days) an extreme rain event re-flooded a historically drained wetland. Locations include Ditch 2 (D2) and Wetlands 1-2 (W1 and W2, sampling points within locations denoted by letters). See text for explanation of abbreviated binding fraction names (Methods: Sequential P Extraction).

Table 2.6 Sequentially extracted sediment P binding fractions in sediments collected from seven locations in a historically drained wetland immediately prior to (Sept 3, 2008) and immediately following (Sept 17, 2008) a flooding event, expressed per gram dry weight. The p-value is for the difference between pre- and post-flood individual binding fractions (F-test).

P Binding Fraction	Pre-Flood ($\mu g\ P\ g^{-1}$)	Post-Flood ($\mu g\ P\ g^{-1}$)	Post minus Pre-Flood ($\mu g\ P\ g^{-1}$)	p-value
SedTP	1488 ± 23	1373 ± 212	-115	0.842
H_2O~P	5 ± 1	7 ± 2	2	0.651
BD~SRP	378 ± 10	161 ± 26	-217	**0.019**
BD~DNRP	312 ± 11	31 ± 6	-281	**< 0.001**
NaOH~SRP	95 ± 7	159 ± 28	65	0.101
NaOH~DNRP	66 ± 3	197 ± 42	132	**0.006**
HA~P	119 ± 6	284 ± 44	165	**0.009**

the BD~P from the sediments could explain the observed increase in P in surface waters.

Intact Core Experiment

In an experiment testing the effects of surface water dissolved oxygen on sediment P release to surface waters in intact cores, sediments from Wetland 1 exhibited greater P release rates than Wetland 3 (Table 2.7). Wetland 1 sediments under the Low O_2 treatment released P at a significantly higher rate than under High O_2. Although the result was not significant, there was also a trend towards higher SRP release in surface waters of Low O_2 cores collected from Wetland 3 (Figure 2.7, Table 2.7).

Discussion

Restoring wetland hydrology to historically drained lands may have unintended consequences for nutrient cycling. Upon re-flooding of a historically drained wetland, we observed rapid release of inorganic P from sediments to surface waters. This nutrient release established a high-P environment in surface waters that coincided with the proliferation of duckweed and filamentous algae. In addition, considerable amounts of P were likely exported to downstream ecosystems in the months following re-flooding.

Sediment P Release

In both field and laboratory studies, re-flooding historically drained sediments has been shown to lead to P release from sediments to surface waters, although estimated rates vary (Table 7.8). The rates of sediment P release we observed (6.5 mg SRP and

Table 2.7 Mean P release rates (plus or minus one standard error) from intact cores from two locations in the Fort Custer Area 2 Wetland in which surface water was aerated (High O_2) or un-aerated (Low O_2). N=6.

Site	Treatment	P Release (mg P m^{-2})	p-value	P Release Rate (µg P d^{-1} m^{-2})	p-value
Wetland1	High O_2	2.3 ± 0.94		36 ± 13	
	Low O_2	5.0 ± 1.10	0.09	118 ± 27	**0.022**
Wetland3	High O_2	0.11 ± 0.02		1.8 ± 0.8	
	Low O_2	0.20 ± 0.06	0.16	4.0 ± 1.5	0.3

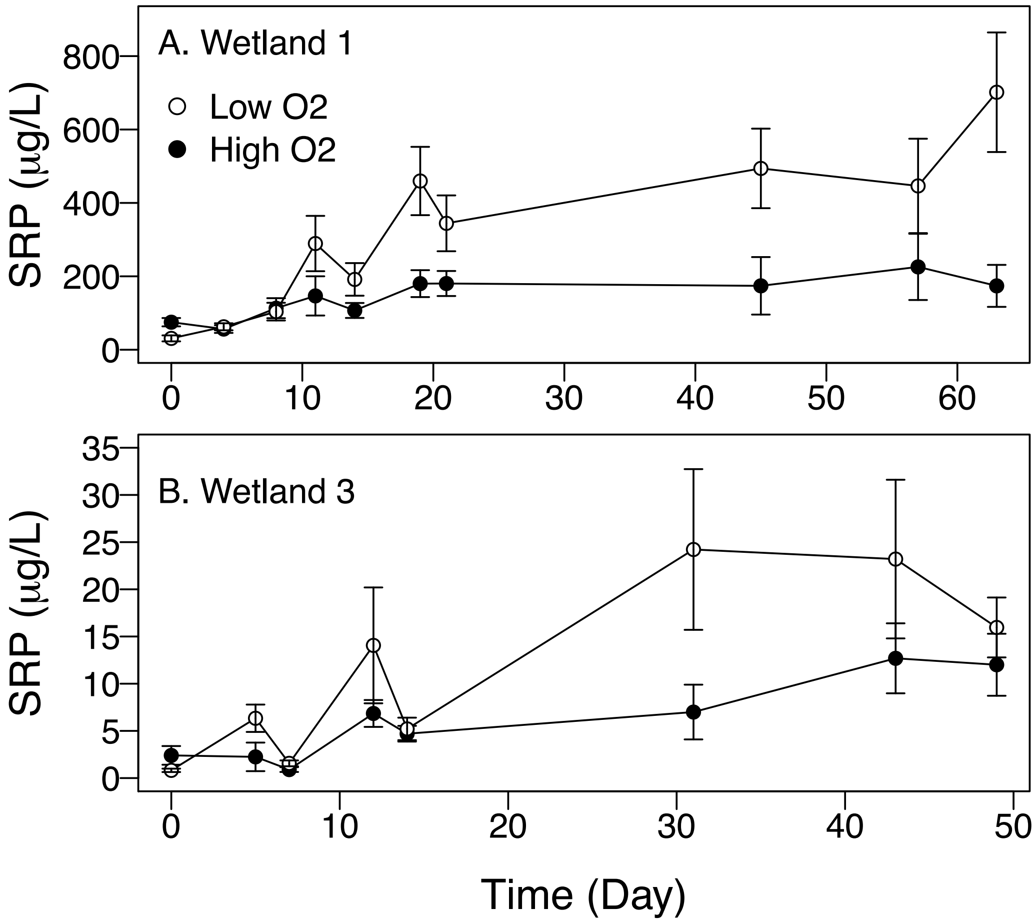

Figure 2.7 Soluble reactive phosphate (SRP) concentrations in surface water of intact cores sampled from two locations in the Fort Custer Area 2 Wetland. Surface waters in "High O_2" cores were constantly aerated, while those in "Low O_2" cores were left un-aerated. Values are means of 6 replicate cores plus or minus one standard error.

Table 2.8 Published P release rates from re-flooded historically drained sediments measured in situ (A) or in intact cores (B) sampled from drained or recently re-flooded wetland ecosystems.

A. Field Studies

Source	Location	SRP Release ($mg\ m^{-2}\ d^{-1}$)	TP Release ($mg\ m^{-2}\ d^{-1}$)
Ardón et al. 2010	Timberlake Restoration Project, North Carolina, USA (440 ha)	0.03	0.15
Coveney et al. 2002	Marsh Flow-Way Demonstration Project, Florida, USA (210 ha)	10-20	
Wong et al. 2011	Williamson River Delta, Oregon, USA (2,200 ha)		10
Duff et al. 2009	Wood River Wetland, Upper Klamath Basin, Oregon, USA (1,300 ha)	19.2-72	
This Study	Fort Custer Area 2 Wetland, Michigan, USA (9 ha)	6.5	9.1

B. Intact Core Studies

Source	Sediment Source	SRP Release ($mg\ m^{-2}\ d^{-1}$)	TP Release ($mg\ m^{-2}\ d^{-1}$)
Aldous et al. 2005	Restored wetlands near Upper Klamath Lake, Oregon, USA	8.6-55	
Bostic and White 2007	Blue Cypress Marsh Conservation Area, Florida, USA	5.74-43	8.69-26.6
Corstanje and Reddy 2004	Blue Cypress Marsh Conservation Area, Florida, USA	0.7-109	2.8-436
Martin et al. 1997	Everglades Nutrient Removal Project Wetland, Florida, USA	1.2-6	
Pant and Reddy 2003	Artificially drained agricultural (dairy) land slated for a constructed wetland, Florida, USA	11-22	
Zak et al. 2010	Drained fens, Germany & Poland	0-52.3	

9.1 mg TP m^{-2} d^{-1}) immediately following re-flooding was in the range of observed values from past studies (Table 7.8). Even after the initial pulse of sediment P release after re-flooding, we observed high surface water P concentrations and filamentous algal blooms during the ensuing year, suggesting that sediment P release continued to occur but the released P was effectively sequestered in biomass.

Initial sediment P release was rapid (within days) and most of this was inorganic; nearly 100% of the TDP was SRP, and over 70% of total released P was SRP. The amount of PO_4^{3-} sorbed to sediment minerals is partially controlled by equilibria with dissolved PO_4^{3-} in surrounding pore water and overlying surface water (Froelich 1988). When sediments of the wetland were suddenly inundated with low-nutrient surface water from precipitation-driven flooding, they presumably released large amounts of accumulated PO_4^{3-} into pore and surface waters in response.

In addition to the immediate release of SRP from the re-flooded sediments into overlying water, summer flooding probably caused rapid oxygen depletion in these highly organic sediments, establishing conditions for microbial reduction of oxidized iron and subsequent mobilization of the formerly Fe-sorbed PO_4^{3-}. We measured high dissolved Fe(II) concentrations in pore waters, and both the loosely sorbed and redox-sensitive iron-bound inorganic P pools (H_2O~P, BD~SRP) measured in sediments prior to the flooding event were large enough to explain the observed increase in surface water P. The rapid decline observed in sediment BD~SRP after flooding also suggests release of Fe-bound P, and the higher rates of P release in experimental intact cores with low-oxygen surface water provide further evidence for the sediment's propensity to release Fe-bound P under anoxic conditions. Rapid, high rates of inorganic P release such as those we observed are often proximately attributed to desorption of Fe-bound P due to Fe(III) reduction under anoxic conditions (Mortimer 1941, 1942, Marsden 1989).

Ultimately, high rates of sediment P release occurred because the drained sediments contained large amounts of easily mobilized P forms. Wetland sediments typically store large amounts of P in organic matter, which is slow to decompose under flooded, anoxic conditions. However, when wetland sediments are drained, mineralization rates increase as organic sediments are exposed to atmospheric oxygen (McLatchey and Reddy 1998), converting organic P to more readily mobilized inorganic P. In addition to the conversion of existing sediment organic P to more reactive inorganic P, large amounts of readily mobilized P are typically added to drained wetland areas when they are used for agriculture. The specific agricultural management history of the Area 2 Wetland is unknown because the area was converted to a military training area in 1917, and it may have been farmed subsequent to conversion as well as before. Although it is unlikely that the site experienced high loads of industrial P fertilizer, it may have experienced P supplements through the use of manure or other practices.

The high-iron nature of sediments in this wetland likely led to even higher rates of inorganic P accumulation than may have otherwise occurred because of the high iron sorption capacity of the sediment. Even though sediment TP values were also high compared to most wetlands, Fe:P ratios were still lower (~7) than then the suggested ratios of 8-10 at which sorption capacity is low and sediments are likely to release P

(Jensen et al. 1992, Geurts et al. 2008, Zak et al. 2010). In addition, a high proportion of sediment total Fe was in the amorphous, poorly crystalline form (i.e., oxalate extractable), which has a higher sorption capacity than more crystalline forms of oxidized iron due to high surface areas and more –OH groups for PO_4^{3-} ions to exchange with (McLaughlin et al. 1981, Axt and Walbridge 1999). The high sorption capacity conferred by large amounts of poorly crystalline iron minerals may have prevented some accumulated PO_4^{3-} from being exported from the ecosystem during brief periods of hydrologic connection and/or inundation due to storms or beaver activity. Thus, the wetland likely contained large amounts of easily mobilized P due to (1) mineralization of organic P in flooded anoxic sediments, (2) a history of external P additions during agricultural use of the drained soils, and (3) high sorption capacity allowing for accumulation of PO_4^{3-}.

The Role of Humic Substances

One notable aspect of the sediments in the wetland was the relatively large amount of humic-acid associated P (on average, 8% prior to re-flooding, 20% after) as compared to other wetland sediments in the area (Chapter 3, this dissertation). We detected humic-associated P by using a sequential fractionation scheme that precipitates humic-associated P out of the NaOH organic-P extraction solution (Paludan and Jensen 1995). The importance of humic-acid associated P in this wetland is likely due to the nature of the sediment, being both highly organic and high in iron. During the year after flooding, surface water in the wetland contained high DOC concentrations (average = 33 mg L^{-1}), and, thus, humic-associated P may also be an important component of surface and soil water P, as well as in the solid sediment phase.

Metals, most importantly aluminum and iron oxides, complex strongly with humic substances. Phosphate, in turn, can sorb to humic-metal complexes via the same process by which it sorbs to metal oxyhydroxides (exchange with –OH groups). Under certain conditions, humic-metal-associated P can be a quantitatively important form of P in lake water, soil solutions (Gerke 2010), and wetland and lake sediments (Paludan and Jensen 1995).

Although the processes surrounding retention and release of humic-associated P are largely unknown, they likely play an important role in governing the P cycling of this wetland. Humic substances compete with PO_4^{3-} ions for anionic sorption sites on Fe- and Al-hydroxides and may inhibit inorganic P sorption (Nagarajah et al. 1970, Bhatti et al. 1998), but the PO_4^{3-} sorption capacity of humic-metal complexes is higher than for purely inorganic crystalline and poorly crystalline iron and aluminum oxides (Gerke 2010). Thus, the net effects of soil organic matter and humic acids tend to improve PO_4^{3-} sorption capacity (Gerke 2010). In addition, organic acids may inhibit crystallization of Fe oxides, further improving sediment PO_4^{3-} retention by maintaining oxic Fe in poorly crystalline forms with higher PO_4^{3-} sorption capacity (Schwertmann 1966, Kodama and Schnitzer 1977).

It is likely that humic-acid associated PO_4^{3-} is a dominant feature in many drained wetland areas as drained organic soils decompose and become more humified.

How humic-metal- PO_4^{3-} complexes react to environmental changes like re-flooding is an important area of potential study. They may be relatively unreactive in sediments, but when in surface waters, P associated with humic-metal complexes can be liberated by UV irradiation, which reduce Fe(III) to Fe(II) (Francko and Heath 1982). Humic-metal-associated P is mobilized at low pH (Jones et al. 1993), and some plants may acquire P in this way through acidic organic root exudates (Gerke 2010). The response of humic-metal-associated P to changing redox conditions is especially poorly known, although a few studies suggest that the oxidation of reduced iron may be inhibited if it is associated with organic matter, theoretically diminishing the P sorption capacity of anoxic sediment (Koenings and Hooper 1976). An improved understanding of how P associated with humic-metal complexes behaves differently than P sorbed to purely inorganic metals may improve our ability to predict how highly humic sediments will respond to environmental changes like flooding.

P Dynamics in the Years After Initial Flooding

Surface water P concentrations in the wetland remained relatively high throughout the first year after re-flooding, except for a brief decline in early spring 2009. The inverse relationship between dissolved oxygen and SRP concentrations during the first year of our study suggests two non-mutually exclusive processes controlling surface water SRP concentrations during this time period. When oxygen is high, surface water SRP may be low because (1) PO_4^{3-} is retained by sorption to oxidized Fe and/or (2) algae and aquatic plants have taken up most available SRP and produce high O_2 by photosynthesis. Higher rates of P release in intact cores with low surface water oxygen in the absence of algal growth provide evidence that when oxygen is consumed in the water column, iron at the sediment-water interface is microbially reduced, and previously sorbed PO_4^{3-} is mobilized into the water column. Low surface water P concentrations under high oxygen conditions can also be explained by photosynthetic uptake, and we observed high biomass P in filamentous algae that coincided with low SRP concentrations throughout the wetland. Thus, it is likely that a combination of algal P uptake during high O_2 periods and P desorption from Fe under low O_2 conditions together led to the observed pattern of high SRP concentrations under lower dissolved oxygen conditions.

Despite periodic re-occurrence of low dissolved oxygen concentrations during the second year after re-flooding, surface water SRP concentrations remained generally low after September 2009. It is possible that sediment P release rates remained high and photosynthetic organisms like algae and duckweed took up P to maintain P concentrations at low levels. This is unlikely to completely explain the low SRP concentrations because we did not observe elevated SRP concentrations in surface water under ice in winter months (although sediment P release rates may be lower at low temperatures). Thus, we suspect that sediment P release rates slowed considerably during the second year after re-flooding.

Fate of Sediment-Released P

Slower sediment P release rates may have occurred in Year 2 because 1) most of the readily mobilized P was released during initial re-flooding and left the system via the outflow, 2) the readily mobilized P was somehow transformed into more recalcitrant forms and stored in sediments, and/or 3) changing conditions in the sediment environment slowed sediment P release rates. Differences in the distribution of P binding fractions before and after flooding provide some evidence that at least a portion of readily mobilized inorganic P was transformed to less-reactive forms, even in the days following re-flooding.

A large amount of sediment-released P was stored in the biomass of filamentous algae, although the longevity of this storage is uncertain. Our filamentous algal biomass estimates are high (96-204 g m^{-2} d.w.), but within the range of the few published values for this type of algal assemblage (Schoenberg and Oliver 1988, Robinson et al. 1997). Our mid-range estimate of P stored in algal biomass in early spring after re-flooding (21 kg P) far exceeds our estimate that the wetland initially released 7 kg of total P initially upon flooding, as well as the estimated amount of P in surface waters at the end of winter (3 kg P), suggesting either inaccurate assumptions, high sediment SRP release that we did not detect after ice-off and before algal growth, or that algae were exhibiting luxury uptake of P.

The uncertainties in our estimate of algal P include the P concentration in algal biomass, which we did not directly measure, and the spatial variation of algal biomass in un-sampled areas of the wetland. The published range of algal P content is about 0.1-0.5% of dry weight (Reddy and DeLaune 2008). If we use those values as extremes, the possible range of P content in filamentous algal biomass on the day we sampled was about 8.2-41 kg P. Thus, even at the lowest potential algal biomass P content, algal biomass P more than explains our observed P release based on differences in surface water concentrations. It is likely that algal biomass P was higher than the lowest end of the range, as algae are known to exhibit luxury uptake in high-P environments (Reddy and DeLaune 2008).

Regardless, it is evident that large amounts of sediment-released P can be stored in algal biomass. Algal uptake may even drive higher rates of sediment P release. The concentration of dissolved PO_4^{3-} in pore or surface waters controls the rate of PO_4^{3-} desorption from benthic or suspended sediment particles (Froelich 1988). Thus, by lowering PO_4^{3-} concentrations in the water column, algae may enhance PO_4^{3-} desorption and diffusion from pore waters into surface waters. Thus, algal growth and P uptake may initiate a positive feedback loop, enhanced by algal luxury P uptake (Reddy and DeLaune 2008) that would increase net release of P from sediments to surface waters. However, this feedback may be prevented if algal growth increases oxygen concentrations at the sediment-water interface, improving sediment P sorption. The role that filamentous algal growth plays in controlling rates of sediment P release and storage in wetlands remains poorly understood.

Sediment P release and storage may also be affected by duckweed in the genera *Lemna* and *Wolffia*, rapidly reproducing plants that were particularly prolific in the re-flooded wetland. Although we did not directly quantify the abundance of duckweed when sampling, this small, floating plant displayed nearly 100% coverage of wetland surface waters in spring and early summer, even when SRP concentrations were relatively low. Duckweed has a high capacity to take up P (Reddy and De Busk 1985), and it is possible that much of the released P ultimately was re-stored in the sediment as buried dead duckweed biomass. Although there are numerous studies on the purposeful use of duckweed to sequester P (Korner et al. 2003), its role in controlling P cycling in natural systems (in which it is not harvested and used in the system) is less well-known. Duckweed is likely to play an important role in re-flooded historically drained wetlands, especially when establishment of shading macrophytes is limited by eutrophic conditions, phytotoxin accumulation in pore waters, or other conditions (Roelofs 1991, Smolders and Roelofs 1993).

Ecosystem export of total P via the wetland outflow was also very high, and exceeded the estimate of initial P release, suggesting that sediments continued to release P after the initial release we observed. Most of the export was as total P, although the initial P release was largely as SRP. There also is uncertainty around our estimates of export because of a deficiency of discharge data early on in the study. However, concentrations of conservative ions, such as Mg^{2+}, as well as the lack of observed internal wetland flow suggest that mixing between most of the newly re-flooded “Wetland” zones and Ditch 1 and the Outflow Pond was minimal. Thus, a substantial portion of sediment-released P in the wetland zones was likely not flushed from the wetland but rather was ultimately stored in sediments or continues to be internally cycled. The balance between P stored in sediments and transported to downstream ecosystems is important in ecosystem management and depends on the morphometry and hydrology of a particular wetland.

Longevity of P Release

We observed declines in surface water SRP concentrations over the longer term, suggesting that P release after re-flooding historically drained areas in this wetland restoration may have been transient. Continued monitoring is required, however, to be certain that P release has slowed, as several studies have shown that re-flooded historically drained agricultural areas can continue to release P for at least 10 years following re-flooding (Montgomery and Eames 2008, Duff et al. 2009, Steinman and Ogdahl 2011). Why SRP concentrations declined in our wetland is uncertain, but filamentous algae and duckweed remained dominant members of the plant community even when SRP concentrations were low, suggesting that there was still ample available SRP to fuel internal eutrophication. These results may not extend to wetlands with different sediment properties (Chapter 3, this dissertation). For example, our sediments contained relatively low amounts of AVS and $CaCO_3$, both of which play important roles in P retention & release processes, but which may respond differently to the same hydrologic change.

Restoring wetland hydrology to historically drained land caused rapid release of large amounts of inorganic P from sediments to surface waters. Rates of P release slowed after one year, but in the meantime substantial concentrations of P were exported to downstream ecosystems. In addition, high growth of filamentous algae and duckweed still occurred, even when surface water SRP concentrations were lower. These observations demonstrate that when restoring wetlands by re-flooding historically drained areas, managers should incorporate longer-term monitoring of water quality and consider the potential for sediment P release to jeopardize restoration goals and affect downstream ecosystems.

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CHAPTER 3: TEMPORARY DESICCATION OF BIOGEOCHEMICALLY DIVERSE SEDIMENTS CAUSES VARIABLE SEDIMENT PHOSPHORUS RELEASE

Abstract

Humans invest considerable resources in protecting, restoring, and constructing wetland ecosystems to retain phosphorus (P), particularly through sediment storage. However, sediment P-retaining processes are highly vulnerable to natural and human-driven changes in wetland hydrology. Net sediment-water P exchange is controlled by several biogeochemically distinct processes including sorption-desorption, mineral co-precipitation, and assimilation and mineralization of organic matter, all of which are controlled to varying degrees by sediment moisture and oxygen conditions. Despite recognition of this biogeochemical complexity, few studies have investigated the effects of altered hydrology on a diverse range of wetland sediments. In this study we experimentally investigated how hydrologic regime and sediment biogeochemistry interactively influence net sediment-water P exchange. Using sediment-water microcosms with sediments from 16 biogeochemically diverse wetlands, we tested the effects of two hydrologic regimes (drying and re-inundation versus continuous inundation) on sediment P release to pore and surface waters. The effects of hydrologic regime on both the direction and magnitude of sediment-water P exchange depended significantly on sediment identity, and treatment effects on P release to pore and surface waters differed. In the majority of sediments (ten), dried and re-inundated sediments released more P into pore and/or surface waters than continuously inundated references. In contrast, two sediments released more P to pore waters in continuously inundated references than their dried and re-inundated counterparts, but this difference was not observed concurrently in surface waters. In six sediments, hydrologic regime did not significantly influence P release to surface or pore waters. Sediment properties controlling P release to pore waters differed from those controlling P release to surface waters. The effect of drying on net sediment P release to pore waters after re-inundation was positively related to sediment iron and loosely sorbed phosphate, and the effect on P release to surface waters was negatively related to acid volatile sulfides and total calcium carbonate. We observed high P release in dried and re-inundated sediments with relatively high amounts of loosely sorbed phosphate, suggesting that drained sediments with a legacy of high P loads will be most likely to release P and experience internal eutrophication when re-flooded. The differential responses of diverse wetland sediments to hydrologic manipulation show that hydrologic management of wetlands for nutrient removal must be evaluated on a case-by-case basis with knowledge of sediment biogeochemistry.

Introduction

Phosphorus (P) pollution and the eutrophication it causes present a pervasive problem in many aquatic ecosystems, especially within or downstream of agricultural and urban areas. In many landscapes, wetlands retain nutrients and can reduce P movement to sensitive downstream water bodies (Reddy and DeLaune 2008). For this reason, wetlands are often constructed or restored to mitigate P pollution (Kadlec and Wallace 2009). However, the capacity of wetlands to retain P is variable (Richardson 1985) and, at times, wetlands can be sources of P to downstream ecosystems (Richardson 1985, Coveney et al. 2002, Dunne et al. 2012).

Wetlands commonly experience seasonal and inter-annual fluctuations in water levels, which may be exacerbated by human actions such as water withdrawals, engineered land drainage, and controlled re-flooding of historically drained areas (Ardón et al. 2010, Steinman and Ogdahl 2011). In addition, global climate change and predicted shifts in temperature, precipitation, and seasonality of hydrologic flows will drastically alter the hydrologic regimes of many aquatic systems (Poff et al. 2002). Shallow ecosystem sediments are especially sensitive to hydrologic change because relatively small changes in water level may inundate or expose large areas of sediment, with consequent changes in oxygen and moisture condition, which will have large effects on the biogeochemistry of elements, including P. Sediments play a central role in wetland P cycling because they most often represent the largest potentially available pool of P (Dunne et al. 2007), and nutrient release from sediments strongly affects concentrations in shallow overlying water columns.

Sediment-water P exchange is simultaneously controlled by several distinct biogeochemical processes. These processes include abiotic sorption-desorption, mineral co-precipitation, biotic assimilation, and organic matter mineralization, and all are influenced differently by oxygen and moisture conditions. The longstanding paradigm is that oxidized iron at the sediment-water interface is the primary driver of sediment P retention by preventing phosphate (PO_4^{3-}) dissolved in pore water from entering overlying water (Mortimer 1941, 1942). Poorly crystalline, hydrated oxidized iron minerals (also referred to as amorphous iron oxyhydroxides) strongly sorb PO_4^{3-} ions (McLaughlin et al. 1981). However, oxygen depletion followed by microbially driven iron reduction at the sediment-water interface mobilizes reduced iron and previously sorbed PO_4^{3-}, and is often the primary mechanism of PO_4^{3-} desorption and release from sediments into surface waters (Marsden 1989). Drying influences this sorption-desorption process by exposing sediments to oxygen, presumably transforming reduced iron into oxidized forms that more strongly sorb PO_4^{3-} and diminishing rates of P release.

Although iron sorption is often considered the dominant form of P retention in aquatic ecosystems, other sediment processes involving sulfur, calcareous minerals, and organic matter are also important in controlling net sediment-water P exchange (Boström et al. 1988, Hupfer and Lewandowski 2008). In sediments where both iron and sulfur are important constituents, reduced sulfur (free sulfide, hereafter called H_2S) produced by anaerobic sulfate reduction reacts with both oxidized and reduced iron to form insoluble iron sulfide minerals (FeS_x), thereby diminishing the P-sorption capacity of the sediments (Roden and Edmonds 1997). In these types of sediments, oxidation of FeS_x during drying of previously inundated sediments can potentially reconstitute iron-P binding capacity (Smolders et al. 2006b). In calcareous sediments, PO_4^{3-} can co-precipitate with or sorb to calcium carbonate ($CaCO_3$) minerals, retaining large amounts of P (Hamilton et al. 2009). These calcareous minerals tend to precipitate at higher pH (most often due to removal of carbon dioxide from water by photosynthesizing organisms) and/or temperature, and dissolve under low pH conditions, releasing associated P (Boström et al. 1988). Finally, organic matter often represents the largest pool of P in wetland sediments (Dunne et al. 2007). The release of P from organic matter occurs during microbial mineralization, which is stimulated when previously anoxic sediments are drained and exposed to atmospheric oxygen. Inorganic P sorption and co-precipitation and organic P assimilation and mineralization are all directly and indirectly influenced by biotic and abiotic processes, which in turn are strongly controlled by oxygen and moisture conditions.

The net exchange of P between sediments and water thus depends both on the dominant chemical forms of P present and environmental conditions. Despite evidence for considerable biogeochemical variability in regulation of sediment P retention and release mechanisms in wetlands (Richardson 1985, Boström et al. 1988), the controlling factors are incompletely understood, and it remains difficult to predict how a given wetland sediment will respond to hydrologic fluctuations. In part this is because few studies have conducted comparative experiments to test the effects of controlled hydrologic alterations on multiple wetland sediments with widely varying characteristics.

To address this, we experimentally tested the effects of sediment drying and re-inundation on sediment-water P exchange using sediments from 16 shallow freshwater ecosystems of diverse biogeochemistry. We hypothesized that the direction and magnitude of sediment-water P exchange in response to experimental drying and re-inundation would be affected by sediment biogeochemical characteristics (Table 3.1, Figure 3.1). We predicted 1) that sediments with considerable amounts of iron and/or FeS_x would release less P after drying and re-inundation than when continuously inundated due to oxidation of reduced iron; 2) that primarily organic sediments would release more P when dried and re-inundated than continuously inundated due to enhanced mineralization rates, and 3) that in calcareous sediments, $CaCO_3$-associated

Table 3.1 Hypothesized effects of four main sediment properties on amount of phosphate (PO_4^{3-}) released from sediments to pore and surface waters after drying and re-inundation, and lists of measured variables that are potential predictors of each sediment property of interest. Variables are defined in Table 4.

Sediment Property	Measured Indicator Variables	Hypothesized effect on PO_4^{3-} release
Organic P	Org. Matter, Organic~P, NaOH~DNRP, HA~P	positive
Iron-bound P	Total Fe, Ox-Fe, BD~SRP, Fe:P	negative
Oxidizable FeS_x	AVS	negative
$CaCO_3$-associated P	Total $CaCO_3$, HCl~SRP	none

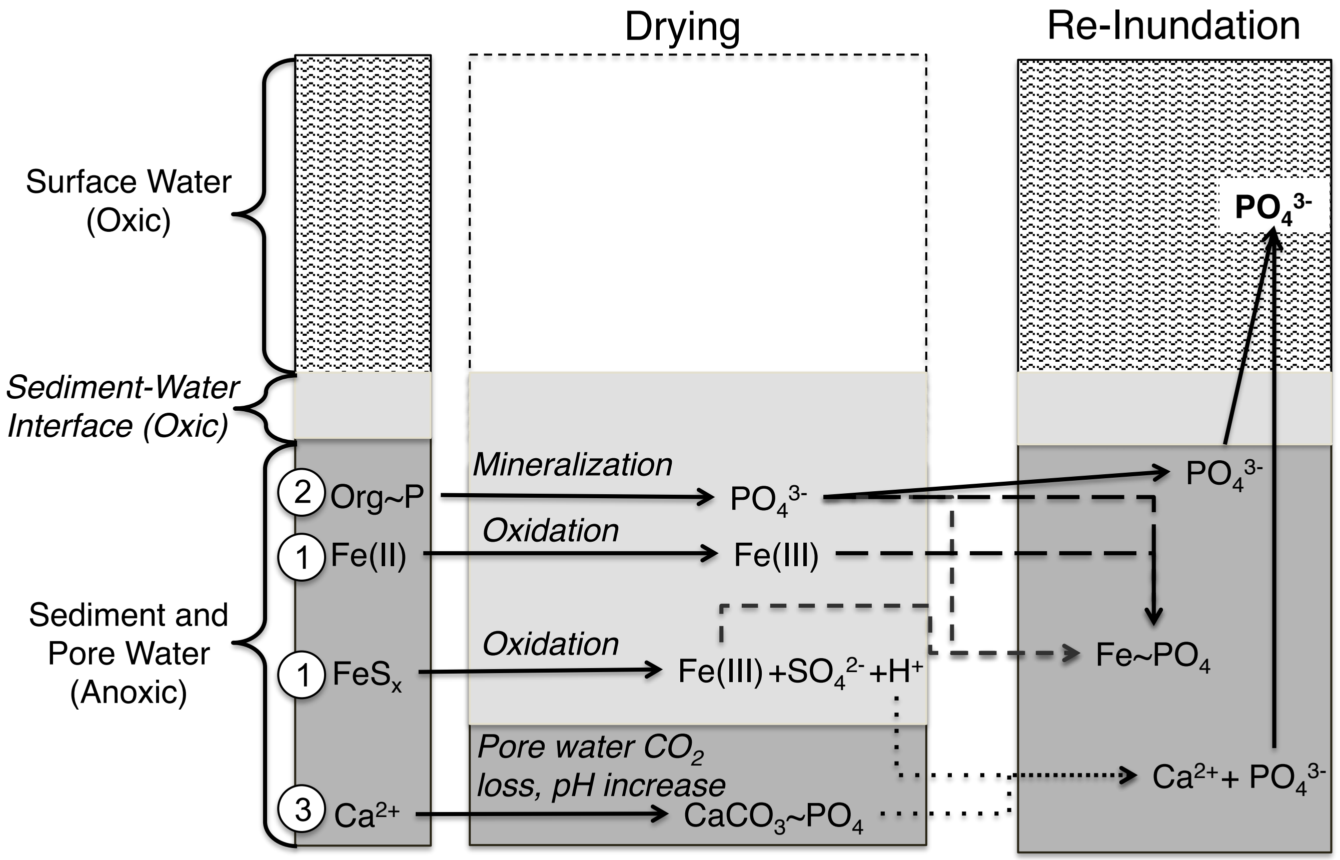

Figure 3.1 Conceptual diagram illustrating hypothesized biogeochemical effects of experimental sediment drying and re-inundation on phosphorus retention mechanisms. In this diagram, "~P" indicates P binding, such as sorption, coprecipitation, or covalent bonding, that retains P in association with the sediments. We predicted that 1) sediments with considerable amounts of iron and/or FeS$_X$ would release less P when dried and re-inundated than when continuously inundated due to oxidation of reduced iron; 2) that primarily organic sediments would release more P when dried and re-inundated than continuously inundated due to enhanced mineralization rates, and 3) that CaCO$_3$-associated P would increase due to loss of pore water carbon dioxide sediment drying, unless simultaneous oxidation processes produced sufficient acid to exceed the sediment's buffering capacity

P would increase during drying due to loss of pore water carbon dioxide, unless simultaneous oxidation processes produced sufficient acid to exceed the sediment's buffering capacity (Table 3.1, Figure 3.1).

Methods

Experimental Design

To test the effects of sediment biogeochemistry, hydrologic change, and their interaction on sediment-water P exchange, we collected sediment from 16 wetlands in southwest Michigan near the W.K. Kellogg Biological Station of Michigan State University, spanning a gradient groundwater influence (Table 3.2). Each of the 16 sediments was homogenized and then distributed into microcosms with an overlying water column. We were primarily interested in sediment processes, and because surface water chemistry (especially P concentration) is an important driver of sediment-water P exchange, we used a single water source to eliminate initial surface water chemistry as a source of variation. We incubated sediments in sediment-water microcosms for 2-5 weeks in the dark at room temperature, then subjected microcosms to one of two hydrologic treatments: either continuous inundation ("Reference") or temporary drying followed by re-inundation ("Dry"). For each of the 16 sediments, the two treatments were replicated three times, for a total of 96 experimental units (microcosms).

To test for the effects of hydrologic change on sediment-water P exchange, we measured soluble reactive phosphate (SRP) in pore and surface waters through time

Table 3.2 Sediment sampling site information. All sediments were collected in water of 0.5-1 m depth. Sites are all located in Universal Transverse Mercator Zone 16. Percent groundwater influence (GW) was calculated from magnesium concentrations (Whitmire and Hamilton 2005) using a mixing model assuming groundwater and precipitation represent the only inputs of dissolved Mg^{2+} to the ecosystems and using Mg^{2+} concentrations of 0.05 mg L^{-1} for precipitation (1979-2002 NADP/NTN 2003) and 23 mg L^{-1} for groundwater (Kalamazoo County mean). Conductivity was temperature-corrected to its value at 25°C. An * indicates sampling sites that likely were not continuously flooded over the past 10 years.

Site Name	**Abbreviation**	**UTM Easting**	**UTM Northing**	**Cond. (µS cm^{-1})**	**GW (%)**
Brook Lodge Marsh	BL	0634585	4690718	455	92
Douglas Lake	DL	0634596	4690688	382	94
Eagle Marsh	EM	0638009	4687350	341	79
Fort Custer Area 2 Wetland*	FCTC	0641163	4687408	291	37
Jackson Hole	JH	0635460	4685882	376	90
Loosestrife Fen	LF	0634931	4691989	396	92
Lux Arbor Pond 10*	P10	0626455	4703201	39	5
Lux Arbor Pond 18*	P18	0626637	4704091	57	13
Lux Arbor Pond 23*	P23	0627091	4705624	239	57
Lux Arbor Pond 6*	P6	0625855	4703265	77	18
Lux Arbor Pond 9*	P9	0626419	4703237	33	3
Osprey Bay*	OB	0626837	4703733	298	57
Sheriffs Marsh	SM	0637557	4695693	400	81
Turkey Marsh*	TM	0631750	4696366	353	75
Whitford Lake	WL	0635404	4685816	382	92
Wintergreen Lake	WG	0632473	4695097	343	84

after hydrologic treatment. We considered sediment P “retention” to be any process by which P was maintained in solid, relatively unavailable forms, including sorption, co-precipitation, and covalent bonding (i.e., in organic material), and “release” to be any situation in which P shifts from sediment-bound forms to dissolved, reactive PO_4^{3-} in either pore or surface water.

Sediment Sampling

We used an Eckman dredge to collect sediments under 0.5-1 m of water. We sieved wet sediments through 2 mm mesh and sub-sampled them for pre-treatment chemical analyses. We did not attempt to keep sediments under anoxic conditions during homogenization because sediments were not intended to represent processes occurring in the specific wetlands from which they were sampled, but rather to provide a diversity of characteristics for experimental purposes.

Experimental Methods

The experiment had three phases: equilibration, drying, and re-inundation. After homogenization, we distributed the water-saturated sediments into experimental microcosms to a sediment height of 15 cm (after 24 h of settling). Microcosms were made of clear, 7.3 cm diameter acrylic tubing cut to 46 cm length and sealed on the bottom. Within four days of aliquoting sediment, we carefully added one liter of low nutrient, filtered (Whatman GF/F 0.7 μm) lake water (Lower Crooked Lake, MI, with an SRP concentration of 1.5 μg L^{-1}) over the wet sediment to create a 23 cm overlying water column to initiate the equilibration phase. For each of the 16 sediments, we established six microcosms, allowing for three replicates of each hydrologic treatment, for a total of 96 experimental units.

Following initial inundation, we allowed sediments to equilibrate with the overlying water column for 2-5 weeks, during which oxygen in the surface water was kept close to atmospheric equilibrium using aquarium bubblers to mimic oxic water column conditions in a shallow, productive wetland. After observing that P concentrations in overlying water changed relatively little after the first 2 weeks of equilibration, a shorter equilibration phase was used for the second set of eight sediments tested.

After equilibration, we imposed the hydrologic treatments (drying phase). For each sediment, we removed the surface water from three of the six microcosms, and these were placed in a forced-air drying oven set at 30°C (Dry microcosms). The other three replicates of each sediment remained inundated and aerated at room temperature as during the equilibration phase (Reference microcosms). We weighed the Dry microcosms to determine soil moisture at the end of the drying phase. Sediments contained variable levels (3-50%) of soil moisture by the end of the drying phase due to textural differences among sediments collected from different sites.

To begin the re-inundation phase after the 4-5 week drying phase, we removed surface water from Reference microcosms and replaced it with fresh low-nutrient filtered lake water. Sediment in Dry microcosms was rewetted with 100-300 mL of de-ionized water to replace moisture lost in evaporative drying, and subsequently re-inundated with one liter of low-nutrient filtered lake water. Water columns in Dry treatments microcosms were removed prior to evaporative drying, so by re-wetting sediments with de-ionized water and re-inundating them with filtered lake water after drying, Dry microcosms should have contained a similar ion balances to continuously inundated controls.

Measuring Response to Hydrologic Treatment

We assessed sediment-water P exchange and related processes by sampling microcosm surface and pore waters for SRP, sulfate (SO_4^{2-}), nitrate (NO_3^-), and reduced iron (Fe(II)) concentrations during the re-inundation phase. After re-inundation, we measured surface water SRP twice a week for at least the first four weeks, and weekly thereafter. We measured pore water SRP at least three times during the re-inundation phase for the first eight sediments tested (P18, P23, P9, FCTC, BL, DL, LF, & TM, tested between June 2009 and December 2009), and weekly for the other eight sediments (between September 2009 and February 2010). Surface water SO_4^{2-} and NO_3^- concentrations were measured in the first set of microcosms once at the end of the re-inundation phase, and three times during the re-inundation phase (weeks 2, 7, & 10) in the second set. Pore water SO_4^{2-} and NO_3^- concentrations were measured in week 9 for sediments P9, P23, P18, and FCTC, week 4 for TM, LF, BL, and DL, and in weeks 0, 2, and 7 for other sediments. Reduced Fe(II) in the pore water was measured approximately weekly for the first five weeks in sediments P9, P23, P18, and FCTC, in weeks 0, 5, and 10 for sediments TM, LF, BL, and DL, and every other week in the remaining sediments.

We sampled surface water by removing 30 mL of water from the top of the water column using a plastic syringe and filtering this water through a 0.45 µm cellulose-acetate filter with a glass fiber pre-filter (Steriltech). We sampled sediment pore water by using a plastic syringe to remove 10 mL of water through a sediment pore water sampler (Rhizon; Rhizosphere Research Products, Wageningen, The Netherlands) with a nominal pore size of ~0.2 mm installed 8 cm above the bottom of each microcosm, at the vertical midpoint of the sediment column. We replaced the total volume of water sampled with low nutrient filtered lake water added to the surface water. After 9-11 weeks of inundation, we recorded the sediment height in each microcosm to calculate bulk density, siphoned off surface water for water chemistry analysis, and removed the sediment, homogenized it by hand, and analyzed it for sediment chemistry.

Water Analyses

We measured SRP concentrations in surface and pore water samples using the molybdate blue colorimetric method (Murphy and Riley 1962), and NO_3^- and SO_4^{2-} using membrane-suppression ion chromatography. We measured Fe(II) in pore waters using reaction with ferrozine based on a method modified from Lovley and Phillips (1987) and Stookey (1970), in which the pore water sample was immediately added to a solution of 50 mM HEPES buffer containing ferrozine (1 g L^{-1}). After color formation, we measured sample absorbance on a spectrophotometer at 562 nm.

Sediment Analyses

We sampled homogenized moist sediment prior to hydrologic treatment to measure organic matter, total phosphorus (Total P), total iron (Total Fe), total carbonate ($CaCO_3$), acid volatile sulfides (AVS), oxalate-extractable iron (Ox-Fe), and sequentially extracted P binding fractions. We sub-sampled moist sediment into 1-3 g wet weight aliquots for dry weight to wet weight ratios, Ox-Fe, and sequential P extraction. We used dry weight to wet weight ratios to estimate dry mass of sediment in each core, bulk density of the sediments, and soil moisture of the Dry treatments at the end of the drying phase. To measure Ox-Fe, an indicator of poorly crystalline iron minerals, we extracted ~0.4 g moist sediment in a 0.2 M acid ammonium oxalate solution for four hours in darkness (Walbridge et al. 1991).

To prepare sediment for measurement of organic matter, Total P, Total Fe, and $CaCO_3$, we dried a sub-sample of sediment to a constant weight and homogenized it with a mortar and pestle. We then combusted triplicate sub-subsamples (~1 g d.w.) of dried and ground sediment for analysis of organic matter as loss on ignition (550°C). We extracted combusted samples for 10 min in boiling 1 M hydrochloric acid (HCl) to extract Total P and Total Fe (Andersen 1976). We measured $CaCO_3$ in triplicate sub-samples of dried and ground sediment (0.1-1 g d.w.) by acidifying in a sealed container, measuring carbon dioxide produced using a pressure transducer, and calculating the original carbonate concentration using the Ideal Gas Law. We measured iron in Ox-Fe and Total Fe extracts was using flame atomic absorption spectrophotometry.

We froze approximately 100 g of moist sediment for analysis of AVS. We measured AVS following US EPA Method 821-R-91-100 by acidifying sediment samples with HCl to convert AVS to H_2S, which was then trapped as sulfide (S^{2-}) in an alkaline solution (0.5 M NaOH) and measured colorimetrically after reaction with a mixed diamine reagent (H_2SO_4, N,N-dimethyl-p-phenylenediamine oxalate, and ferric chloride hexahydrate) (Allen et al. 1991). Analytical sulfide standards were prepared from a stock solution standardized versus thiosulfate.

Sequential Extraction of P Binding Fractions

We used a sequential extraction procedure (Paludan and Jensen 1995) to measure operationally defined P binding fractions in our 16 sediments (Table 3.3). These measurements allow for a more mechanistic understanding of dominant P retention and release processes in each sediment, because different chemical forms of P respond differently to changes in biological, physical, and chemical conditions. Many sequential extraction procedures have been used to extract individual P binding fractions in soils and sediments, although all provide operationally defined results that are useful indicators, but not pure samples, of chemical forms of P (Pettersson et al. 1988).

We sequentially extracted P binding fractions in triplicate samples of wet pre-treatment sediment (0.5-5 g d.w.). The first step used de-oxygenated de-ionized water to extract loosely bound P (H_2O~P). Next, bicarbonate-buffered dithionite (0.11 M) extracted PO_4^{3-} bound to redox-sensitive oxidized iron minerals (BD~P) and some non-reactive (mostly organic) P (BD~DNRP) (Reitzel et al. 2006). This step also may extract some apatite-bound P in calcareous sediment (Reitzel 2005). The third step used sodium hydroxide (0.1 M NaOH) to extract PO_4^{3-} bound to redox-insensitive aluminum and iron oxides that undergo anionic exchange with hydroxide (NaOH~SRP) and non-reactive organic and inorganic P (pyro- and polyphosphates). Non-reactive P extracted by NaOH was acidified to separate out precipitating humic-acid associated P (HA~P) from other non-reactive P molecules (NaOH~DNRP). After NaOH extraction, HCl (0.5 M) extracted acid-soluble P, mostly bound to apatite and other calcareous

Table 3.3 Expected forms of soluble reactive and dissolved non-reactive phosphorus (SRP and DNRP, respectively) extracted by sequential fractionation (Paludan and Jensen 1995).

Extractant	P Fraction	Expected P forms
Deoxygenated water	H_2O~P	Loosely sorbed phosphorus
0.11 M sodium bicarbonate and sodium dithionite	BD~SRP	Phosphate associated with redox-sensitive iron minerals
	BD~DNRP	Non-reactive (mostly organic) phosphorus extracted by BD solution
0.1 M sodium hydroxide	NaOH~SRP	Phosphate associated with aluminum oxides and non-redox sensitive iron minerals
	NaOH~DNRP	Non-reactive phosphorus including organic P and pyro- and poly-phosphates
0.1 M sodium hydroxide + 1 M sulfuric acid	HA~P	Phosphorus associated with humic acids that precipitate from acidified NaOH extract
0.5 M hydrochloric acid	HCl~P	Phosphate associated with pH-sensitive apatite and calcareous minerals
Hot 1 M hydrochloric acid post-combustion	Res~P	Refractory organic and inorganic phosphorus

minerals (HCl~P). Residual P in the sediment pellet following the preceding chemical treatments was presumed to be bound in highly recalcitrant organic matter or crystalline mineral substances, and was extracted by combustion followed by ten minutes in boiling 1 M HCl (Res~P). Reactive P in each operationally defined fraction was detected using standard colorimetric methods, and non-reactive P was measured as the difference between reactive P and colorimetrically detected P following persulfate digestion (total P). We did not detect any non-reactive H_2O~P or BD~P, so results are not reported for those fractions.

Statistical Analyses

To detect P release differences between hydrologic treatments among sediments, we used reduced maximum likelihood mixed-effects models with hydrologic treatment (Trt), sediment (SedType), and the interaction between them (Trt x SedType) as fixed effects and each individual microcosm as a random effect to account for repeated measures through time within individual microcosms. We used likelihood ratio tests to assess the significance of including microcosm as a random effect by comparing two models, identical in all terms except the random effect, revealing whether inclusion of this term produced a model with significantly more likelihood (Pinheiro and Bates 2004).

We calculated initial P release rates to surface waters within treatments and sediments using average surface water SRP concentrations for the first three sampling events after re-inundation. Observed SRP patterns through time were variable, so we chose the first three sampling events arbitrarily for consistency among treatments. We used linear regression analysis to detect if the release rate slope was significantly different from zero ($\alpha = 0.05$). There was not sufficient sampling frequency to calculate meaningful initial release rates to pore water.

We did not observe a net removal of P from surface water by sediments in either hydrologic treatment, most likely because of the low SRP of experimental water used to inundate microcosms. Rather, in most cases we observed either no net change in or increasing SRP, suggesting that P either remained in sediment pools or was mobilized into pore and/or surface waters. The SRP concentration of the water used to inundate the treatments was low (1.5 μg L^{-1}), encouraging sorption equilibria to shift towards release of P from sediment pools into initially low-SRP surface waters.

To identify which sediments responded to hydrologic treatment (i.e., whether or not Dry was significantly different from Reference) and the direction of the response (i.e., whether Dry was greater or less than Reference) we tested for significant differences between Dry and Reference treatments within each of the 16 sediments. To detect significant treatment differences in water chemistry (concentrations of SRP, dissolved Fe(II), SO_4^{2-} and NO_3^-) within sediments, we averaged values within replicate microcosms (n=3) through time during the re-inundation phase (sampling frequency varied for dissolved species and by sediment). For each water chemistry response variable, we used 16 individual analysis of variance tests with treatment (Dry or Reference) as a fixed factor to test for differences within each sediment sample. We detected significance at $\alpha = 0.05$ with the Benjamini & Hochberg (1995) correction for multiple comparisons that minimizes the risk of making Type II Errors by controlling the false discovery rate, rather than the family wise error rate (Verhoeven et al. 2005).

To obtain a single index of how sediment-water P exchange responded to drying in each of the 16 sediment types that could be compared with sediment characteristics, we calculated the difference between hydrologic treatments in average P concentration following re-inundation. To investigate the role of sediment biogeochemistry in determining sediment responses, we used this index as a response variable predicted from sediment characteristics in linear models. We averaged water chemistry measurements through time within microcosms and across replicate treatment microcosms within sediments, and then calculated the difference between paired sediments (Dry-Reference). For each response (pore and surface water), we used stepwise forward regression analysis to identify sediment chemistry variables that best predicted water chemistry responses. To avoid the influence of multicollinearity among predictors in models, variables with a variance inflation factor of greater than two were not incorporated into the models (Graham 2011). We chose predictor variables from all sediment variables measured, including absolute values of sequentially extracted P binding fractions. To best meet linear model assumptions of equal variance and normality, pore water Dry-Reference SRP values were left untransformed, and surface water Dry-Reference SRP values were natural log(x+min value)-transformed.

To test whether or not our results supported our hypotheses of how drying influences sediment P retention mechanisms (Figure 3.1, Table 3.1), we compared stepwise regression-generated "best" models to univariate hypothesis-based models with indicators of four sediment properties which we predict to be important: organic P (organic matter, organic~P, NaOH~DNRP, HA~P), iron-bound P (Total Fe, Ox-Fe, BD~SRP, Fe:P ratio), oxidizable FeS_x (AVS), and $CaCO_3$-associated P (Total $CaCO_3$, HCl~SRP) as predictors (Table 3.1). Within each set of measured indicators of the four sediment properties, the model with the lowest Akaike Information Criterion (AIC) was selected as a candidate “hypothesis” model for calculation of AIC weights. Thus, a set of candidate models for each of the two response variables (surface water Dry-Reference SRP and pore water Dry-Reference SRP) included one model for each sediment property hypothesis, and one stepwise regression-selected “best” model. We used Akaike’s Information Criterion (AIC) to assign weights to each model that predict the probability that, given our data, each individual model is the best within the group of models (Burnham 2004).

For all chemical analyses, values lower than detection limit were replaced with zeros prior to analysis. Unless otherwise stated, sediment percent organic matter was arcsine-square root transformed, and all other variables were natural log-transformed. All statistical analyses were completed in R version 2.13.2 (R Development Core Team 2011).

Results

Sediment Chemistry

The 16 sediments used to investigate effects of drying on sediment-water P exchange encompassed a broad range of biogeochemical characteristics (Table 3.4). Sediment total P was significantly positively correlated with sediment organic matter, total Fe, Ox-Fe, and all sequentially extracted P binding fractions (Table 3.5). Molar ratios of Fe:P, which are sometimes used to predict sediment propensity to release P, ranged from 3.5-37, with a mean of 10. Both absolute and relative amounts of P binding fractions showed substantial variability among sediments (Figure 3.2).

Table 3.4 Biogeochemical characteristics of 16 experimental wetland sediments measured prior to hydrologic regime manipulation. Total phosphorus (Total P), total iron (Total Fe), oxalate-extractable iron (Ox-Fe), acid volatile sulfides (AVS) and total calcium carbonate ($CaCO_3$) are reported as per gram dry weight of sediment. Organic matter was measured as loss on ignition. Dry Soil Moisture is the moisture content of the sediment after experimental desiccation, prior to re-inundation. Sediment type abbreviations are defined in Table 3.2.

Sediment Type	Bulk Density (g cm^{-3})	Dry Soil Moisture (%)	Organic Matter (%)	Total P (µg g^{-1})	Total Fe (mg g^{-1})	Fe:P Molar ratio	Ox-Fe (mg g^{-1})	AVS (µmol g^{-1})	$CaCO_3$ (mg g^{-1})
BL	0.20	32	18	443	11.84	14.8	0.16	4.6	396
DL	0.08	50	55	523	9.44	10.0	4.3	2.4	190
EM	0.99	10	4	130	3.89	16.6	1.8	1.5	62
FCTC	0.16	50	80	1440	11.88	4.6	8.6	0.21	2
JH	0.82	8	5	105	3.66	19.3	0.46	1.0	5
LF	0.24	38	40	773	4.96	3.6	1.3	0.82	9
OB	0.52	9	8	167	1.60	5.3	0.3	0.17	4
P10	0.26	9	17	537	4.98	5.2	2.0	0.13	12
P18	0.21	14	24	512	4.69	5.1	1.6	0.27	12
P23	0.29	21	19	366	4.09	6.2	0.69	0.22	4
P6	0.63	3	6	177	3.43	10.7	1.3	1.5	12
P9	0.41	8	21	556	3.99	4.0	1.0	0.48	7
SM	0.23	22	30	1910	36.88	10.7	40.1	7.5	23
TM	0.41	19	15	459	4.54	5.5	0.69	1.7	265
WL	1.33	3	1	28	1.87	37.1	0.50	0.91	9
WG	0.30	27	24	528	3.37	3.5	0.70	0.37	3

Table 3.5 Pearson's r correlation coefficients between sediment characteristics measured upon collection, including organic matter as loss on ignition (OM), total phosphorus (TP), total iron (TFe), oxalate-extractable iron (OxFe), acid-volatile sulfides (AVS), total carbonate (CO_3) and sediment P binding fractions of 16 sediments. OM in % was arcsine-square root transformed and other variables were natural log-transformed prior to correlation analyses. Correlation coefficients in boldface are statistically significant ($p < 0.05$).

	OM	TP	TFe	OxFe	AVS	$CaCO_3$	H_2O~SRP	BD~SRP	NaOH~SRP	NaOH~DNRP	HA~P	HCl~P	Res~P
OM	1.00												
TP	**0.80**	1.00											
TFe	**0.61**	**0.75**	1.00										
OxFe	**0.57**	**0.60**	**0.71**	1.00									
AVS	-0.09	0.04	**0.54**	0.20	1.00								
$CaCO_3$	-0.08	0.04	0.34	-0.07	**0.70**	1.00							
H_2O~SRP	0.32	**0.54**	0.41	0.20	0.09	-0.08	1.00						
BD~SRP	0.50	**0.71**	**0.81**	**0.78**	0.41	0.02	**0.60**	1.00					
NaOH~SRP	0.49	**0.79**	0.51	**0.65**	-0.13	-0.38	**0.54**	**0.75**	1.00				
NaOH~DNRP	**0.70**	**0.91**	**0.52**	0.46	-0.14	-0.17	**0.72**	**0.66**	**0.84**	1.00			
HA~P	**0.71**	**0.88**	0.48	0.47	-0.33	-0.28	**0.51**	**0.51**	**0.84**	**0.89**	1.00		
HCl~P	**0.63**	**0.62**	**0.71**	**0.66**	0.34	0.02	0.43	**0.69**	0.48	0.45	**0.41**	1.00	
Res~P	**0.81**	**0.84**	**0.60**	0.28	0.04	0.22	0.39	0.46	0.43	**0.76**	0.69	0.38	1.00

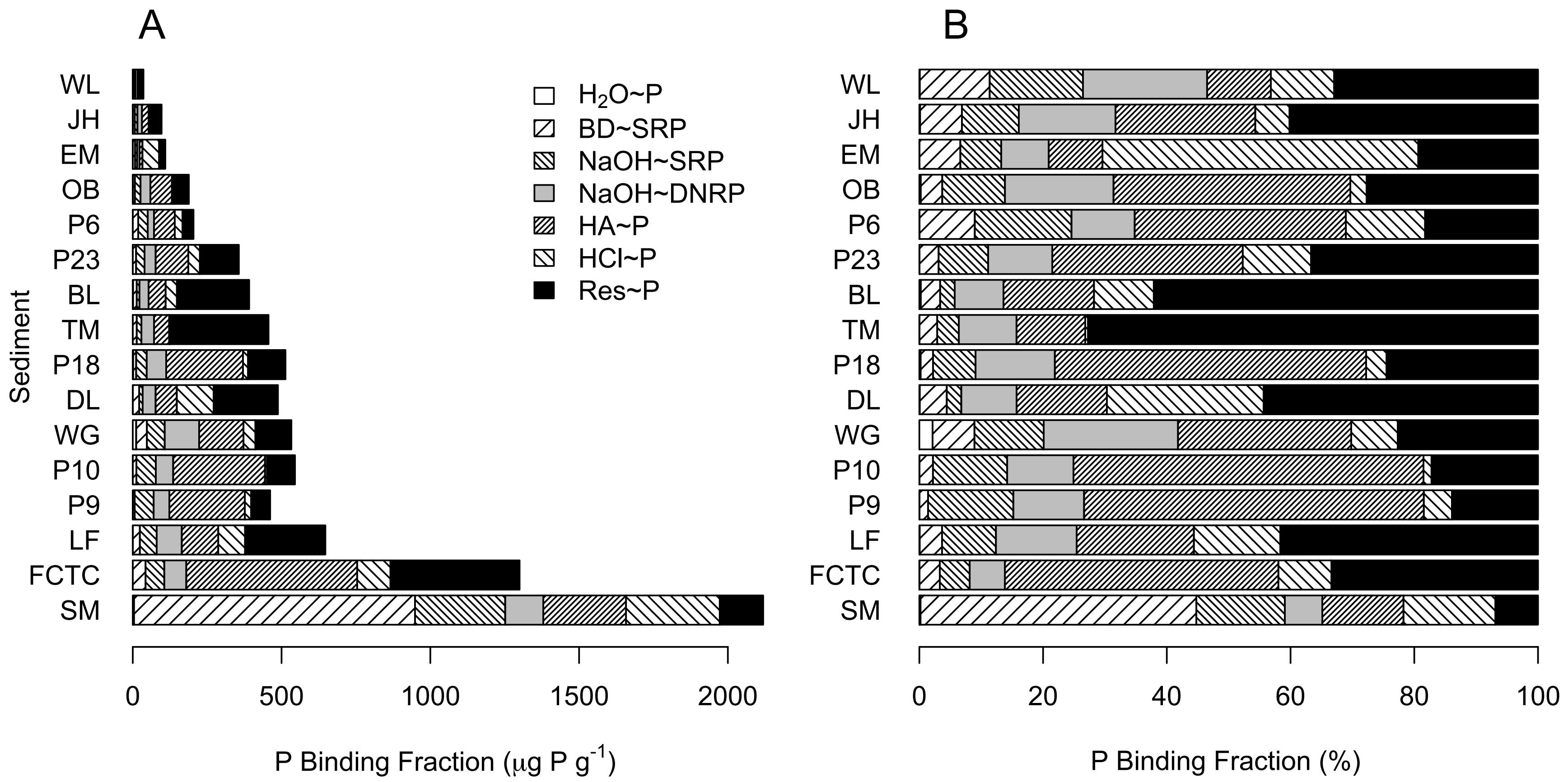

Figure 3.2 Absolute values (A) and proportional amounts (B) of operationally defined phosphorus binding fractions (Table 3.3) in the 16 sediments used in the experimental drying and re-inundation.

Effects of Hydrologic Treatment on Water Chemistry in Sediment-Water Microcosms

The effect of hydrologic treatment on sediment-water P exchange significantly varied among the 16 sediments, both in terms of magnitude of P release and direction of response (Table 3.6). Both hydrologic treatment and sediment identity interacted to significantly affect pore and surface water SRP, pore and surface water SO_4^{2-}, pore water Fe(II), and surface water NO_3^- ($p < 0.001$).

Sediment-Water P Exchange

Significant differences in pore water SRP were not always accompanied by significant differences in surface water SRP of the same sediment, and vice versa (Figure 3.3; Table 3.6). In seven of the 16 sediments, average pore water SRP was greater in Dry than Reference treatments, whereas in two sediments, pore water SRP was higher in the Reference than Dry treatments (Figure 3.3; Table 3.6). Average surface water SRP was higher in Dry than Reference treatments of six sediments, and no sediments had significantly higher surface water SRP in the Reference than Dry treatments. In several sediments, hydrologic treatment caused no significant difference in SRP concentrations in pore water and/or surface water (Figure 3.3; Table 3.6). Average SRP concentrations were generally much higher in pore water than surface water (mean ± std error: 439 ± 40 and 15 ± 1.8 µg P L^{-1}, respectively; Table 3.6).

The highest amounts of sediment P release to surface water were observed in Dry sediments (Figure 3.3; Table 3.6), but initial P release rates were generally low, even when total P release over the course of the experiment was high. Only seven

Table 3.6 Sediment P flux response to drying and re-inundating (Dry) or continuously inundated (Ref.) treatments. SRP concentrations in pore waters (PW) and surface waters (SW) were averaged through time and among cores within hydrologic treatment and sediment. Significant differences between mean PW and SW SRP concentrations in Dry and Ref. treatments were tested using an F test on means within microcosms through time (n=3). P values in bold represent significance after Benjamini & Hochberg's (1995) correction for multiple comparisons (α=0.05). Final SW SRP is the SRP concentration measured on the final day of the re-inundation phase, averaged across cores within treatment and sediment (n=3 unless otherwise noted). Data are mean ± standard error.

	Mean PW SRP (µg P L^{-1})			Mean SW SRP (µg P L^{-1})			Final SW SRP (µg P L^{-1})	
Sediment	Dry	Ref.	*p*	Dry	Ref.	*p*	Dry	Ref.
BL	12±4	34±18	*0.070*	1±0	1±0	*0.369*	0±0	4±3
DL	21±6	2±1	***0.001***	1±0	2±1	*0.059*	1±1	0±0
EM	39±3	101±11	***0.001***	1±0	1±0	***0.001***	2±1	1±0
FCTC	571±121	496±134	*0.653*	56±7	3±2	***0.002***	71±10	17±16
JH	371±51	49±7	***0.002***	3±1	1±0	***0.005***	1±0	1±0
LF	15±8	30±12	*0.288*	1±0	1±0	*0.149*	2±0	1±0
OB	773±92	201±40	***0.013***	30±7	1±0	***0.001***	33±29	1±0
P10	126±11	47±8	***0.011***	2±0	1±0	*0.399*	1±0	2±1
P18	276±78	71±12	*0.144*	5±1	1±0	*0.026*	14±6	2±0
P23	221±54	124±21	*0.466*	3±1	1±0	*0.054*	1±0	1±0
P6	294±32	40±9	***<0.001***	2±0	1±0	***0.010***	2±1	1±0
P9	275±57	100±23	***0.017***	15±2	1±0	*0.035*	21†	1±1
SM	183±26	1080±124	***0.006***	4±1	7±1	*0.049*	1±0	5±2
TM	10±3	18±4	*0.086*	2±1	1±0	*0.303*	0±0	0±0
WL	139±13	27±8	*0.058*	1±0	1±0	*0.606*	1±1	1±0
WG	3877±332	2076±154	***<0.001***	294±25	7±1	***<0.001***	340±25	1±0

*indicates slope significantly different from zero (p<0.05)
† n=1 because of core leakage

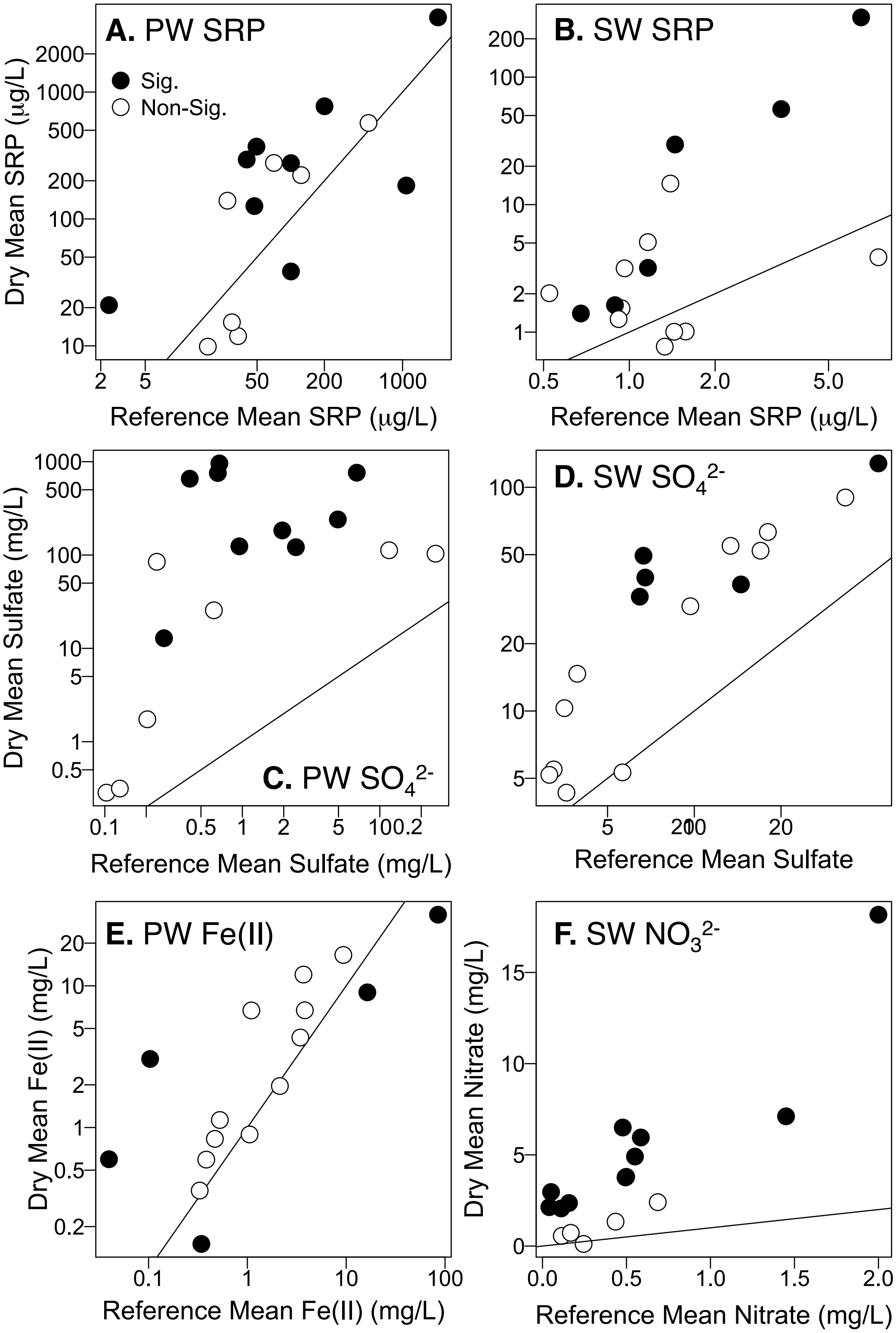
A. PW SRP
Sig.
Non-Sig.
Dry Mean SRP (µg/L)
2000
1000
500
200
100
50
20
10
2
5
50
200
1000
Reference Mean SRP (µg/L)
B. SW SRP
200
100
50
20
10
5
2
1
0.5
1.0
2.0
5.0
Reference Mean SRP (µg/L)
C. PW SO$_4^{2-}$
Dry Mean Sulfate (mg/L)
1000
500
100
50
10
5
1
0.5
0.1
0.5
1
2
5
100.2
Reference Mean Sulfate (mg/L)
D. SW SO$_4^{2-}$
100
50
20
10
5
5
200
20
Reference Mean Sulfate
E. PW Fe(II)
Dry Mean Fe(II) (mg/L)
20
10
5
2
1
0.5
0.2
0.1
1
10
100
Reference Mean Fe(II) (mg/L)
F. SW NO$_3^{2-}$
Dry Mean Nitrate (mg/L)
15
10
5
0
0.0
0.5
1.0
1.5
2.0
Reference Mean Nitrate (mg/L)

Figure 3.3

Figure 3.3 (continued) Mean concentrations of SRP (A, B), SO_4^{2-} (C, D), Fe(II) (E), and NO_3^- (F) in temporarily desiccated (Dry) sediments plotted against continuously inundated controls (Reference) in sediment pore waters (PW: A, C, E)) and in overlying surface waters (SW: B, D, F) of microcosms containing16 biogeochemically diverse sediments, compared to 1:1 lines. Note that both axes in panels A through E are plotted on log scales, and the differences in scale between panels. Solid black circles denote statistically significant differences between the two hydrologic treatments, and hollow circles denote no significant difference. Sediment points lying above the 1:1 line had higher average concentration in dried and re-inundated treatments than continuously inundated references.

initial P release rates to surface water were significantly different from zero (three Dry and four Reference, data not shown). Of these, initial P release rate was greater than 1 μg P L^{-1} d^{-1} only in the Dry WG sediment, at 15 μg P L^{-1} d^{-1}. Initial P release rates were generally poor predictors of final SRP concentration, reflecting the inconsistency of P flux patterns through time among sediments (Figure 3.4).

Patterns of change in SRP concentrations through time were highly variable (Figure 3.4). In surface water, SRP concentrations were usually low (<10 μg L^{-1}) with episodically higher concentrations observed on some sampling days. Only two sediments (FCTC and WG) showed a sustained increase in SRP concentration in surface water without re-uptake by sediments (Figure 3.5). In both cases, Dry sediments released significantly more SRP than the Reference sediments, and final SRP concentrations were substantial (71 ± 10 and 340 ± 25 μg P L^{-1}, respectively; Figure 3.5).

P Flux Process Indicators: Fe(II), SO_4^{2-}, and NO_3^-

Pore water dissolved Fe(II) was significantly different between treatments in only 5 of the 16 sediments (Figure 3.3), and pore water Fe(II) response mirrored SRP response in only 3 sediments (EM, JH & SM). In two sediments, Dry treatments had significantly more pore water dissolved Fe(II) than their Reference counterparts, whereas three sediments contained higher Fe(II) in Reference than Dry sediments (Figure 3.3).

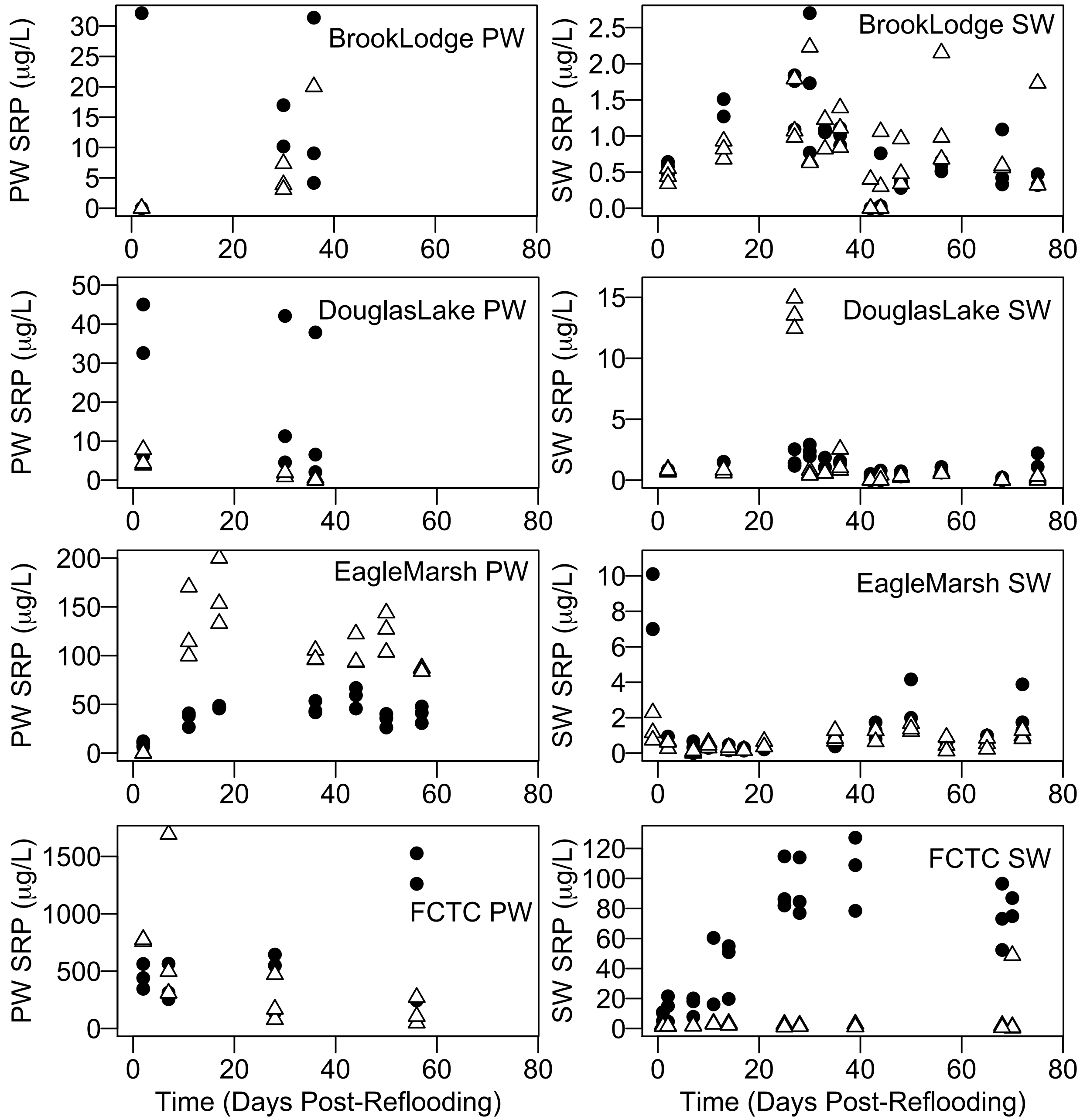

Figure 3.4 SRP concentrations measured in pore water (PW) and surface water (SW) of sediment-water microcosms after hydrologic manipulations. Three replicate microcosms of each sediment temporarily desiccated and re-inundated (“Dry” treatment, closed circles), while three replicates were continuously inundated (Reference, open triangles).

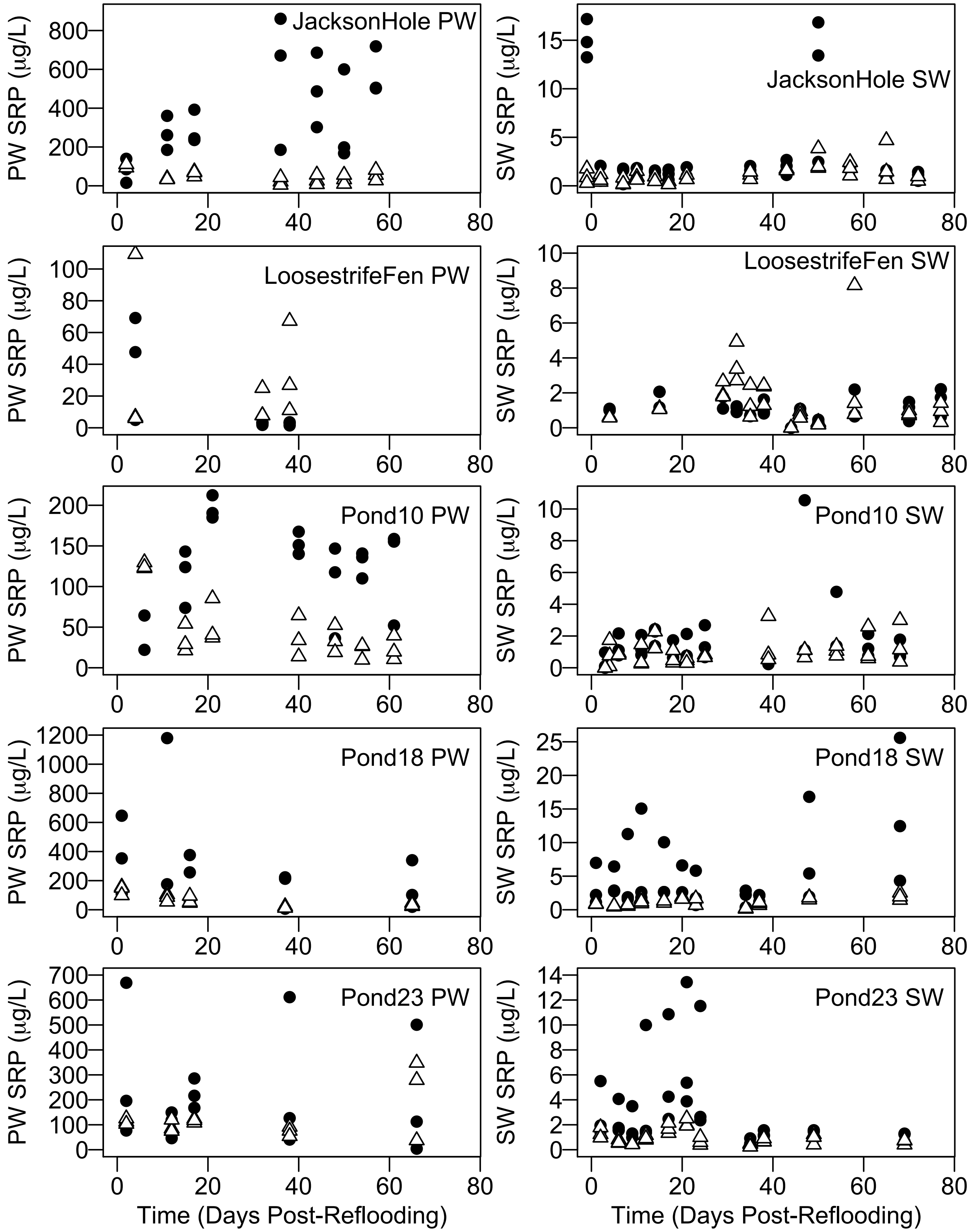

Figure 3.4, continued

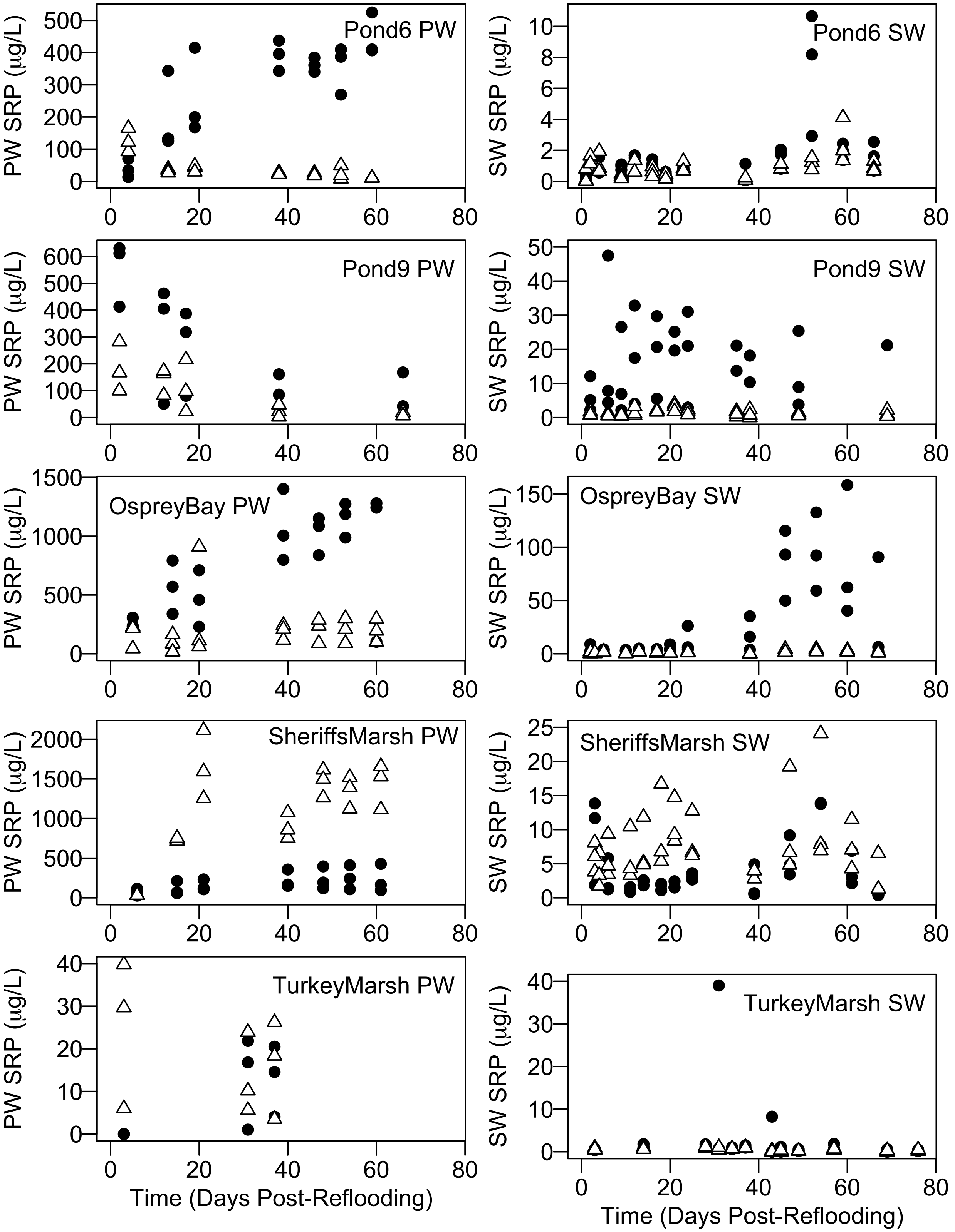

Figure 3.4, continued

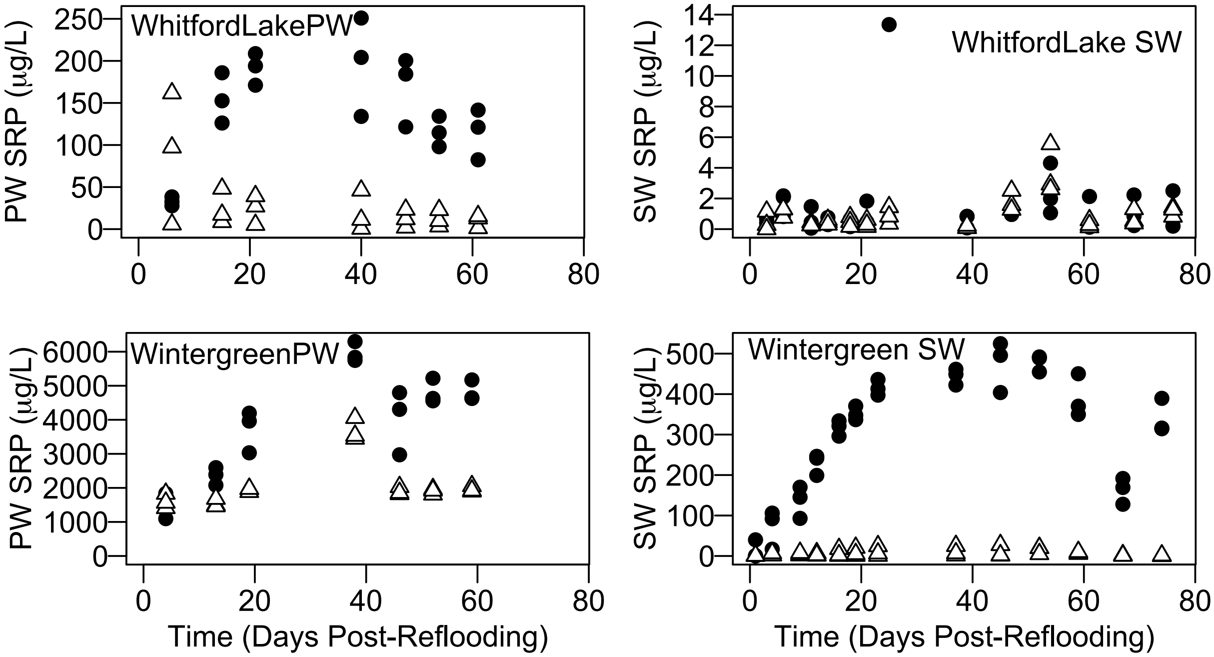

Figure 3.4, continued

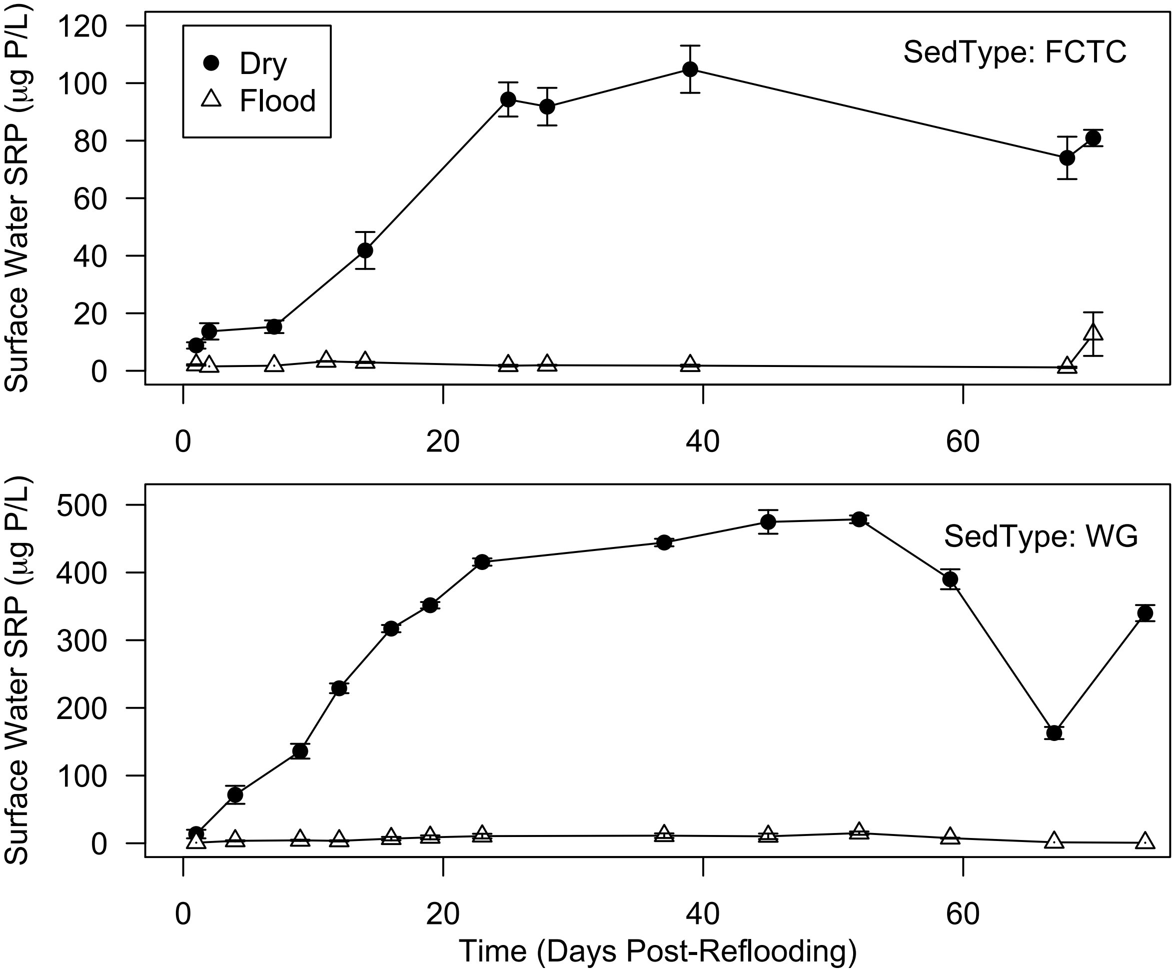

Figure 3.5 Post-re-inundation SRP concentrations in surface waters overlying dried and re-inundated (circles) and continuously inundated (triangles) sediments for the only two of 16 experimental sediments that showed consistently and strongly elevated SRP concentrations in surface waters after re-inundation. Each data point is an average of three replicate experimental microcosms, with standard error bars.

In the case of SO_4^{2-} and NO_3^-, the direction of response to hydrologic treatment was uniform across sediments in that concentrations were either higher in Dry than Reference sediments or not significantly different between hydrologic treatments; Reference sediments never contained more SO_4^{2-} or NO_3^- than Dry sediments (Figure 3.3). In most cases (9 out of 16 sediments), surface water NO_3^- was significantly higher in Dry than Reference treatments (Figure 3.3). In pore waters, NO_3^- was uniformly very low (usually below our detection limit of ~0.01 mg N L^{-1}) with no significant differences between treatments (data not shown). Sulfate differences were especially pronounced in pore water, in which nine of the 16 sediments had significantly greater SO_4^{2-} in Dry than Reference pore waters, and the magnitude of these differences was often high (Figure 3.3).

Relationships between Sediment Chemistry and Dissolved P Dynamics

To investigate how sediment biogeochemical properties influenced sediment P release in response to hydrologic treatment, we tested relationships between post-inundation SRP release and sediment variables. In pore waters, we identified total Fe and H_2O~SRP as the “best” predictors of P release using stepwise forward regression, and Akaike weights indicate a 98% chance that of the five candidate models compared, this is the best model (Table 3.7). Total Fe was negatively related to pore water Dry-Reference SRP, while H_2O~SRP was positively related (Figure 3.6). These

Table 3.7 Comparison of linear models predicting sediment SRP release in response to drying and re-inundation from sediment characteristics. Sediment-water microcosms containing 16 diverse sediments were subjected to drying and re-inundation (Dry) or continuous inundation (Reference). Average post-inundation SRP concentrations in Reference treatments were subtracted from the Dry treatment of the same sediment (“Dry-Reference SRP”). Univariate models predicting Dry-Reference SRP from indicator variables were compared using AIC, and the “best” model within each set of sediment property indicators was selected for model comparison. A single “best” model was identified by selecting parameters from all measured sediment variables using stepwise forward selection and included as a candidate model (in boldface). Candidate models were compared by calculating Akaike weights (ω_i). Predictor variables were natural-log or arcsine square root transformed, PW Dry-Reference SRP response was left untransformed, and SW Dry-Reference SRP response was natural log+4 transformed. Models that were not identified as the “best” model, but were significant at $p<0.05$ are italicized.

	Sediment Property	Predictor(s)	Direction	R^2	p-value	ΔAIC_c	ω_i
PW SRP Response Variable: PW Dry-Reference SRP	Organic P	Org. Matter	negative	0.01	0.68	14.50	<0.01
	Iron-Bound P	*Total Fe*	*negative*	*0.34*	*0.02*	*9.46*	*0.01*
	Oxidizable FeS_x	AVS	negative	0.23	0.06	11.15	<0.01
	$CaCO_3$-Associated P	Total $CaCO_3$	negative	0.15	0.14	12.46	<0.01
	Stepwise Model	**Total Fe, H_2O~SRP**	**negative, positive**	**0.68**	***<0.01***	**0.00**	**0.98**
SW SRP Response Variable: natural log (SW Dry-Reference SRP +4)	Organic P	Organic~P	positive	0.04	0.43	5.87	0.03
	Iron-Bound P	Fe:P	negative	0.24	0.06	2.98	0.14
	Oxidizable FeS_x, Stepwise Model	**AVS**	**negative**	**0.41**	***<0.01***	**0.00**	**0.64**
	$CaCO_3$-*Associated P*	*Total* $CaCO_3$	*negative*	*0.26*	*0.04*	*2.54*	*0.18*

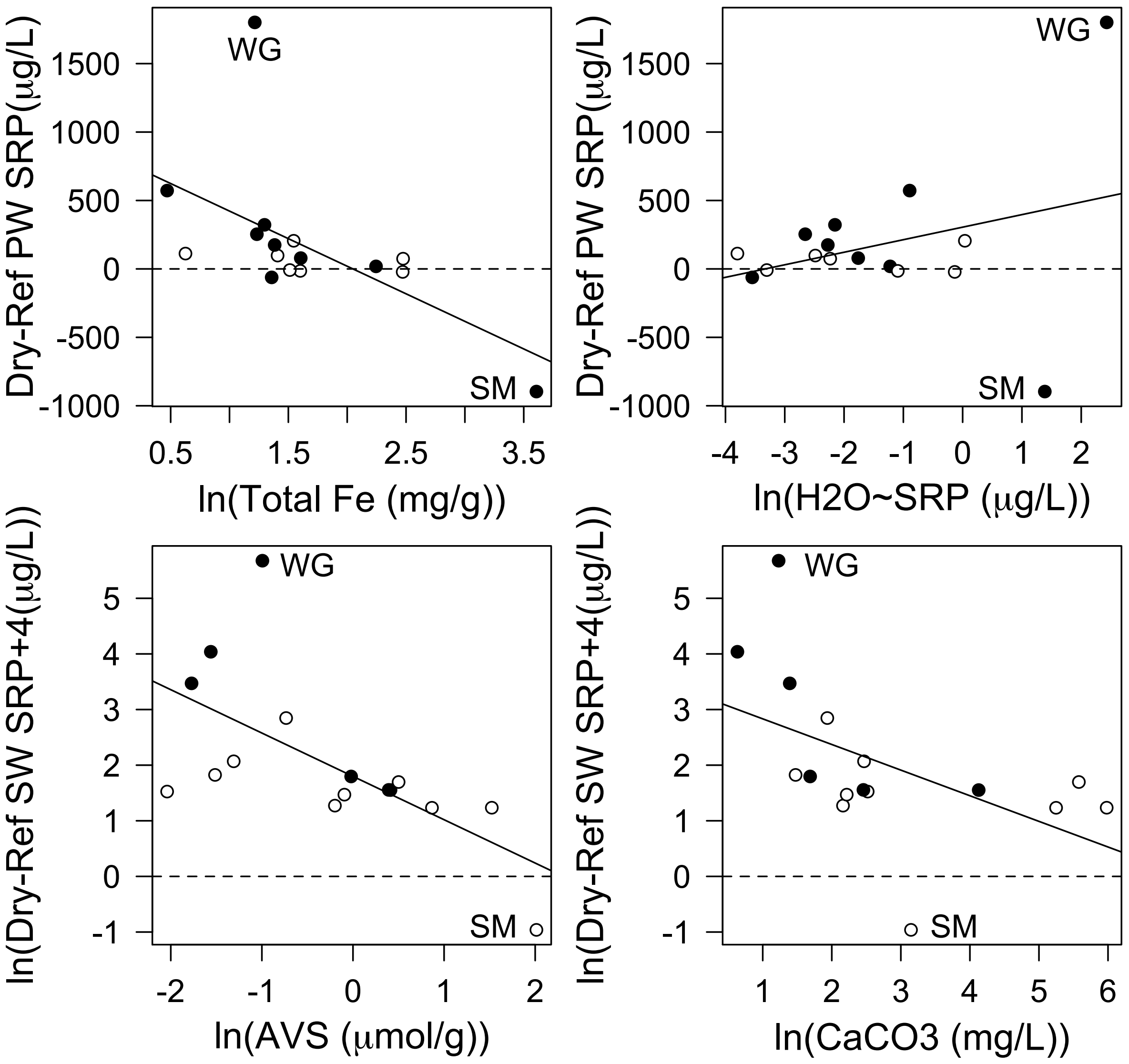

Figure 3.6 Relationships between sediment-water P exchange and sediment characteristics in 16 sediments subjected to hydrologic manipulation in sediment-water microcosms. P exchange response to drying is measured as the difference between average SRP in surface or pore waters (SW, PW, respectively) in dried then re-inundated (Dry) treatments compared to that in continuously inundated (Ref) reference sediments of the same sediment. Solid black circles denote statistically significant differences between hydrologic treatments based on Benjamini & Hochberg's (1995) correction for multiple comparisons, and hollow circles denote no significant difference. Solid line is based on least-squares linear regression model. To best meet linear model assumptions of equal variance and normality of errors, Dry-Ref PW SRP was left untransformed, Dry-Ref SW SRP was ln+4 transformed, and sediment predictors were ln-transformed. Sediments that exhibited extreme responses (WG and SM, see Table 3.2) are labeled.

directional relationships support our hypotheses of how drying influences sediment P biogeochemistry (Figure 3.1, Table 3.1). The univariate model predicting pore water Dry-Reference SRP from Total Fe (negative relationship) was also statistically significant ($p<0.05$), but much less well-supported than the stepwise model (Table 3.7). In surface waters, we identified the best model predicting P release as the univariate model with AVS, and there was a 64% chance that of the four candidate models compared, this model garnered the most support from the data. The relationship between surface water SRP response and AVS was negative, or sediments with high AVS tended to release less P to surface waters when dried and re-flooded compared to when continuously flooded (Figure 3.6). There was also a significant negative relationship between total sediment $CaCO_3$ and surface water P release (Figure 3.6) with an 18% chance that this was the “best” of the four models compared (Table 3.7). In our sixteen sediments, AVS and $CaCO_3$ were significantly positively correlated (Pearson’s $r=0.70$, $p=0.002$). We hypothesized a negative relationship between surface water Dry-Reference SRP and AVS, but hypothesized no relationship with $CaCO_3$, yet the tested models reveal a significant negative relationship (Tables 3.1, 3.7).

Discussion

Biogeochemical characteristics determined the magnitude and direction of P release from dried and re-inundated wetland sediments. Of the sediments that showed significant differences in P release, most released more P in dried and re-inundated treatments than in continuously inundated reference treatments. Sediment characteristics were important in determining the amount of P released to pore and to surface waters. Amounts of sediment total Fe and loosely sorbed P determined the effect of drying on P release to pore waters, whereas sediment AVS and $CaCO_3$ were most important in determining the effect on P release to surface water. Control of sediment PO_4^{3-} release by redox-driven iron sorption is a long-standing paradigm, yet sediment-water P exchange may also be controlled by other processes including co-precipitation and organic matter mineralization, which is likely why we observed variable responses among sediments (Boström et al. 1988, Hupfer and Lewandowski 2008).

Relating Sediment-Water P exchange to Sediment Characteristics

The negative relationship between drying-induced P release to pore water and total sediment Fe supports the hypothesis that drying causes oxidation of Fe(II) below the sediment-water interface and thereby improves sediment P retention by creating Fe(III) sorption sites for PO_4^{3-}. This suggests that in some instances, drying of high-iron sediments may improve their ability to retain P, as has also been suggested elsewhere (Smolders et al. 2006a, 2006b). This result was especially pronounced in sediment from SM, which contained by far the most sediment Fe by all measures and was one of only two sediments in which significantly more P was released to pore waters in Reference than Dry treatments.

We also observed a significant positive relationship between pore water P release and H_2O~P. H_2O~P measures PO_4^{3-} that is “loosely sorbed” to sediments and would include any dissolved PO_4^{3-} in pore water that remained in the moist sediment sample used for sequential P extraction. WG sediment had by far the highest concentration of H_2O~P and its Dry treatment released the highest amount of SRP to both pore and surface waters, but the significant relationship between surface water P release and sediment H_2O~P is robust to omitting the WG data point (not shown). There were several other sediments that were very similar to WG in terms of sediment chemistry (e.g., P23), but responded differently to drying. The high H_2O~P in sediment from WG may indicate that its P sorption capacity is at or near saturation and likely reflects the legacy of P inputs and eutrophication in the sediment's source ecosystem, Wintergreen Lake, which has a long history of heavy waterfowl use as part of the W.K. Kellogg Bird Sanctuary (Manny et al. 1994). Although H_2O~P represents a relatively small proportion of total sediment P, even in sediment from WG (~3%), it is theoretically by far the most reactive sediment fraction, and may explain the substantial P release response to drying in this sediment. However, the mechanism of how drying and re-inundation would influence this binding fraction differentially than continuous inundation remains unclear.

Predictors of surface water P response were different from those of pore water response. In fact, although Fe was the best predictor of pore water P release, the relationships between surface water P release and Fe indicator variables were only marginally significant ($p > 0.06$). Instead, sediment AVS was the best predictor of surface water P response, and P release was also significantly related to $CaCO_3$. The strong negative relationships between surface water P release and both AVS and $CaCO_3$ are confounded due to the strong positive correlation between AVS and total

$CaCO_3$ in our 16 study sediments. Either or both relationships predicting surface water P release from AVS and $CaCO_3$ may be causal, and not simply due to correlation, because 1) we expected drying to oxidize FeS_x (measured as AVS) in sediments, creating new iron oxyhydroxides and increasing sediment PO_4^{3-} sorption and 2) via co-precipitation and/or sorption, $CaCO_3$ may act as a secondary sink for PO_4^{3-} released by other mechanisms during drying (organic matter mineralization, loss of sorption capacity, etc.).

The role of sulfur and FeS_x in wetland sediment P cycling has long been known (Caraco et al. 1989), but the potential for SO_4^{2-} pollution to cause internally driven eutrophication is increasingly being recognized. Wetlands receiving high SO_4^{2-} inputs can become internally eutrophied as SO_4^{2-} enters anoxic wetland sediments, is microbially reduced to H_2S, and reacts with iron to form insoluble FeS_x minerals, decreasing Fe~P binding and leading to PO_4^{3-} release to surface waters (Caraco et al. 1989, Lucassen et al. 2004). Thus, it has been suggested that such wetlands could benefit from temporary drying that oxidizes FeS_x minerals and re-creates iron oxyhydroxide binding sites, thus improving PO_4^{3-} sorption capacity (Lucassen et al. 2005, Smolders et al. 2006b), although the improvement may occur only in well-buffered sediments. In poorly buffered sediments, the oxidation of FeS_x lowers sediment pH and may cause dissolution of calcareous minerals and any PO_4^{3-}

associated with them (Lucassen et al. 2005). In this experiment, significantly higher SO_4^{2-} in surface and pore waters of many Dry treatment sediments compared to the corresponding Reference sediment provides strong evidence that FeS_x oxidation occurred in many of our Dry sediments. Thus, our results provide some evidence that temporary drying may improve sediment P retention capacity via oxidation of FeS_x, but this relationship is confounded in some of the sediments with the presence of $CaCO_3$. Further studies are needed to understand the complex relationships between iron, sulfur, calcium carbonate, and sediment-water P exchange.

Comparisons With Other Studies

Previous studies on the effects of drying and re-wetting on sediment-water P exchange have yielded variable conclusions, just as different sediments responded differently to hydrologic treatment in this experiment. Several experimental studies have shown P release upon re-inundation of dried sediments (Qiu and McComb 1994, Olila et al. 1997, Corstanje and Reddy 2004), yet most of these were conducted on sediments of limited biogeochemical variability. Some studies (Qiu and McComb 1994, Young and Ross 2001, Lucassen et al. 2005, Smolders et al. 2006b, Zak et al. 2010) have investigated effects of drying and re-inundation on sediment-water P flux in sediments of varying characteristics, but wide variability in experimental design and methods makes it difficult to compare across studies.

Although many published studies show P release from sediments to surface waters upon re-inundation of drained or dried sediments (Qiu and McComb 1994, Olila et al. 1997, Corstanje and Reddy 2004), there is evidence that released P originates from both organic and inorganic sediment pools and the relative importance of these sources in diverse sediments remains difficult to predict, due to remaining lack of mechanistic understanding. Organic P can be mineralized during dry phases (Qiu and McComb 1994, Olila et al. 1997, Corstanje and Reddy 2004) and/or released due to microbial cell lysis upon re-inundation (Qiu and McComb 1994). However, we observed no significant relationships between indicators of organic P and P release in our sediments, suggesting that if these processes occurred they were obscured by interactions with processes controlled by other sediment characteristics.

Release of inorganic P, despite potential iron oxidation, may be due to changes in mineralogy. It has repeatedly been shown that drying soils and sediments reduces their inorganic PO_4^{3-} sorption capacity, even under aerobic conditions (Twinch 1987, Schoenberg and Oliver 1988, Qui and McComb 2002). The mechanism behind this effect remains uncertain, although shifts in particle size distribution to larger particles (Twinch 1987, de Vicente et al. 2010) and aging of minerals from amorphous to more crystalline forms (Baldwin 1996, Qui and McComb 2002) have been suggested. Either of these mechanisms would result in a loss of surface –OH groups in iron oxyhydroxides for PO_4^{3-} ions to exchange with, thus decreasing the overall sorption capacity of the sediment and potentially explaining our observation of higher PO_4^{3-} in many temporarily desiccated sediments than the same continuously inundated sediments.

Several studies report higher P release rates than we measured. Most initial P release rates in our study were not significantly different than zero, and even the highest observed release rate (WG, 15 mg P m^{-2} d^{-1} in the Dry treatment) was moderate compared to the range of published results (Table 3.8). Many previous estimates of sediment P release rates were made for sediments that had historically elevated P loads from agricultural activity (Olila et al. 1997, Corstanje and Reddy 2004, Bostic and White 2007). It is likely that areas with a legacy of P enrichment would contain a higher proportion of reactive P than less impacted areas, leading to greater release rates upon re-inundation. Most of our sample sites, although located in a largely agricultural landscape, are only moderately impacted by agricultural P inputs due to the dominance of groundwater flow paths in this landscape.

Several studies have used sediment and/or pore water Fe:P ratios as predictors of an ecosystem's propensity to release P into surface waters. For example, Jensen et al. (1992) found that aerobic shallow lake sediments tended to release P only if their sediment Fe:P molar ratios were below 8.3. Geurts et al. (2008) showed that in fens across Europe, P release occurred where pore water Fe:PO_4^{3-} values were lower than 3.5 and total sediment Fe:P lower than 10. In experimental inundation of historically heavily drained peat, Zak et al. (2010) found that P release rates were significantly related to peat Fe:P molar ratios, predicting net P release when sediment Fe:P is less than 10.

In our study, pre-treatment sediment molar Fe:P ranged from 3.5 to 37. Substantial concentrations of surface water SRP (>10 µg L^{-1}) were observed only upon re-inundating sediments with molar Fe:P ratios less than ~6. However, not all sediments with sediment Fe:P below this "threshold" released P into surface waters

Table 3.8 Published P release rate values for re-flooded sediments in intact cores.

Source	Location	Study Description	P release rate range (mg P m^{-2} d^{-1})
Bostic & White 2007	Florida, USA	Re-inundating marsh sediment	-3.68 - 43
Corstanje & Reddy 2004	Florida, USA	Re-inundating marsh sediment	0.7 - 109
Olila et.al. 1997	Florida, USA	Re-inundating marsh sediment	7.6 - 334.2
Qiu & McComb 1994	Perth, Australia	Air drying and re-inundating littoral lake sediments	37
Zak et al. 2010	Germany and Poland	Re-inundating historically drained fen sediment	0.1 - 52.3
Steinman et al. (unpublished)	Michigan, USA	Re-inundating sediment along moisture gradient from upland to 1 m deep	0.4 - 37.9
This study	Michigan, USA	Experimental drying and re-inundating wetland sediment	0 - 15

(P18, TM, P10, LF), suggesting that something other than sorption to iron minerals retained P below the sediment-water interface in these sediments. Overall, it seems that low Fe:P ratios may be useful in identifying ecosystems that may be vulnerable to P release, but do not necessarily indicate that P will be released from or retained in sediments.

The two sediments that displayed large, sustained P release during re-inundation following drying (FCTC, WG) were collected from ecosystems with a history of higher P loading than the other sediments. FCTC sediments were collected from historically drained agricultural land that was recently re-flooded as part of a wetland restoration (Chapter 2, this dissertation), and WG sediments were collected from riparian areas of hypereutrophic Wintergreen Lake. The sustained increase and high final surface water SRP concentrations in these two sediments, despite constant aeration, suggests that sediments such as these are likely to release P to surface waters regardless of whether they are oxic or anoxic as long as surface water PO_4^{3-} concentrations remain below their relatively high equilibrium phosphorus concentrations (the concentration at which sediments display neither net sorption nor release of P). It also emphasizes that the amount of reactive forms of P, rather than total sediment P or Fe:P ratio, determines the propensity of sediments to release P into surface waters, as these two sediments did not contain the highest total P concentrations by any means. The large amount of released P from these sediments could cause considerable eutrophication in a shallow water column.

Caveats and Conclusions

Our ability to relate lab-scale microcosm studies such as this to whole ecosystems is limited. Oxidation of originally anoxic sediments during pre-experiment homogenization may have lead to an unnaturally high initial P sorption capacity (Hupfer and Lewandowski 2008). In addition, our microcosms lacked many important ecosystem components, most notably photosynthetic plants and algae that would take up available P in natural ecosystems and macroinvertebrates that could alter sediment-water interface conditions via bioturbation and bioirrigation. Furthermore, our decision to use low-nutrient inundation water, while providing the ability to interpret results from a wide variety of sediments, likely led to higher rates of P release than if we had used water with higher PO_4^{3-} concentrations.

Nonetheless, this experiment demonstrates that sediment P retention and release following drying and re-inundation is a highly variable phenomenon that is subject to multiple controls. Although sediment-water P exchange may be controlled by redox-driven iron-PO_4^{3-} sorption, this process in itself is complex due to variable mineralogy of iron solids, which range from poorly crystalline with high PO_4^{3-} sorption capacity to highly crystalline with much lower sorption capacity. In addition, many other processes control sediment P cycling including organic matter storage and mineralization, co-precipitation or dissolution with carbonate minerals, and uptake and release by biota. All of these processes are differentially influenced by drying and consequent changes in oxygen and moisture conditions. Thus, a one-size-fits-all approach to managing wetlands for mitigation of P pollution is unlikely to be successful, and individual wetlands must be evaluated on a case-by-case basis to predict the response of sediment-water P exchange to hydrologic variability.

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CHAPTER 4: NATURALLY OCCURRING CHEMICAL STRESSORS IN UNCONTAMINATED FRESHWATER SEDIMENTS: SULFIDE, AMMONIA, AND IRON

Abstract

Potentially toxic levels of three naturally occurring chemical stressors (sulfide, ammonia, and iron) are prevalent in freshwater sediments, and their roles in shaping ecosystem structure (i.e., plant and animal communities) and function (e.g., biologically mediated elemental cycles) should not be overlooked. Although freshwaters contain far less sulfate than marine waters, sulfate reduction is still significant and the resultant sulfide can exist at toxic concentrations in freshwater sediments. Freshwater sediments often are exceedingly high in ammonium, so even at pH levels where unionized, toxic ammonia is a small fraction of total ammonium plus ammonia, toxic levels to vulnerable organisms like unionid mussels may still exist. Pore water, and sometimes surface water, concentrations of reduced iron can reach levels that may be toxic to wetland plants and animals. The toxic effects of reduced iron—as well as the reoxidation of that iron and formation of smothering floc—are known to constrain wetland plant communities, but their effects on benthic invertebrates are less well-understood. In addition to their roles as toxicants, reactive forms of reduced iron and sulfide can influence the toxicity, fate, and transport of anthropogenic contaminants (e.g. heavy metals, Hg, As). To assess the prevalence of and biogeochemical controls on toxic levels of sediment sulfide, ammonia, and iron, we sampled sediments, pore waters, and surface waters from 42 shallow freshwater locations across 24 shallow (< 2 m) ecosystems in southwest Michigan and compared our measured concentrations to water quality criteria established by the U. S. Environmental Protection Agency (EPA)

and toxic thresholds in the published literature. The benthic environment of almost every freshwater ecosystem we measured was theoretically toxic or stressful to some component of aquatic life in some area or at some time, based on EPA criteria for aquatic life for dissolved sulfide, iron, and ammonia. Organismal tolerances to chemical stressors vary, so the toxicant concentrations that we measured are likely shaping benthic ecological communities and influencing rates of ecosystem function in ways that need to be better understood to provide a context for ecotoxicological studies of other contaminants in fresh waters.

Introduction

Naturally occurring substances in aquatic ecosystems can be just as, or more, toxic to organisms as anthropogenic contaminants. These substances, particularly reduced, dissolved sulfide, iron, and ammonia, tend to accumulate in anoxic sediment pore waters (Ponnamperuma 1972) and overlying water, where they can be toxic to rooted plants (Pezeshki 2001), benthic animals (Vuori 1995, Wang and Chapman 1999, Mummert et al. 2003), and other organisms. Ecotoxicologists typically assess the prevalence of potentially toxic concentrations of naturally occurring chemicals in ecosystems that also contain anthropogenic contaminants. Biogeochemists measure these reduced species in freshwater ecosystems to investigate elemental cycles, but sometimes overlook their potential toxicity and consequent effects on community composition and ecosystem function (Lamers et al. 2012).

The toxic effects of sulfide, ammonia, and iron are variably understood. Dissolved sulfide is highly toxic and reactive, but its prevalence and toxicity to specific organisms, especially in freshwater ecosystems, are poorly understood (Wang and

Chapman 1999). The toxicity of ammonia, on the other hand, is better understood, especially to vulnerable freshwater organisms like unionid mussels (Augspurger et al. 2003, U.S. Environmental Protection Agency 2009). Iron can be toxic at high concentrations, but toxicity investigations have been limited, and at times contradictory (Vuori 1995). Water quality criteria for protection of aquatic life for all three of these species are established by the U.S. Environmental Protection Agency (EPA) (U.S. Environmental Protection Agency 1976, 1986, 2009). While criteria for ammonia, especially with regard to freshwater mussels, is strong and science-based (Augspurger et al. 2003, U.S. Environmental Protection Agency 2009), criteria for sulfide and reduced iron are comparatively weakly supported (Thurston 1979, State of Ohio Environmental Protection Agency 1998, Wang and Chapman 1999).

It is important to understand the role that dissolved sulfide, iron, and ammonia play as toxicants in uncontaminated sediments to assess their potential to confound bioassessments, impede restoration goals, and lead to misuse of remediation resources in areas where natural, rather than anthropogenic, contaminants are the true culprit constraining organismal behavior and survival.

In this paper, we briefly review the literature on these three naturally occurring toxicants, including controls on their concentrations in sediments and water, and then present the results of a survey of their concentrations in diverse freshwater sediments of southwestern Michigan where historical industrial contamination with toxicants is essentially absent, although elevated nutrient concentrations are common. Our objective is to examine the potential for toxic levels of toxicants under natural conditions, and to explore the biogeochemical settings in which toxic levels are observed.

Background

Controls over Environmental Concentrations

Hydrogen sulfide, dissolved iron, and ammonia are all reduced substances that tend to accumulate in sediment pore waters, where their transformation to oxidized, less toxic forms is inhibited by a lack of oxygen (Ponnamperuma 1972). All three chemical species interact with one another and are strongly coupled to other biogeochemical cycles (Lamers et al. 2012). Environmental concentrations of dissolved sulfide, iron, and ammonia are controlled by a combination of geochemistry and biologically mediated transformations, although to some degree entire landscapes are enriched with nitrogen and sulfur from diffuse sources including atmospheric deposition.

The chemical forms and thus the toxicity of hydrogen sulfide, iron, and ammonia depend not only on their concentration, but also on pH. For many toxicants, electrochemically neutral dissolved species tend to be more toxic than charged ions, presumably because they can more easily cross cell membrane lipid layers (Newman and Clements 2008). The dissociation of dissolved species into unionized and ionic components is often strongly controlled by pH. Dissolved free sulfide (ΣH_2S), which is typically measured by colorimetric or gas-chromatographic methods, is the sum of unionized hydrogen sulfide (H_2S), the bisulfide ion (HS^-), and the sulfide ion (S^{2-}). Concentrations of S^{2-} are negligible under most natural environmental conditions (Schoonen and Barnes 1988), and the relative distribution of H_2S and HS^- depends mostly on pH. At pH 7, the fractions of H_2S and HS^- are roughly equal, but at pH 6, the more-toxic H_2S comprises most (about 91%) of total free sulfide (Millero et al. 1988, Wang and Chapman 1999). Ammonium occurs as both the ammonium ion (NH_4^+) and unionized ammonia (NH_3). Unionized NH_3 makes up a higher proportion of total ammonium at higher pH (Emerson et al. 1975), and is typically associated with most of the toxic effects of ammonium. To complicate matters, overall ammonium toxicity is sometimes greater at lower pH regardless of dissociation rates between ammonium's two forms (Thurston et al. 1981). Ammonia and ammonium are typically measured in a colorimetric assay that provides the sum concentration of the two forms, sometimes referred to as total ammonium nitrogen. Hereafter, we will use ΣNH_4^+ to refer to total ammonium nitrogen ($NH_4^+ + NH_3$), NH_4^+ to refer to the ammonium ion, and NH_3 to refer specifically to unionized ammonia. Dissolved iron tends to be more toxic at low pH (e.g., more toxic to mayflies at pH 4.5 than 7, Gerhardt 1992), partially because reduced iron (Fe(II)) is more prominent at low pH than oxidized iron (Fe(III)) (Gerhardt 1992). Oxidized Fe(III) tends to precipitate out of waters, and thus dissolved, reduced Fe(II) is more likely to be encountered and/or be taken up by organisms, where it can have direct toxic effects.

Sulfide is produced in sediment pore waters by microbially mediated organic matter decomposition and dissimilatory sulfate (SO_4^{2-}) reduction (King and Klug 1982, Megonigal et al. 2004, Reddy and DeLaune 2008). Dissolved sulfide is volatile and easily oxidized, and thus ΣH_2S can be highly transient in oxic or shallow waters

(Jorgensen 1977). The transient nature of ΣH_2S as well as methodological difficulties often preclude its systematic measurement at appropriate temporal and spatial scales, especially in fresh waters (Bagarinao 1992). Even in environments where oxygen is not present, numerous processes decrease ΣH_2S concentrations. Sulfide can be transformed to more oxidized, non-toxic forms (e.g., thiosulfate) by oxidized inorganic molecules like nitrate (NO_3^-) or Fe(III) (Jorgensen 1990), dissolved organic matter (Heitmann and Blodau 2006), and organisms with detoxifying adaptations (Hauschild et al. 1999). Radial oxygen loss by plant roots (Azzoni et al. 2001) and bioirrigation by burrowing invertebrates (Goldhaber et al. 1977) can decrease ΣH_2S concentrations in sediment surface layers. Sulfide reacts rapidly with iron to form insoluble iron sulfide complexes, decreasing ΣH_2S concentrations (Wetzel 2001, Morse and Rickard 2004, Rickard and Morse 2005). Despite its transience, accumulation of small amounts of ΣH_2S for short periods of time can have substantial ecological effects due to its high acute toxicity (Evans 1967). In addition, the prevalence of toxic ΣH_2S accumulation may be increasing (Lamers et al. 1998) because SO_4^{2-} concentrations have increased to polluting levels in many freshwater ecosystems for a variety of reasons, including fertilizer use (Orem et al. 2011), atmospheric deposition (Gorham 1976, Shah et al. 2000), and oxidation of geologic iron sulfide during drought, wetland drainage, and by increasing groundwater nitrate loading (Bates et al. 2002).

Ammonium concentrations are controlled by microbially mediated redox transformations that produce and consume ΣNH_4^+, and by uptake by plants and microorganisms. Mostly originating from organic matter decomposition, ΣNH_4^+ concentrations can reach exceedingly high values in organic, high-nutrient sediments. Ammonium tends to accumulate in anoxic pore waters where the lack of oxygen prevents its nitrification to NO_3^- and organismal uptake is lower, but is frequently also measurable in oxygenated surface waters albeit at lower concentrations (Frazier et al. 1996, Wetzel 2001). The toxic effects of NH_3 have largely been studied in areas contaminated with sewage and other organic pollution, and ΣNH_4^+ is often identified as the main toxicant even in sediments containing many novel anthropogenic contaminants (Ankley et al. 1990). In addition to point-source organic pollution, widespread non-point source inorganic N pollution from agricultural fertilizer use and industrial atmospheric deposition may contribute to increased incidences of ΣNH_4^+ toxicity (Camargo and Alonso 2006).

Reduced Fe(II) is a prevalent form at low pH and low redox potential (Reddy and DeLaune 2008), and the concentration of dissolved Fe(II) in the environment is strongly controlled by microbial oxidation and reduction (Lovley 1993). Fe(II) is produced in sediments after oxygen has been consumed and iron-reducing bacteria transform Fe(III) to Fe(II) in anaerobic respiration. Iron reduction can represent a substantial proportion of total anaerobic respiration and organic matter mineralization in freshwater wetland sediments (Whitmire and Hamilton 2008). The mineralogy and crystallinity of iron solids in sediments strongly influences concentrations of dissolved Fe(II). Solid iron exists in forms ranging from highly structured and crystalline minerals to less crystalline, poorly ordered “amorphous gels.” Iron-reducing bacteria can more readily transform the "amorphous," poorly crystalline iron oxides than the more structured, crystalline iron oxides to the reduced Fe(II) form (Lovley and Phillips 1986). If sufficient ΣH_2S is present, its reaction with iron to yield insoluble iron sulfides can deplete pore water iron concentrations (Wetzel 2001). Iron pollution is typically caused by mining and industrial activities, but iron can also reach naturally high concentrations in areas with the iron-rich geology. Iron concentrations also can increase where historically flooded areas are drained for agricultural and other uses, through the oxidation of iron sulfides that had accumulated under anoxic conditions (Vuori 1995).

Interactions with other toxicants

Sulfide, iron, and ammonia are not only directly toxic in their own right, but all three influence general sediment toxicity by interacting with one another and other toxicants. Sulfide toxicity can diminish plant ΣNH_4^+ uptake, increasing ΣNH_4^+ concentrations and potentially NH_3 toxicity (Koch and Mendelssohn 1989). Iron and ΣH_2S essentially detoxify one another if present in the proper ratios due to their formation of insoluble iron sulfides in reduced sediments (van der Welle et al. 2006).

Iron and ΣH_2S also both play important roles in determining the toxicity of heavy metals, particularly arsenic and mercury. Sulfide is well known to reduce overall sediment toxicity by binding with heavy metals (such as nickel and cadmium) in anoxic sediments (Di Toro et al. 1990). Arsenic (As) sorbs to iron oxides under oxic conditions and co-precipitates with ΣH_2S under high-ΣH_2S reducing conditions, leading to complex, spatially and temporally heterogeneous interactions between these three toxicants, which control one another's bioavailability (Smedley and Kinniburgh 2002, O'Day et al. 2004). Arsenic desorption from iron oxides under reducing conditions is one of the main causes of large As "release" events, which are of particular concern in anoxic aquifers that supply drinking water (Smedley and Kinniburgh 2002).

Mercury in various forms associates both with Fe(III) and ΣH_2S, and both iron and sulfur play direct roles in biogeochemical mercury transformations (Morel et al. 1998, Ullrich et al. 2001). Most notably, SO_4^{2-} reducing bacteria have been implicated in the formation of methylmercury (Gilmour and Henry 1991, Coleman Wasik et al. 2012), which is the form of mercury that is the most toxic, bioaccumulates in organisms, and can persist in the environment. Thus, situations that are conducive to toxic ΣH_2S concentrations may also enhance mercury toxicity.

Ammonium does not react geochemically with other toxicants as conspicuously as iron and sulfur, but it plays an important role as a plant nutrient. The addition of ΣNH_4^+ to a nitrogen-limited system potentially enhances its overall rates of photosynthesis and respiration, indirectly stimulating heterotrophic microbial activity and increasing an ecosystem's propensity for sediment and bottom-water anoxia, which influences the partitioning and bioavailability of all redox-sensitive toxicants (Camargo and Alonso 2006). Excess ΣNH_4^+ in oxic water is converted by bacterial nitrification to NO_3^-, a chemolithoautotrophic process that consumes oxygen and can also contribute to oxygen depletion in sediments and water (Wetzel 2001, Cébron et al. 2003).

Sensitivity of Aquatic Biota

Variable organismal tolerances to stressors are well-known, and are often taken advantage of in bioassessment, in which managers use benthic community composition as an indication of ecosystem health (Kerans and Karr 1994). However, sensitive organisms are also often limited by high concentrations of naturally occurring chemical stressors, even in uncontaminated environments lacking anthropogenically produced toxicants. In naturally extreme environments, such as deep-sea hydrothermal vents, hot springs, and groundwater seep sinkholes, evolved adaptations to toxic conditions have led to highly unique and specialized communities (Somero et al. 1989). Even in environments with moderately toxic conditions, such as salt marshes, variable organismal tolerances and high concentrations of natural stressors shape animal and plant communities (Ingold and Havill 1984, Geurts et al. 2009).

Organisms can have physiological, structural, and/or behavioral adaptations that allow them to cope with ΣH_2S, dissolved Fe(II), and ΣNH_4^+ exposure, although these adaptations are poorly understood for most taxa and toxicants. Many of these adaptations involve transforming reduced, toxic species into more benign oxidized forms, either by altering the oxygen conditions in their immediate environment or taking up reduced substances and transforming them to less toxic substances. Most wetland plant species have adapted to low-oxygen, phytotoxic flooded conditions by evolving aerenchymal tissue that transports oxygen to the roots (Ernst 1990). Wetland plant roots leak oxygen to varying degrees (Armstrong 1980, Armstrong and Armstrong 1990), allowing for oxidation of toxic reduced species. Many benthic animals either passively or actively increase movement of oxygenated surface water into the sediments by bioirrigating burrows at the sediment-water interface (Gallon et al. 2008). These and other adaptations are limited in their ability to detoxify in conditions of high toxicant concentrations, however, and sediments high in ΣH_2S, dissolved Fe(II), and/or ΣNH_4^+ may support fewer species than similar sediments with lower concentrations of these toxicants.

Altering Ecosystem Processes

In addition to shaping plant & animal communities, the toxic effects of natural stressors directly and indirectly alter ecosystem processes. For example, sulfide interferes with enzyme function, and thus can directly inhibit many microbially mediated biogeochemical transformations, such as shifting the product of nitrate reduction from N_2 gas (denitrification) to ammonium (DNRA) by inhibiting NO-and N_2O-reductases (Brunet and Garcia-Gil 1996). High concentrations of natural stressors may also select for plant species with high rates of root radial oxygen loss (Smolders and Roelofs 1996) and increase rates of bioirrigation (Miron and Kristensen 1993), greatly altering redox conditions at the sediment-water interface and in shallow sediment, and influencing sediment-surface water solute exchange. The effects of ΣH_2S, dissolved Fe(II), and ΣNH_4^+ toxicity on biogeochemical processes are seldom recognized, but could be especially important in wetlands where organic matter inputs and/or production are high and pore and surface waters frequently experience anoxia.

Study Objectives

The objective of this study was to assess the prevalence of and biogeochemical controls on toxic levels of ΣH_2S, dissolved Fe(II), and ΣNH_4^+ in a biogeochemically and ecologically diverse set of fresh water ecosystems. We sampled sediments, pore waters, and surface waters from 42 locations across 24 uncontaminated, shallow freshwater bodies in southwestern Michigan and compared our measured concentrations to EPA-established water quality criteria as well as toxic thresholds in the literature.

Methods

Within each water body, we sampled one to three locations that represented hydrologically and/or chemically distinct areas. Of the 42 locations sampled, we simultaneously sampled the full suite of pore water, surface water, and sediment variables of interest in 27 locations.

Site Selection

Lakes, wetlands and rivers were sampled in Kalamazoo and Barry counties of southwestern Michigan, in a glacial landscape with deciduous forest, row-crop agriculture, and low-density residential development. To represent as wide a range of biogeochemical variability as possible, we chose to sample water bodies along a gradient of water sources from nearly 100% groundwater to a dominance by precipitation, as indicated by magnesium ion (Mg^{2+}) concentrations, which serve as a conservative tracer for groundwater in this region of Michigan (Table 4.1) (Whitmire and Hamilton 2005). We sampled in shallow (< 2 m deep) waters, either by wading or from a canoe, in late summer (July-October) in 2009 and 2010. Thus, deeper lakes were sampled in littoral zones. In some water bodies, we sampled multiple locations along known hydrochemical gradients.

We also sampled surface and pore waters monthly for two years at five locations in a recently restored wetland. The Fort Custer Area 2 Wetland was restored in 2008 by by re-flooding a historically drained 22-acre area. We sampled surface and pore waters in three newly flooded areas (Wetlands 1, 2, and 3) and two historically flooded drainage ditches (Ditches 1 and 2) approximately monthly for two years following the September 2008 re-flooding event. Throughout our sampling period, pore water ΣH_2S concentrations were low or below detection limit, so we only report time series of Fe(II) and ΣNH_4^+ measurements.

Field Sampling

At each location, to minimize disturbance and contamination, we sampled surface water, then pore water, and finally sediments. If the water column was well mixed (as indicated by temperature and dissolved oxygen), we measured surface water variables at a single location and from a single grab sample to represent the surface water chemistry of all sediment sampling locations within a site. We used a Hydrolab multisonde to measure temperature, dissolved oxygen, pH, and conductivity near the surface, above the bottom of the sediments, and at mid-depth (if total depth >1 m) in surface water. We collected a surface water sample from mid-depth to measure soluble

Table 4.1 Natural chemical stressor survey sampling site information, including number of locations sampled (No.) within each ecosystem and whether or not surface water (SW), pore water (PW), and sediment (Sed) chemistry were measured. All samples were collected in water of 0.5-2 m depth in late summer (July-October). Sites are located in Universal Transverse Mercator Zone 16. Percent groundwater influence (GW%) was calculated from magnesium (Mg^{2+}) concentrations (Whitmire and Hamilton 2005) using a mixing model assuming groundwater and precipitation represent the only inputs of dissolved Mg^{2+} to the ecosystems and using Mg^{2+} concentrations of 0.05 mg L^{-1} for precipitation (1979-2002 NADP/NTN 2003) and 23 mg L^{-1} for groundwater (Kalamazoo County mean). The groundwater percentage exceeding 100% likely reflects spatial variability in groundwater in the watershed of the Kalamazoo River.

Ecosystem	**Type**	**No.**	**Year(s) Sampled**	**Easting**	**Northing**	**Cond (µS cm^{-1}, 25°C)**	**GW %**	**SW**	**PW**	**Sed**
LTER Kettle Pond	Pond	1	2010	0633783	4696919	32	5	x	x	x
Lux Arbor Pond 10	Pond	1	2009	0626455	4703201	27	7	x	x	x
Lux Arbor Pond 26	Pond	1	2009	0626220	4703702	72	13	x	x	x
Lux Arbor Pond 6	Pond	1	2009	0625855	4703265	105	21	x	x	x
Lower Crooked Lake	Lake	1	2009	0626394	4702941	265	41	x	x	
Fort Custer Area 2 Wetland	Wetland	2	2009, 10	0641163	4687408	289	50	x	x	x
Lux Arbor Pond 23	Pond	1	2009	0627091	4705624	290	55	x	x	x
Middle Crooked Lake	Lake	2	2010	0627670	4704862	388	57	x	x	x
Eagle Lake	Lake	1	2009	0638009	4687350	388	76			x
Sheriffs Marsh	Wetland	2	2010	0637578	4695697	436	83	x	x	x
Jackson Hole Lake	Lake	2	2009	0635460	4685882	371	86			x
Wintergreen Lake	Lake	2	2009	0633089	4695399	362	87	x	x	x
Whitford Lake	Lake	2	2009	0635404	4685816	419	88			x
Turkey Marsh	Wetland	2	2009	0631750	4696366	439	89	x	x	x
Douglas Lake	Lake	1	2009	0634596	4690688	447	92	x	x	x
Kellogg Forest Pond	Pond	3	2009, 10	0635540	4691393	501	95	x	x	x
Gull Lake	Lake	2	2010	0631307	4696133	353	97	x	x	x
Kalamazoo River	River	3	2010	0617028	4692581	667	102	x	x	

Table 4.1, Continued

Ecosystem	**Type**	**No.**	**Year(s) Sampled**	**Easting**	**Northing**	**Cond (µS cm^{-1}, 25°C)**	**GW %**	**SW**	**PW**	**Sed**
Loosestrife Fen	Wetland	2	2009	0634931	4691989	313	103	x	x	x
Gun River	River	1	2010	0616295	4705546	525	103	x	x	x
Bellingham Drain	Ag. Drain	2	2010	0616354	4705575	586	104	x	x	x
Augusta Creek	Stream	1	2010	0635416	4691719	525	107	x	x	x
Three Lakes	Lake	3	2010	0628761	4689661	602	116	x	x	x
Windmill Pond	Pond	3	2009	0631599	4695837	467	118	x	x	x

reactive phosphate (SRP), total phosphorus (TP), ΣNH_4^+, NO_3^-, SO_4^{2-}, calcium (Ca^{2+}), and Mg^{2+}.

After sampling surface waters, we sampled pore water from a known depth beneath the sediment-water interface using a plastic syringe and tubing connected to a screened filter at the end of a drive-point sampler. To account for fine scale spatial heterogeneity in pore water chemistry, we withdrew and combined pore waters from at least three arbitrarily selected locations from within a 1 x 1 m area to obtain a composite 60 mL sample. We filtered (0.45 µm) pore water samples and added reagents in the field to analyze dissolved Fe(II) and ΣH_2S, and transported filtered samples to the lab to analyze SRP, TP, ΣNH_4^+, NO_3^-, SO_4^{2-}, Ca^{2+}, and Mg^{2+}. We measured pore water pH in the field, excluding gas exchange from the sample by injecting a small amount of pore water from a syringe into tubing over a pH electrode (Orion Combination pH Electrode, Thermo Scientific).

In most locations, a single sample was collected, integrating pore water from ~5-15 cm below the sediment-water interface, but at seven locations (Three Lakes, 3L1, 3L2, 3L3; Augusta Creek, AC; Kellogg Forest Pond, KFP3; Sheriffs Marsh, SM1, SM2) where the sediment-water interface was not well defined, pore water was also sampled from within the loose, flocculent top sediment layer by the method described above (3L1-3) or by centrifugation of a sample containing sediment and pore water (AC, KFP3, SM1, SM2). Deeper pore water samples were also collected at the Three Lakes locations (3L1-3, 80 cm below the sediment-water interface) and Augusta Creek (AC, 40 cm). Because of sample loss, we did not measure pore water chemistry at Eagle Lake (EL), Jackson Hole Lake (JH), and Whitford Lake (WL).

After sampling pore water, we took 3 or more cores (inner diameter = 4.6 cm) from within each 1 x 1 m location. We removed any un-decomposed plant material or benthic algae from the core's surface, extruded and discarded the top 2 cm of sediment, and retained sediments from 2-12 cm below the sediment surface, homogenizing multiple cores (3-5) to obtain a composite sample. We extruded cores directly into plastic bags, squeezed out air to minimize oxygen exposure, homogenized sediments by hand, and immediately froze sediments for later chemical analyses of organic matter by loss on ignition (OM), total sediment phosphorus (sedTP), total sediment iron (TFe), oxalate-extractable iron (Ox-Fe), total sediment carbonates ($CaCO_3$), and acid volatile sulfides (AVS). Sediment from the loose, flocculent layer was also sampled at the Kellogg Forest Pond (KFP) and Sheriff's Marsh 1 (SM1) locations by collecting a sediment-water slurry from approximately the top 10 cm of loose sediment using a large syringe with the center of the tip bored out to prevent clogging. Flocculent sediment was separated from pore water by centrifugation and frozen for later analyses of the above chemical characteristics. We measured bulk density of the cored sediments by subsampling homogenized sediment into a container of known volume in 2009 and by sampling and drying an extra core of known volume in 2010.

Chemical Analyses

We measured SRP concentrations using the molybdate blue colorimetric method (Murphy and Riley 1962), and NO_3^-, SO_4^{2-}, Ca^{2+}, and Mg^{2+} ions using membrane-suppression ion chromatography. We measured ΣNH_4^+ using the indophenol blue method (Grasshoff et al. 1983, Aminot et al. 1997) and long-pathlength spectrophotometry. We measured TP after persulfate digestion of unfiltered surface water samples followed by colorimetric SRP analysis.

We measured dissolved Fe(II) in pore waters using reaction with ferrozine based on a method modified from Lovley and Phillips (1987) and Stookey (1970), in which the pore water sample was immediately filtered (0.45 µm) and added to a solution of 50 mM HEPES buffer containing ferrozine (1 g L^{-1}). After color formation, we measured sample absorbance on a spectrophotometer at 562 nm. Iron concentrations lower than 0.2 µM were not detected, and any value lower than this was replaced with this value for data analysis. We measured ΣH_2S using the methylene blue spectrophotometric method (Golterman and Clymo 1969). ΣH_2S concentrations lower than 0.24 µM were not detected, and any value lower than this was replaced with a value of 0.03 µM for data analyses.

To prepare frozen sediment for measurement of OM, sedTP, TFe, and $CaCO_3$, we thawed the sediment and dried a sub-sample of moist sediment to a constant weight and homogenized it with a mortar and pestle. We then combusted triplicate sub-subsamples (~1 g d.w.) of dried and ground sediment for analysis of OM as loss on ignition (550°C). We extracted sedTP, TFe, and TAl from combusted samples using a 10 minute exposure to boiling 1 M hydrochloric acid (HCl) (Andersen 1976). To measure Ox-Fe, an indicator of poorly crystalline iron minerals, we extracted ~0.4 g moist sediment in a 0.2 M acid ammonium oxalate solution for 4 hours in darkness (Walbridge 1991). Phosphorus, iron, and aluminum were measured in HCl extracts and iron in oxalate extracts using inductively coupled plasma atomic emission spectrometry (EPA Method 6010B) by A&L Great Lakes Laboratories (Fort Wayne, IN).

We measured $CaCO_3$ in triplicate sub-samples of dried and ground sediment (0.1-1 g d.w.) by acidifying in a sealed container, measuring carbon dioxide produced using a pressure transducer, and calculating the original carbonate concentration using the Ideal Gas Law. We separately froze approximately 100 g of moist homogenized sediment for analysis of AVS. AVS was later measured following EPA Method 821-R-91-100 by acidifying sediment samples with HCl to convert AVS to ΣH_2S, which was then trapped as S^{2-} in an alkaline solution (0.5 M NaOH) and measured colorimetrically after reaction with a mixed diamine reagent (H_2SO_4, N,N-dimethyl-p-phenylenediamine oxalate, and ferric chloride hexahydrate) (Allen et al. 1991). Analytical sulfide standards were prepared from a stock solution standardized versus thiosulfate.

Assessing Potential Toxicity

The toxicity of ΣNH_4^+ and ΣH_2S depend on their partitioning into ionized and unionized species. We used equations from Emerson et al. (1975) and Millero et al. (1988) to estimate the partitioning of total ammonium nitrogen into NH_4^+ and NH_3 and the partitioning of ΣH_2S into H_2S and HS^-, respectively, based on pH measured in pore waters and temperature measured in overlying surface waters. In seven pore water samples, pH was not directly measured (Gull Lake, GL1, GL2; Fort Custer Area 2

Wetland, FCTCUNA10, FCTCRL10; Kellogg Forest Pond, KFP-Floc, KFP3-10) and we assumed a pH of 7 (mean pH of measured pore water samples was 7.07).

To assess the potential toxicity of our measured concentrations of ΣH_2S, dissolved Fe(II), and ΣNH_4^+, we compared our measured values to EPA water quality criteria and to values in published toxicological studies of benthic invertebrates and wetland plants. EPA criteria correct measured ΣNH_4^+ concentrations for pH and temperature (U.S. Environmental Protection Agency 2009). These data do not represent an exhaustive list of published toxicological studies, but are meant to provide a sense of the potential for our measured concentrations to influence ecosystem communities and function.

Data Analyses

To detect patterns between simultaneously measured pore water, surface water, and sediment parameters and provide insight into biogeochemical conditions leading to high levels of natural chemical stressors in the benthos, we determined correlation coefficients (Pearson's r) between measured variables. Each bivariate correlation contained a slightly different set and amount of sample points because of imbalances in the data set, ranging from 33-52 sample points. Unless otherwise stated, all variables were natural log or arcsine square root (OM) transformed to better meet linear model assumptions prior to statistical analyses.

Results

Concentrations of ΣH_2S, dissolved Fe(II), and/or ΣNH_4^+ exceeded EPA criteria for aquatic life in most of the pore waters we sampled (Figure 4.1). Out of 54 pore water samples, 11 contained concentrations of ΣH_2S, dissolved Fe(II), and ΣNH_4^+ that were all simultaneously higher than EPA criteria. Only seven out of 54 pore water samples were “non-toxic”, in which neither ΣH_2S, Fe(II), nor ΣNH_4^+ concentrations exceeded EPA criteria.

Sulfide

Pore water ΣH_2S ranged from below detection limit to 98 μM (3,100 μg S L^{-1}), with an average of 5.37 μM (172 μg S L^{-1}). Out of 54 pore water samples measured, 40 (74% of samples) contained ΣH_2S concentrations higher than the EPA criterion for freshwater of 0.06 μM (2 μg H_2S L^{-1}) (Figure 4.2) (U.S. Environmental Protection Agency 1986). When the proportion of the more toxic unionized H_2S is estimated, all detectable H_2S concentrations still exceed the EPA criterion for aquatic life (Figure 4.2). Pore water ΣH_2S concentrations were weakly related to most other pore water, surface water, and sediment characteristics (Table 4.2). However, ΣH_2S and dissolved Fe(II) were inversely related ($p=0.092$, $r=-0.23$) (Figure 4.3), such that high ΣH_2S (> 20 μM) never co-occurred with high dissolved Fe(II) (> 200 μM) in the same pore water sample. Pore water ΣH_2S measurements were significantly negatively correlated with

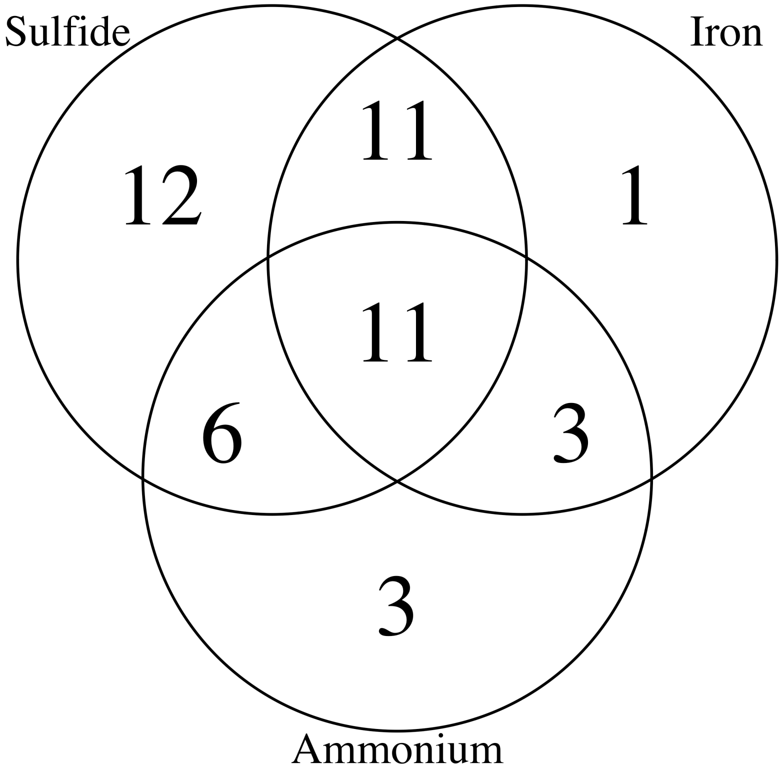

Figure 4.1 Venn diagram illustrating the number of pore water samples taken from 24 shallow (< 2 m deep) freshwater ecosystems in which free hydrogen sulfide, dissolved iron, and/or total ammonium nitrogen were higher than EPA criteria for aquatic life. The chronic threshold for areas where freshwater mussels are present was calculated for ammonium using surface water temperature and measured pore water pH according to the EPA formula (Agency 2009). Out of 54 total pore water samples, only 7 had concentrations below all three criteria.

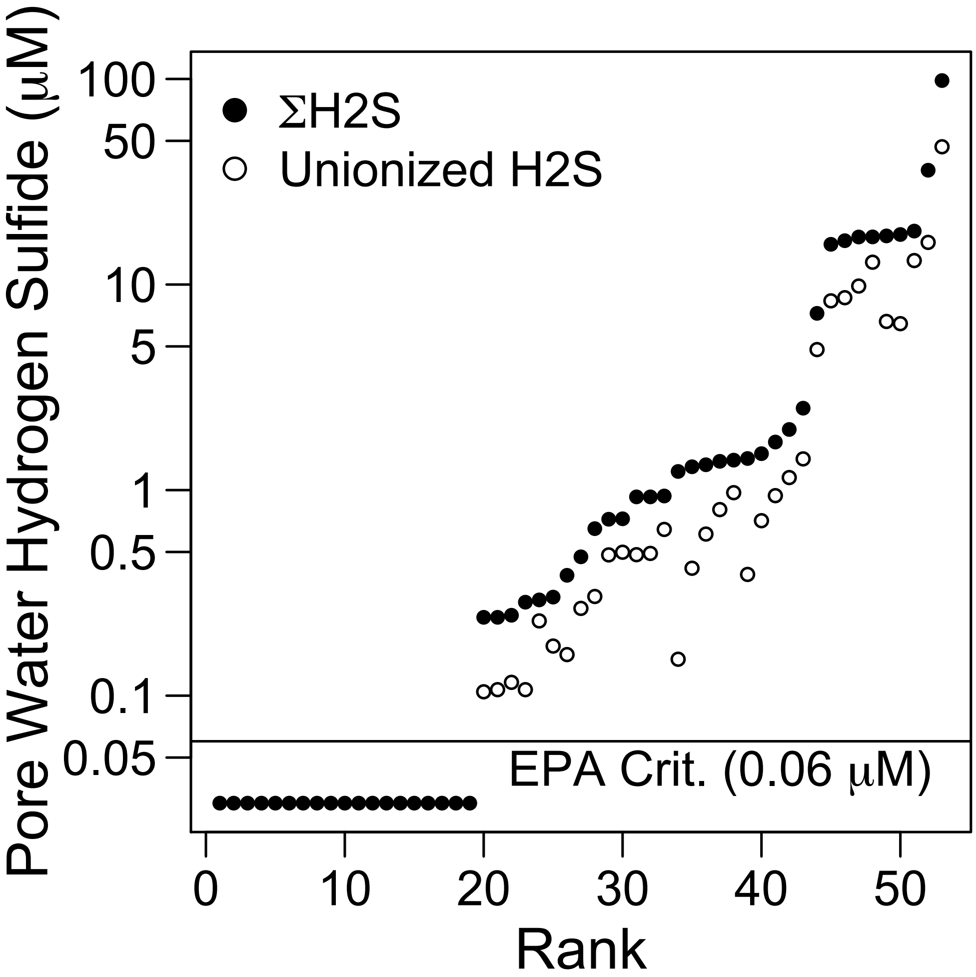

Figure 4.2 Dissolved free hydrogen sulfide (ΣH_2S) concentrations measured in late summer in 54 sediment pore water samples from 24 shallow (< 2 m) freshwater ecosystems and unionized hydrogen sulfide (H_2S) concentrations calculated from simultaneous pH and temperature measurements, assuming a salinity of 0 (Millero et al. 1988). Values < 0.1 µM are non-detects. The horizontal line represents the EPA water quality criterion for aquatic life of 0.06 µM (2 µg H_2S L^{-1}). Note log-scale on the y axis.

Table 4.2 Correlation coefficients (Pearson's r) between pore water dissolved free sulfide (ΣH_2S), dissolved reduced iron (Fe(II)), and total ammonium nitrogen (ΣNH_4^+) and sediment acid volatile sulfide (AVS) and pore water (PW), surface water (SW), and sediment (Sed) characteristics of shallow (< 2 m deep) freshwater ecosystems. Significant correlation coefficients ($p < 0.05$) are in bold, nearly significant ($0.05 < p < 0.1$) are italicized, and the number of data points for each correlation is in parentheses. See text for abbreviations.

	PW ΣH_2S	PW Fe(II)	PW ΣNH_4^+	Sed AVS
PW				
pH	-0.02 (44)	*-0.29 (51)*	**-0.37 (39)**	*0.28 (38)*
ΣH_2S	-	*-0.23 (51)*	0.12 (45)	0.09 (42)
Fe(II)	*-0.23 (51)*		**0.54 (46)**	**0.31 (43)**
SRP	0.08 (46)	**0.43 (47)**	**0.59 (46)**	0.24 (38)
ΣNH_4^+	0.12 (45)	**0.54 (46)**		0.24 (37)
NO_3^-	-0.22 (46)	-0.24 (47)	**-0.43 (46)**	**-0.49 (38)**
SO_4^{2-}	-0.14 (46)	**-0.40 (47)**	**-0.48 (46)**	0.05 (38)
Ca^{2+}	0.15 (45)	0.20 (46)	0.16 (45)	**0.42 (37)**
Mg^{2+}	0.13 (46)	0.14 (47)	0.13 (46)	**0.52 (38)**
SW				
Temperature	**-0.31 (51)**	-0.15 (52)	-0.17 (46)	-0.13 (43)
Conductivity	0.08 (51)	0.16 (52)	0.01 (46)	**0.43 (43**)
pH	0.47 (51)	**-0.29 (52)**	-0.17 (46)	0.01 (43)
Dissolved O_2	0.00 (51)	**-0.32 (52)**	**-0.29 (46)**	-0.06 (43)
SRP	-0.16 (46)	**0.38 (47)**	**0.31 (46)**	0.18 (38)
TP	**-0.27 (51)**	0.23 (52)	0.18 (46)	-0.11 (43)
ΣNH_4^+	-0.12 (46)	**0.45 (47)**	0.06 (46)	**0.47 (38)**
NO_3^-	0.17 (46)	-0.01 (47)	-0.11 (46)	0.16 (38)
SO_4^{2-}	0.20 (46)	-0.22 (47)	-0.15 (46)	**0.38 (38)**
Ca^{2+}	0.21 (46)	0.10 (47)	0.08 (46)	**0.52 (38)**
Mg^{2+}	0.20 (51)	0.07 (52)	0.05 (46)	**0.43 (43)**
Sed				
Bulk Density	-0.02 (38)	**-0.31 (39)**	*-0.31 (33)*	**-0.57 (39)**
OM	-0.09 (39)	**0.45 (40)**	**0.53 (34)**	**0.49 (38)**
sedTP	-0.17 (39)	**0.34 (40)**	**0.52 (34)**	**0.50 (38)**
TFe	-0.18 (39)	**0.33 (40)**	0.21 (34)	**0.64 (38)**
Ox-Fe	-0.26 (39)	**0.46 (40)**	0.22 (34)	**0.61 (38)**
$CaCO_3$	0.18 (38)	-0.16 (39)	-0.08 (33)	0.23 (37)
AVS	0.09 (42)	**0.31 (43)**	0.24 (37)	-

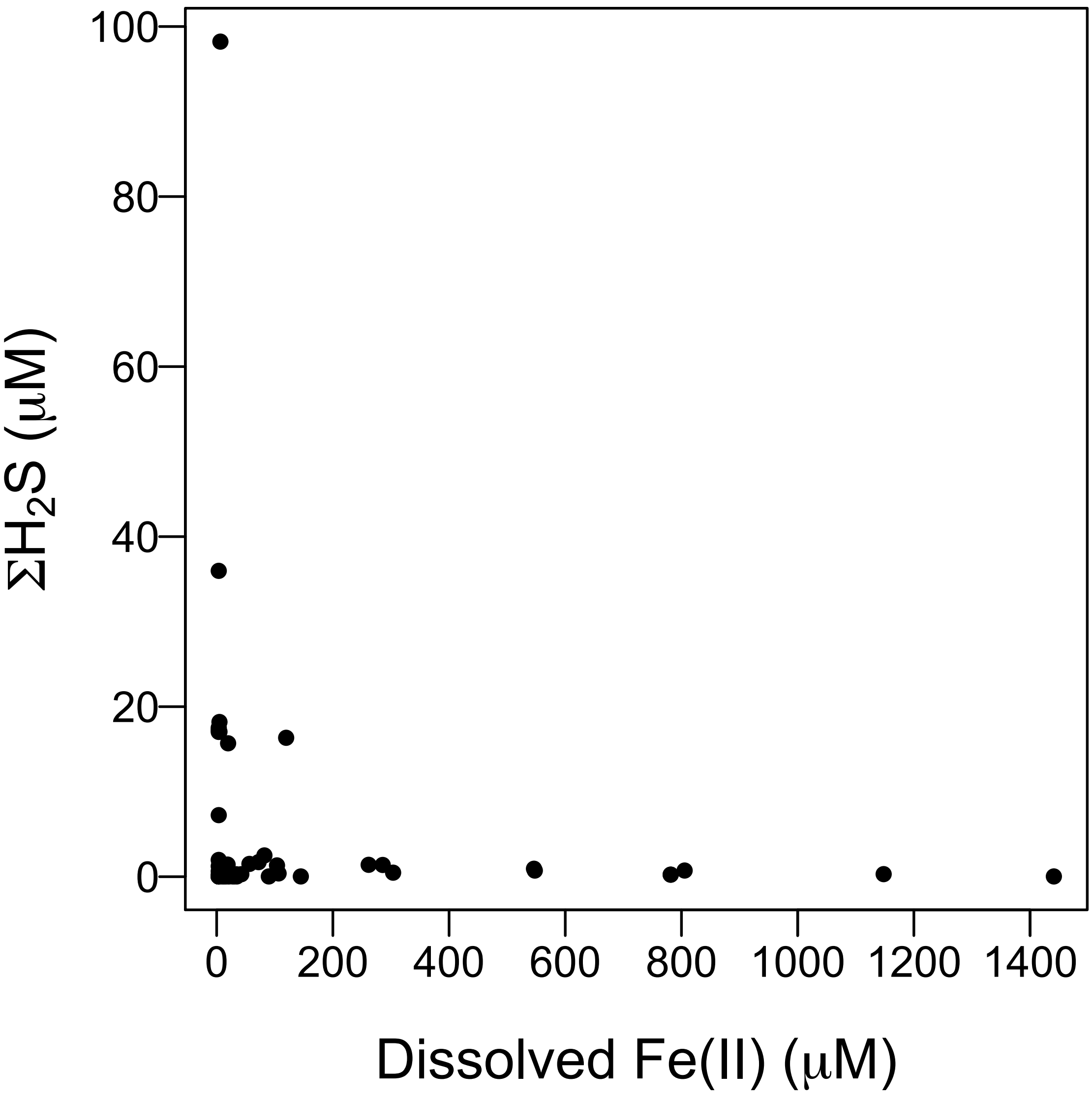

Figure 4.3 Dissolved free hydrogen sulfide (ΣH_2S) and dissolved reduced iron (Fe(II)) measured in late summer in 54 sediment pore water samples from 24 shallow (< 2 m deep) freshwater ecosystems.

surface water temperature and surface water total P (Table 4.2), although the negative relationship with surface water temperature is not strong (p=0.026, r=-0.31), and the negative relationship with surface water total P is driven largely by a single point (SM2) with very high surface water TP and pore water ΣH_2S below detection limit.

Although pore water ΣH_2S concentrations were conspicuously unrelated to any measured solid sediment variables (Table 4.2), sediment AVS, operationally defined as sulfide that is volatilized when treated with acid, may provide more insight into general sediment sulfur biogeochemistry than the more transient ΣH_2S. AVS chemical constituents are complex and have rarely been quantified individually, but are thought to include a variety of metastable metal sulfide minerals, mostly consisting of iron monosulfide aqueous clusters and solids which form due to the strong and rapid reaction between iron and sulfide in reduced sediments, as well as largely unknown amounts of other sulfur constituents including polysulfides and organic sulfur molecules (Wetzel 2001, Morse and Rickard 2004, Rickard and Morse 2005). We did not separate pore water from sediment prior to AVS analysis by centrifugation or filtration, but the amount of dissolved ΣH_2S measured in pore waters sampled in situ was typically much lower than AVS measured in sediments at each location. Dissolved ΣH_2S represented up to 5% of the AVS, except in one location (MCL2) with low AVS (0.08 µmol S g^{-1} d.w.) in which dissolved ΣH_2S represented about 36% of AVS.

In uncontaminated sediments with low amounts of other metals (e.g., Cu, Zn, etc.) such as the ecosystems studied here, iron and sulfide precipitate in roughly equimolar ratios to form iron sulfides. Thus, we predicted that we would only detect free dissolved ΣH_2S in sediments where reduced S (as indicated by AVS) exceeds Fe on a molar basis. In most of the sediments we sampled, poorly crystalline iron greatly exceeded AVS, yet we still sometimes measured ΣH_2S in pore waters of these high iron sediments (Figure 4.4). We detected ΣH_2S at high levels (>10 µM) in sediments where iron exceeded AVS by 1 to 2 orders of magnitude (about 10-100 moles per gram dry weight). AVS was significantly positively correlated with pore water dissolved Fe(II), Ca^{2+}, and Mg^{2+}, surface water conductivity, ΣNH_4^+, SO_4^{2-}, Ca^{2+}, and Mg^{2+}, and sediment OM, sedTP, TFe, and Ox-Fe, and was negatively correlated with pore water NO_3^- and bulk density (Table 4.2).

Few studies have assessed sulfide toxicity to freshwater benthic macroinvertebrates, which are vulnerable to sulfide exposure due to their proximity to low oxygen conditions at and around the sediment-water interface. Invertebrates vary in their tolerance to ΣH_2S, and some can be highly vulnerable to sulfide exposure (Table 4.3). Ninety-six hour LC_{50} values for benthic aquatic invertebrates range from 0.62 µM (*Baetis* mayfly) to 33 µM (*Asellus* isopod) (Oseid and Smith 1974). The “maximum safe

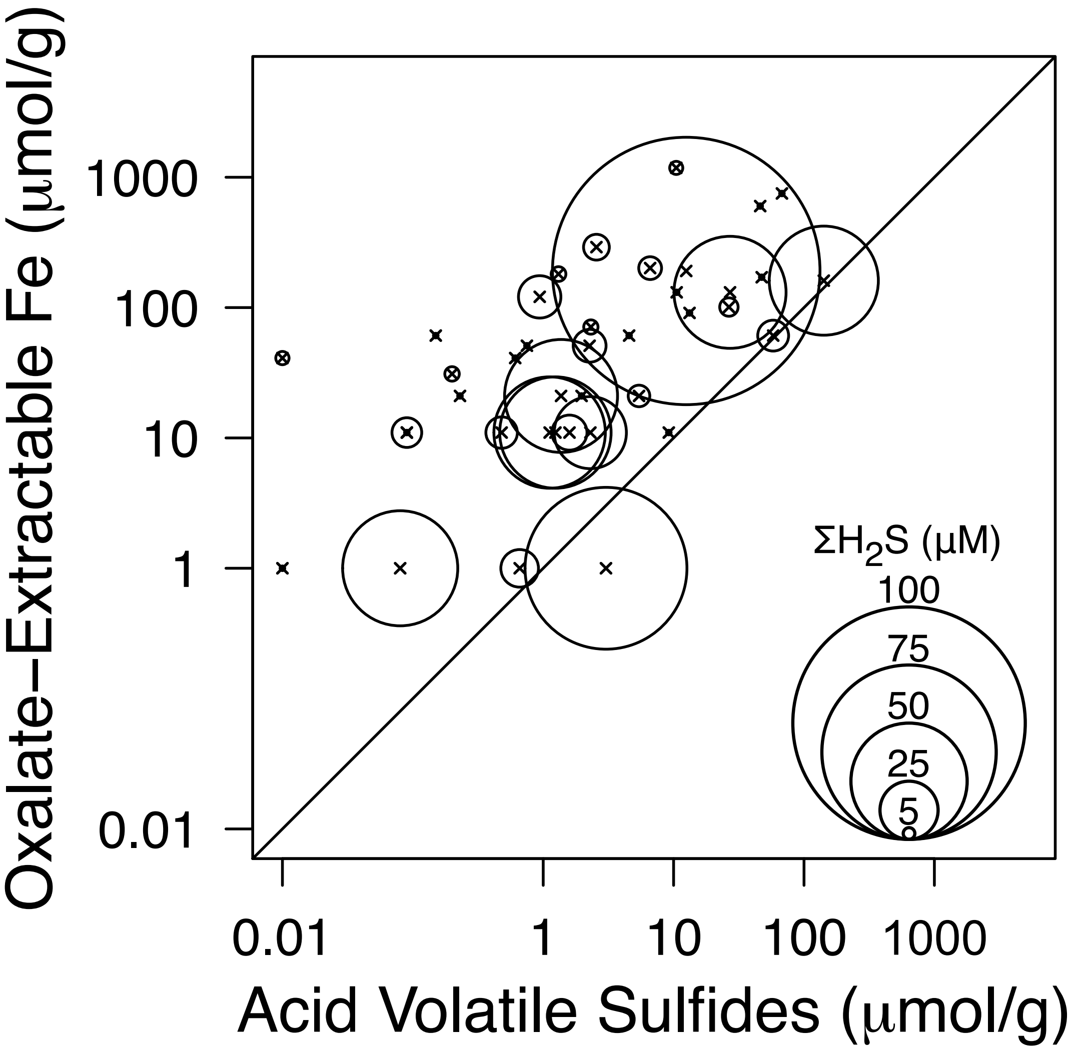

Figure 4.4 Relationship between sediment oxalate-extractable iron (an indicator of poorly crystalline iron oxides) and acid volatile sulfide (an indicator of sulfide bound to metals, particularly iron) measured in shallow (< 2 m deep) sediments compared with dissolved free sulfide (ΣH_2S) measured simultaneously in sediment pore waters. The x at the center of each circle represents the amounts of oxalate-extractable iron and acid volatile sulfide in sediments, while the area of the circle is proportional to ΣH_2S measured in pore waters at the same location. Note log-log scale.

Table 4.3 Published iron, sulfide, and ammonia toxicity thresholds for invertebrates. Unless noted otherwise, concentrations are as dissolved reduced iron (Fe(II)), unionized hydrogen sulfide (H_2S), and unionized ammonia (NH_3). Endpoints include 50% lethal concentration (LC50) assessed as mortality (unless noted otherwise), 50% effective concentration (EC50), and field-based effective concentration at which a 20% decline in abundance is observed (FEC20). Data sources are: (1) Ankley et al. 1995, (2) Arthur et al. 1987, (3) Besser et al. 1998, (4) Biesinger and Christensen 1972, (5) Borgmann et al. 2005, (6) DeGraeve et al. 1980, (7) Dekker et al. 2006 (8) Hickey and Vickers 1994, (9) Küster et al. 2005, (10) Linton et al. 2007, (11) Maltby et al. 1987, (12) Oseid and Smith Jr. 1974, (13) Oseid and Jr 1974, (14) Oseid and Smith 1975, (15) Reinbold and Pescitelli 1982, (16) Scheller 1997, (17) Schubauer et al. 1995, (18) Shuhaimi-Othman et al. 2011, (19) H A Stammer 1953, as cited in US EPA ECOTOX database (U.S. Environmental Protection Agency), (20) Thurston et al. 1984, as cited in US Environmental Protection Agency 1984, (21) Walter 1966, as cited in Gerhardt 1992, and (22) Warnick and Bell 1969.

	Sulfide (µM)	Iron (µM)	Ammonia (µM)	Endpoint	Source
Amphipod					
Crangonyx pseudogracilis			116-401	96 hr LC50	2
Crangonyx richmondensis	26*			96 hr LC50	13
Gammarus pseudolimnaeus	0.06*, 0.69*			Max. safe conc., 96 hr LC50	12
Hyalella azteca		>56**	2.9-435	Iron: 7 d LC50; Ammonia: 96 hr LC50	1,3,5
Caddisfly					
Hydropsyche betteni		287		7 d LC50	22
Philopotamidae		7.9**		FEC20	10
Pycnocentria evecta			29	96 hr LC50	8
Philarctus quaeris			719-726	96 hr LC50 (immobilization)	2
Cladoceran					
Ceriodaphnia dubia			5	48 hr LC50	16
Chydorus sphaericus			136	96 hr LC50	7
Daphnia magna	3.6	172**	291	Iron & Ammonia: 48 hr LC50; Sulfide: 48 hr EC50 (immobilization)	4, 9,15
Daphnia pulicaria			83	48 hr LC50	6

*Total dissolved free sulfide (H_2S+HS^-)

**Total iron

Table 4.3, continued

	Sulfide (µM)	Iron (µM)	Ammonia (µM)	Endpoint	Source
Isopod					
Asellus aquaticus		54, 5368-8361		62 hr LC50, 50 hr LC50	11,21
Asellus racovitzai			353-363	96 hr LC50	2
Asellus militaris	33			96 hr LC50	13
Mayfly					
Baetidae	0.62*	27**		Iron: FEC20; Sulfide: 96 hr LC50	10,13
Callibaetis skokianus			225-344	96 hr LC50	2
Deleatidium spp.			32	96 hr LC50	8
Ephemerellidae	9.9*	5.73, 7.7**	276-420	Iron: 96 hr LC50, FEC20; Sulfide & Ammonia: 96 hr LC50	10,13,20, 22
Hexagenia limbata	0.47, 5.2			Max. safe conc., 96 hr LC50	14
Leptophlebiidae		3.8**		FEC20	10
Zephlebia dentata			>57	96 hr LC50	8
Midge					
Chironomus javanus		11**		96 hr LC50	18
Chironomus tentans			51-985, 38	10 d LC50, 96 hr LC50	3,17
Chironomus riparius	~312-1562			EC50	19
Stonefly					
Acroneuria lycorias		287		9 d LC50	22
Zealandobius furcillatus			>57	48 hr LC50	8

*Total dissolved free sulfide (H_2S+HS^-)

**Total iron

concentrations", as determined by Oseid and Smith (1974, 1975), for the amphipod *Gammarus pseudolimnaeus* and burrowing mayfly nymph *Hexigenia limbata* were found to be even lower, 0.06 and 0.47 µM, respectively. The lowest of these values, 0.06 µM, is below our analytical detection limit. Thus, 74% of our measured pore water samples contained ΣH_2S concentrations that could negatively influence some invertebrate species.

Sulfide is also a known toxicant to plants (Table 4.4), as has been documented particularly in irrigated rice cultivation and salt marshes (Tanaka et al. 1968, Ingold and Havill 1984), although its role is less frequently considered in natural freshwater ecosystems. Negative effects to specific plant species have been observed at pore water sulfide concentrations as low as 5 µM (Smolders and Roelofs 1996). We observed ΣH_2S concentrations higher than this in ten of the 54 pore water samples. When non-sulfidic areas become sulfidic due to eutrophication or increased sulfate inputs, conspicuous die-backs of certain plant species occur, as has been observed for *Phragmites australis* (Armstrong et al. 1996, Armstrong and Armstrong 2001) and *Stratiotes aloides* (Smolders et al. 2003). However, these die-offs and similar conspicuous changes in plant community composition have been observed at pore water sulfide concentrations greater than 200 µM (Table 4.4), much higher than those measured in our survey, in which the maximum observed ΣH_2S concentration was 98 µM.

Table 4.4 Published values of iron, hydrogen sulfide, and ammonia effects on aquatic and semi-aquatic plants in controlled lab experiments and *in situ* field observations. Concentrations are for total free dissolved sulfide (ΣH_2S), total dissolved iron (Fe(II)), and total ammonium nitrogen (ΣNH_4^+) unless noted otherwise. Sources are: (1) Armstrong et al. 1996, (2) Chambers et al. 1998, (3) Clarke and Baldwin 2002, (4) Hill et al. 1997, (5) Howarth and Teal 1979, (6) Koch and Mendelssohn 1989, (7) Lamers et al. 1998, (8) Lucassen et al. 2000, (9) Smolders and Roelofs 1996, (10) Snowden and Wheeler 1993, (11) van der Welle et al. 2006, and (12) Wang 1991.

	Sulfide (µM)	**Study conditions**	**Endpoint**	**Source**
Common reed, *Phragmites australis*				
Rhizome cuttings	1400*	Lab, exposure in rooting medium	Acute die-back symptoms	1
Emerging shoots	375	Lab, roots immersed in solution	Slowed root nutrient uptake	2
Maidencane, *Panicum hemitomon*	360-1020	Lab, intact soil and plant cores	Growth reduction (total, culm, root, rhizome biomass)	6
Carex nigra-dominated community	~200	Lab, intact soil and plant flow-through mesocosms	Regrowth inhibited, esp. *Carex* spp.	7
Characean macroalgae, *Nitella flexilis*	50	Lab, soil and plant mesocosms	Growth reduction (fewer shoots than control)	11
Water soldier, *Stratiotes aloides*	5	Lab, apical 15 cm of root exposed to sulfide solutions	Decreased root survival time	9
Smooth cordgrass, *Spartina alterniflora*				
Emerging shoots	582	Lab, roots immersed in solution	Nutrient uptake not affected	2
Mature plants	670-1440	Lab, intact soil and plant cores	Growth reduction (total and root biomass)	6
Mature plants	200	Lab, plants grown hydroponically	Mortality	5

*Nominal concentration (not directly measured during experiment)

**Concentration of unionized ammonia (NH_3)

Table 4.4, continued

	Iron (µM)			
Water mannagrass*, Glyceria fluitans*	2612	*In situ* observations	Brown necrotic spots on 67% of leaves	8
	806	*In situ* observations	No negative effects	8
Water soldier, *Stratiotes aloides*	418-555	*In situ* observations	Fine root hair death	9
Codlins and cream, *Epilobium hirsutum*	179	Lab, exposure in rooting medium	Reduced relative growth rate	10
Soft rush, *Juncus effusus* and Common cottongrass, *Eriophorum angustifolium*	1790	Lab, exposure in rooting medium	No effects on growth	10
	Ammonium (µM)			
Common rush, *Juncus effusus*	14280	Constructed wetland mesocosms	Growth inhibited	3
Broadleaf arrowhead, *Sagittaria latifolia*	14280	Constructed wetland mesocosms	Growth inhibited	3
Bulrush, *Typha latifolia*	14280	Constructed wetland mesocosms	Growth inhibited	3
Softstem bulrush, *Schoenoplectus tabernaemontani*	7140	Constructed wetland mesocosms	Growth inhibited	3
Common reed, *Phragmites australis*	1430-5700**	Constructed wetland mesocosms	No effect on growth	4
Hardstem bulrush, *Schoenoplectus acutus*	4280**	Constructed wetland mesocosms	Reduced growth (dry matter)	4
Rice, *Oryza sativa*	630**	Lab, concentration renewed daily	27% growth inhibition	12
Common duckweed, *Lemna minor*	510**	Lab, static concentration	50% growth inhibition	12

*Nominal concentration (not directly measured during experiment)

**Concentration of unionized ammonia (NH_3)

Iron

Pore water dissolved Fe(II) concentrations ranged widely, from below detection limit to 1440 µM (0-80 mg Fe L^{-1}), with an average concentration of 150 µM. The EPA's Criterion Continuous Concentration (CCC), or chronic toxicity threshold for aquatic life, is 17.9 µM (1 mg Fe L^{-1}). About half of our sample sites (48%, 26 out of 54) had pore waters with Fe(II) concentrations higher than this (Figure 4.5). In the restored wetland we monitored, iron measured monthly in pore waters was higher than the EPA criterion throughout the 1.5 year sampling period, and reached concentrations as high as 2100 µM (Figure 4.6).

Although iron concentrations are typically highest in anoxic pore waters, we have observed precipitated iron floc and sometimes even measured dissolved Fe(II) in surface waters of some of our highest-iron sites. In the restored wetland, we detected iron in shallow surface waters mostly under ice, but also on one occasion in summer (July). Surface water dissolved Fe(II) concentrations in this wetland ranged from 1-196 µM. In multiple locations on four sampled dates (one of which was in July), the concentration in surface waters exceeded the EPA criterion of 17.9 µM. The high July concentration was measured in surface water under near 100% cover of duckweed with low dissolved oxygen in the water column (0.5 mg O_2 L^{-1}, 5% of atmospheric equilibrium).

Across our 54 pore water samples, high concentrations of Fe(II) tended to co-occur with high concentrations of ΣNH_4^{+} and low concentrations of SO_4^{2}, although

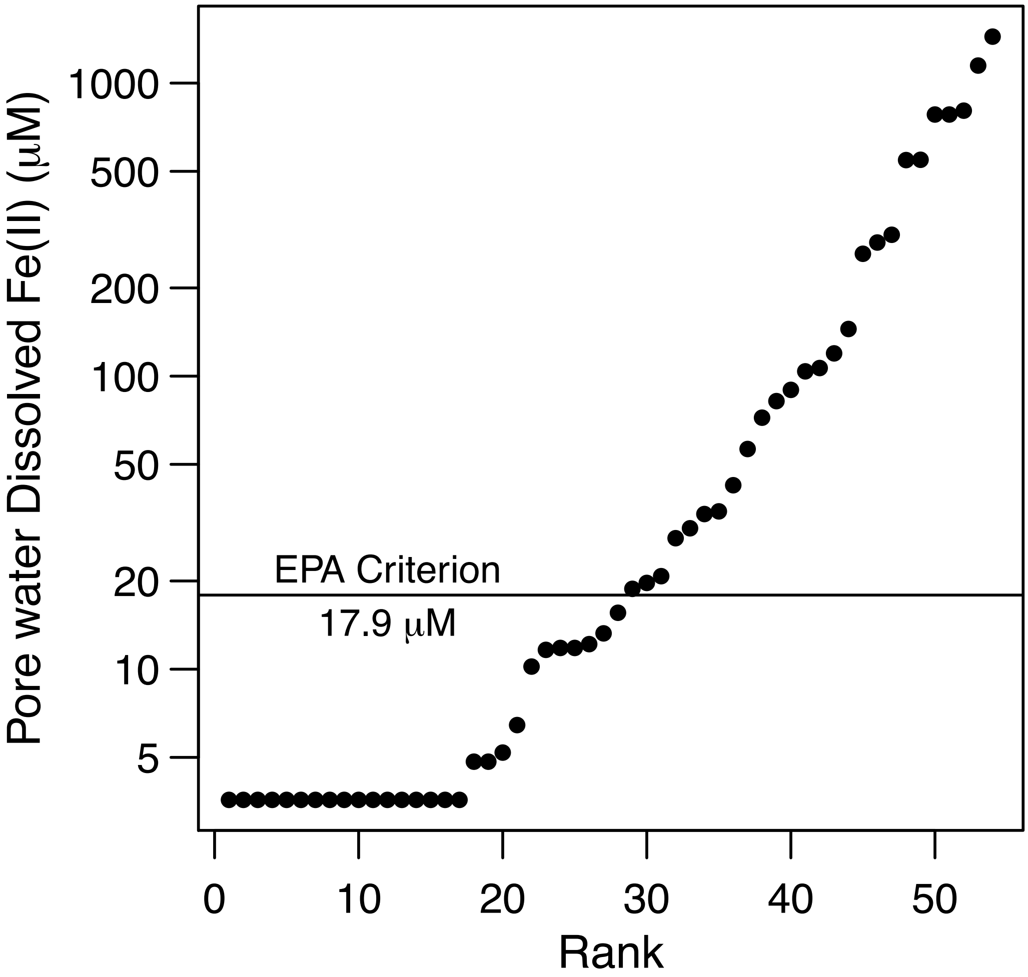

Figure 4.5 Concentrations of dissolved reduced iron (Fe(II)) measured in late summer in 54 sediment pore water samples from 24 shallow (< 2 m deep) freshwater ecosystems, as compared to EPA chronic criteria for aquatic life of 17.9 μM (1 mg L^{-1}) (U.S. Environmental Protection Agency 1976). Note log scale on y axis.

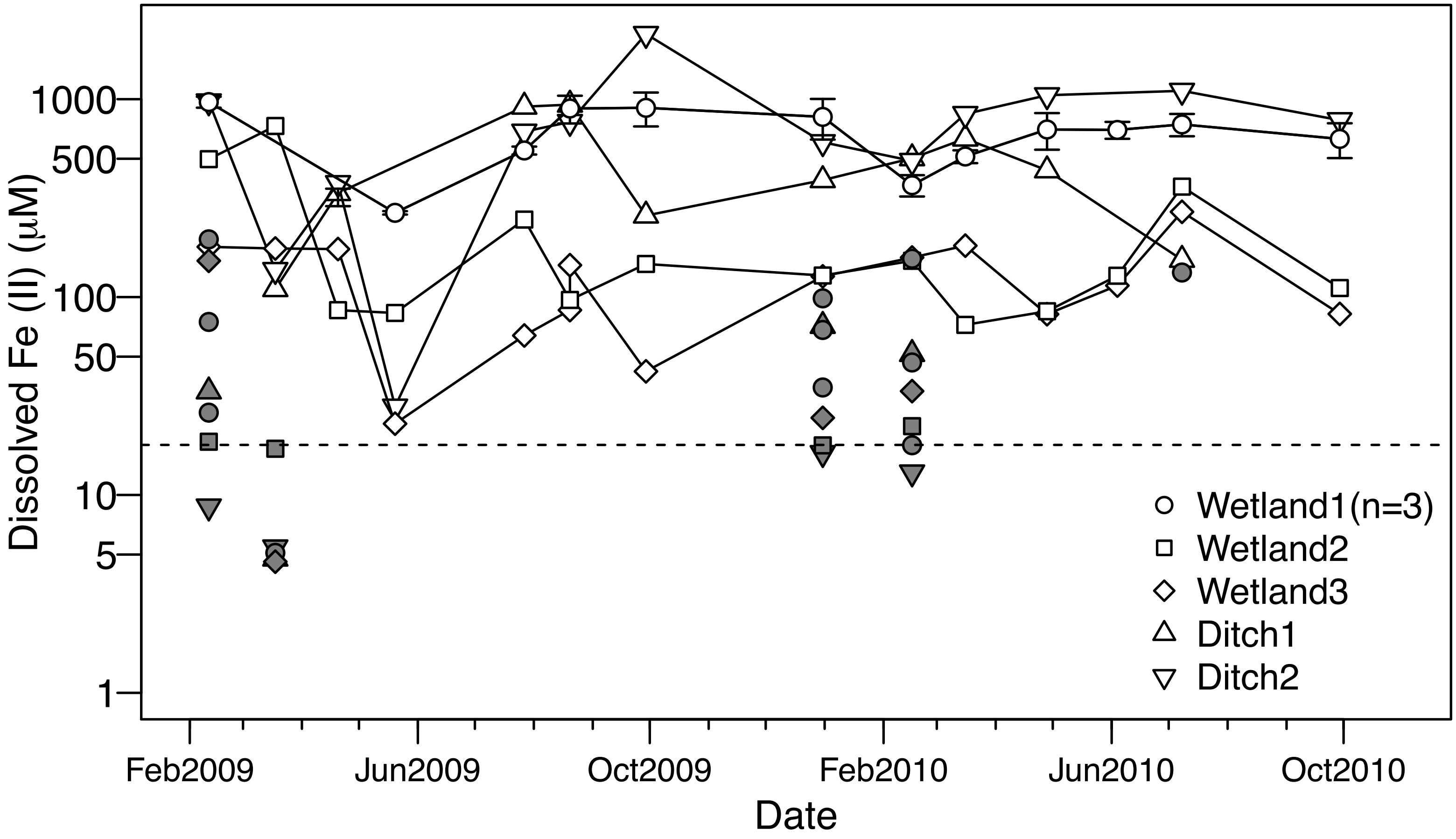

Figure 4.6 Dissolved reduced iron (Fe(II)) measured repeatedly in pore water (open symbols) and during low oxygen events in surface water (grey symbols) in re-flooded wetland areas (Wetlands 1-3) and drainage ditches (Ditches 1-2) of a restored wetland. Wetland 1 represents the average of three locations along a ~100 m transect through the largest re-flooded area of the restored wetland. The horizontal dashed line is the EPA chronic criterion for aquatic life for iron of 17.9 μM (1 mg L^{-1}) (U.S. Environmental Protection Agency 1976). Note log scale on y axis.

statistical relationships are relatively weak (Table 4.2). Pore water dissolved Fe(II) was also significantly ($p < 0.05$) positively correlated with pore water SRP, surface water SRP and ΣNH_4^+, and sediment OM, sedTP, TFe, and Ox-Fe, and negatively correlated with surface water pH and dissolved oxygen concentration, and sediment bulk density (Table 4.2).

Iron toxicity studies are rare and at times contradictory (Vuori 1995). Toxic thresholds range widely, and there is little consistency regarding the method of iron analysis and form (i.e., total dissolved, reduced, or total iron) being measured (Table 4.3). For examples, studies report 62-hr LC50 concentrations for the isopod *Assellus aquaticus* that range from 54 to over 8,000 μM (Table 4.3). Recent studies relating in-stream total iron concentration to benthic macroinvertebrate communities corroborate the EPA water quality criterion of 17.9 μM, suggesting thresholds of 3.8-44 μM to avoid changes in community composition (Linton et al. 2007, Crane et al. 2007, Peters et al. 2011).

The toxicity of iron to plants is less studied (Table 4.4), although toxic effects have been observed at relatively high concentrations. In wetland ecosystems, pore water concentrations of 2579 and 394 μM have been related to *in situ* toxic responses of *Glyceria fluitans* and *Stratiotes aloides*, respectively (Smolders and Roelofs 1996, Lucassen et al. 2000). Six of our 54 pore water samples contained dissolved Fe(II) concentrations exceeding 400 μM, but none were higher than 2579 μM. Out of 44 fen species tested identically, the least tolerant species (*Epilobium hirsutum*) showed growth reduction when grown in media containing 179 μM Fe(II), yet the most tolerant species (*Eriophorum angustifolium* and *Juncus effusus*) showed no negative response to Fe(II) concentrations as high as 1790 µM (Snowden and Wheeler 1993). Nine of our 54 samples contained iron concentrations higher than 179 µM Fe(II).

Ammonium

Pore water ΣNH_4^+ concentrations ranged from 0.2-1170 µM (2.5-16,440 µg N L^{-1}), with a mean of 195 µM (2730 µg N L^{-1}). Unionized NH_3 made up a very small fraction of ΣNH_4^+ in pore waters (maximum percentage= 2.6% of ΣNH_4, maximum concentration = 6.86 µM NH_3). Nonetheless, ΣNH_4^+concentrations were higher than the EPA CCC chronic stress threshold for aquatic ecosystems where freshwater mussels are present (0.26 mg N L^{-1}, or 18.6 µM, at pH 8 and 25°C) in 23 of our 54 pore water samples and above the Criterion Maximum Concentration (CMC) acute threshold for ecosystems where mussels are present (1.8 mg N L^{-1} or 129 µM at pH 8 and 25°C) in 3 pore water samples (Figure 4.7). Some of the highest pore water concentrations were measured in loose, flocculent surface sediment (8 samples, range 21-807 µM ΣNH_4^+, average = 354 µM ΣNH_4^+). Surface water ΣNH_4^+ concentrations were lower than in pore water, ranging from 0.1-52 µM ΣNH_4^+ (1.4-730 µg N L^{-1}), with a mean of 2.7 µM ΣNH_4^+ (37 µg N L^{-1}), all well below (by at least 2 orders of magnitude) chronic and acute EPA criteria for aquatic life.

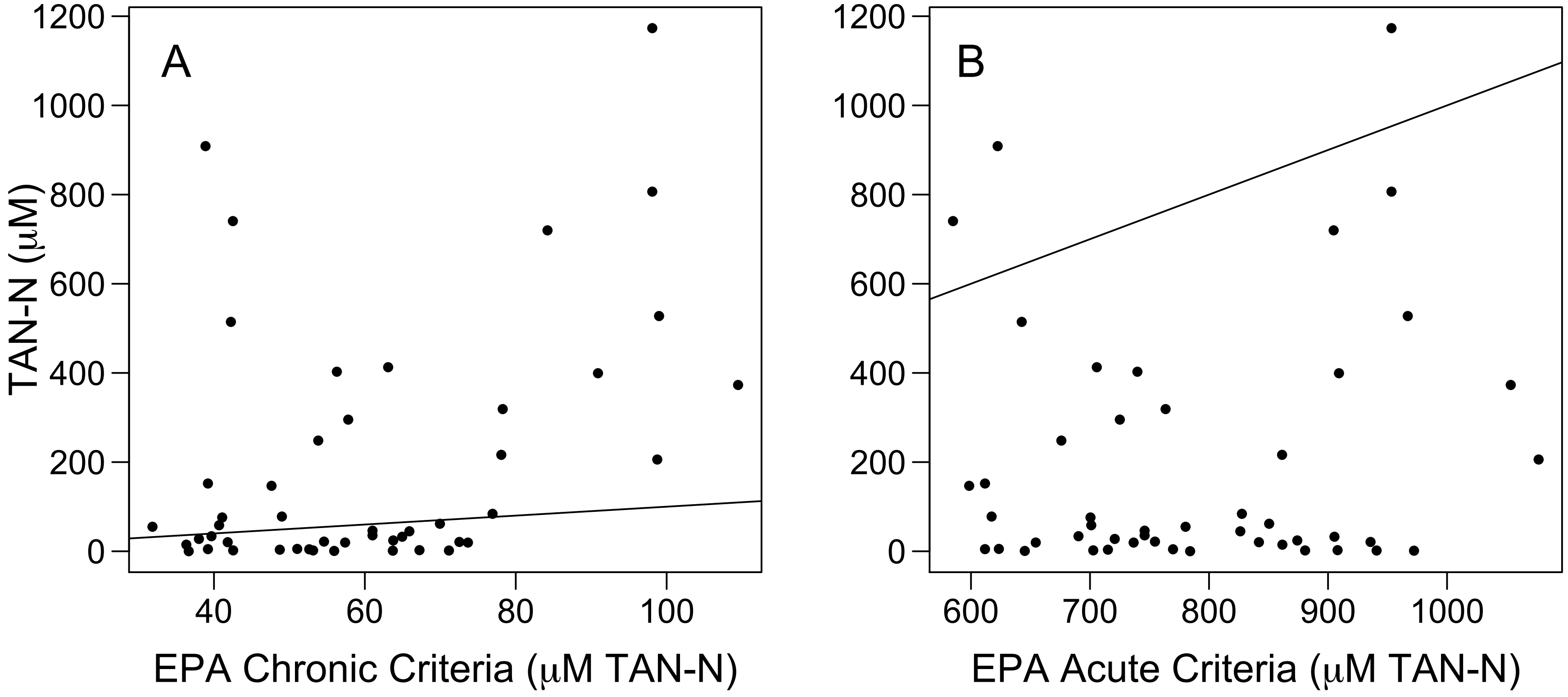

Figure 4.7 Total ammonium nitrogen (TAN-N) concentrations measured in 54 sediment pore water samples from 24 shallow (< 2 m) freshwater ecosystems as compared to EPA Criterion Continuous Concentration (CCC) chronic (A) and Criterion Maximum Concentration (CMC) acute (B) criteria for areas where freshwater mussels are present Criteria are calculated using pore water pH and surface water temperatures measured simultaneously with ΣNH_4^+ using calculations in US Environmental Protection Agency 2009). The dotted line represents the 1:1 line, such that points above the line represent pore water samples that are above the EPA criterion.

In the restored wetland, ΣNH_4^+ concentrations in surface waters were high, but remained lower than the EPA CCC chronic toxicity threshold for mussels (Figure 4.8). However, pore water concentrations were higher, usually above calculated chronic thresholds (which changed over time due to changing pH and temperature) (Figure 4.8). Across our 54 pore water samples, ΣNH_4^+ concentrations were significantly positively correlated with concentrations of pore water dissolved Fe(II) and SRP, surface water SRP, and sediment OM and sedTP, and negatively correlated with pore water pH, NO_3^-, and SO_4^{2-}, as well as with surface water dissolved oxygen concentration (Table 4.2).

The toxicity of ΣNH_4^+, and specifically as NH_3, to freshwater mussels has been investigated earnestly, especially in recent years (e.g. Augspurger et al. 2007). Unionized NH_3 is chronically toxic to juvenile mussels at concentrations as low as 1.57 μM (ΣNH_4^+=26 μM) (Wang et al. 2007), and NH_3 concentrations higher than 0.01 μM have been associated with recruitment failure in stream populations (Strayer and Malcom 2012). Although the proportion of ΣNH_4^+ that existed as NH_3 aried in pore waters we sampled, most (49 out of 54) contained ΣNH_4^+ concentrations greater than 27 μM, and thus likely represent hazardous conditions to some freshwater mussel species and life stages. Other organisms are thought to be generally less vulnerable to NH_3 than freshwater mussels (Table 4.4), although some are affected at comparably low concentrations.

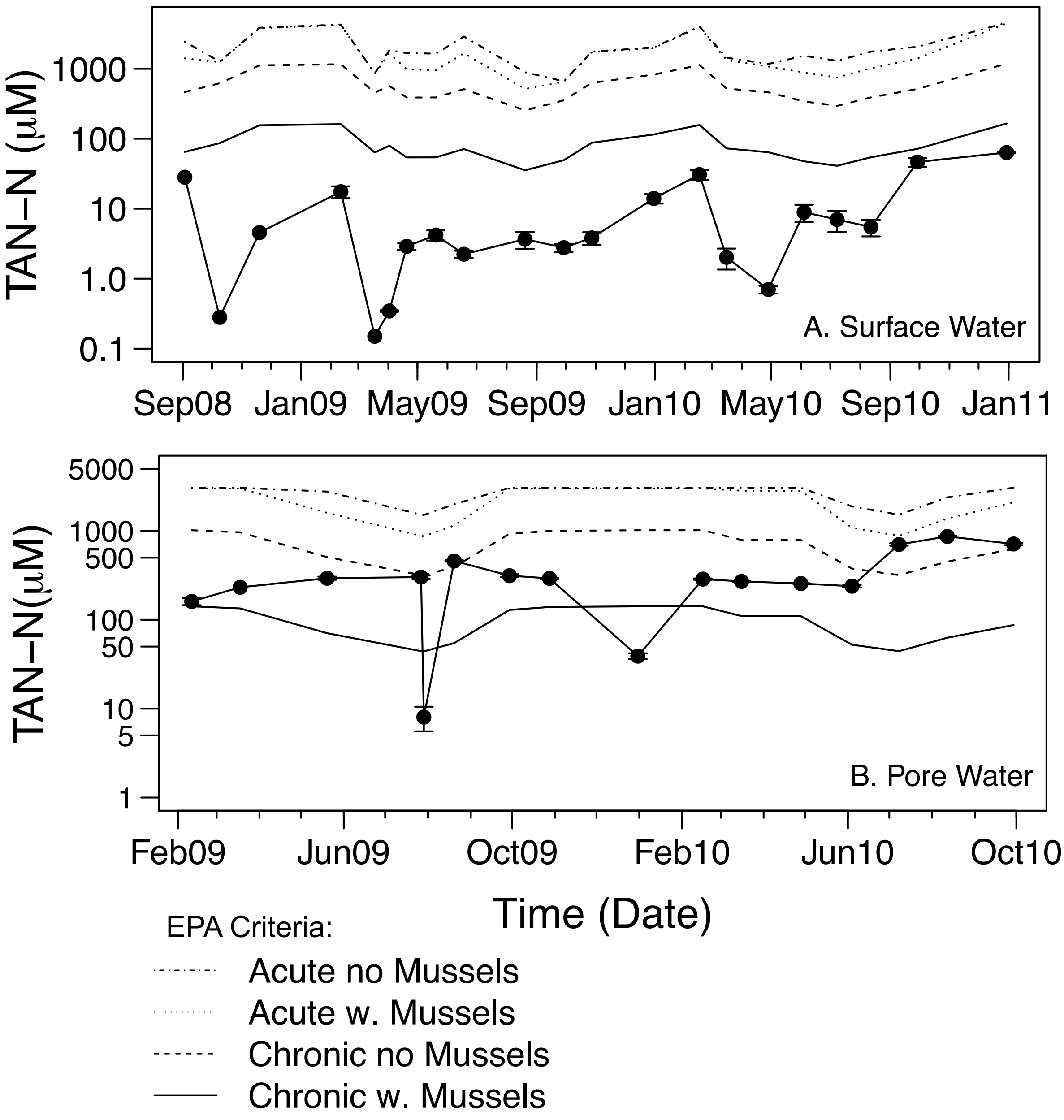

Figure 4.8 Total ammonium nitrogen (TAN-N) concentrations measured repeatedly in surface (A) and pore waters (B) of re-flooded areas in a restored wetland, compared to calculated EPA acute and chronic criteria for areas were mussels are present and not present (calculations based on measured pore water pH and surface water temperatures, Agency 2009). Pore water criteria were calculated based on the measured surface water temperature and an assumed pH of 7.

Higher plants are susceptible to ΣNH_4^+ toxicity due to disruptions in ion uptake mechanisms (Britto et al. 2001, Britto and Kronzucker 2006) in addition to some of the NH_3 toxicity mechanisms experienced by other organisms. Plants are generally less vulnerable to high concentrations of ΣNH_4^+ than animals, and some use NH_4^+ as their preferred nitrogen source. Unionized NH_3 and ΣNH_4^+ at toxic levels (>630 μM NH_3 and 7140 μM ΣNH_4^+), however, has been shown to have negative effects on some plant species (Table 4.4). None of our 54 pore water samples contained NH_3 or ΣNH_4^+ concentrations exceeding these values, and thus ammonium toxicity may not be a common threat to plants in our sampled ecosystems.

Discussion

The benthic environment of almost every freshwater, uncontaminated water body we sampled was theoretically toxic or stressful to some component of aquatic life, based on EPA criteria for dissolved H_2S, Fe(II), and ΣNH_4^+. Organismal tolerances to chemical stressors vary, so the toxicant concentrations that we measured likely shape benthic ecological communities and influence rates of ecosystem function.

In general, ΣH_2S is not commonly measured in pore or surface waters, especially in uncontaminated freshwater ecosystems (Bagarinao 1992). Our measured pore water ΣH_2S concentrations ranging from 0-98 μM are in the mid-range of published measurements from freshwater ecosystems, and one to several orders of magnitude lower than maximum concentrations measured in saline and brackish ecosystems

(Table 4.5). Although our measured concentrations were comparatively low, they are biologically relevant because sulfide is highly toxic, even at micro-molar levels (Evans 1967, Wang and Chapman 1999). Some of our concentrations are comparable to levels (0-24 μM) measured in and above toxic paper fiber sludge deposits that killed walleye eggs and fry in a contaminated river (Colby and Smith 1967).

Ammonium is commonly monitored in surface waters, but measurements in pore water are less common. The range and distribution of surface water ΣNH_4^+ concentrations in this study are similar to those from a larger, broader set of southwestern Michigan freshwater ecosystems we have sampled (Figure 4.9). These ecosystems are embedded in a mixed-use forested, agricultural and low-density development landscape and are representative of many of the moderately impacted aquatic ecosystems throughout the agricultural Midwest. Their nutrient chemistries reflect elevated nitrogen inputs from atmospheric deposition and agricultural fertilizer use (Hamilton LTER Synthesis Chapter), which may lead to increased instances of ammonium toxicity (Camargo and Alonso 2006, Strayer and Malcom 2012).

Our highest pore water concentrations of ΣNH_4^+ (maximum 1173 μM) are higher than some values reported for other freshwaters (e.g., Mississippi River sediments, maximum 714 μM) although our concentrations of unionized NH_3 were lower (our max NH_3 = 6.9 μM, Mississippi River max = 12.5 μM) (Frazier et al. 1996). Some of our samples (7) are in the range of the pore water ΣNH_4^+ concentrations (450-6140 μM) measured in highly contaminated sediments of the Fox River/Green Bay and Illinois

Table 4.5 Published dissolved free sulfide (ΣH_2S) and unionized hydrogen sulfide (H_2S) concentration ranges from freshwater, brackish, and saline ecosystems, in order of maximum value. Unless noted otherwise, values are for sediment pore waters. If multiple ecosystems were studied, the number of systems is provided (n=x).

Ecosystem	ΣH_2S (µM)	H_2S (µM)	Source
Freshwater			
Contaminated River, Minnesota, paper fiber sludge	0-25	0-22*	Colby and Smith Jr. 1967
Sphagnum Big Run Bog, West Virginia	2-40		Wieder and Lang 1988
Restored wetland, California	1-54		Maynard et al. 2011
Southwest MI shallow freshwater ecosystems	**bd-98**	**bd-47**	**this study**
Eutrophic lakes, U.K., hypolimnion (n=3)	0-125		Bark and Goodfellow 1985
Delta Marsh, Lake Manitoba, Canada	0-225		DeVries and Wang 2003
Acidic Lake, north Texas, Surface-7.3 m	40-226	9-71*	Bonn and Follis 1967
Eutrophic Wintergreen Lake, Michigan, hypolimnion, sediment pore waters, summer stratification	~0-240		Smith and Klug 1981
Everglades wetland, Florida, nutrient enriched area	31-468		Orem et al. 2011
Peaty lowlands, Netherlands (n=75)	0-500		Smolders and Roelofs 1996
Pilot-scale constructed wetland, Germany	34-577		Wu et al. 2011
Brackish			
Canvey Island Point salt marsh, United Kingdom	5-400		Ingold and Havill 1984
Charles E. Wheeler Salt Marsh, Connecticut	124-989		Chambers et al. 1998
Limfjorden, coastal estuary, Denmark	0-1100		Jorgensen 1977
Great Sippewissett Salt Marsh, Massachusetts	1.2-1500		Howarth et al. 1983
Baltic Sea, contaminated and reference areas	0-1800		Sundelin and Eriksson 2001
Lake Fërto, Hungary, *Phragmites* die-back area	0-4000		Armstrong et al. 1996
Chesapeake Bay estuary, Maryland	0.5-5600		MacCrehan and Shea 1995
Seagrass meadow in Valle Smarlacca lagoon, Italy	0-7000		Azzoni et al. 2001
Saline			
Long Island Sound, Connecticut, pore waters	0-2800		Goldhaber et al. 1977

bd = below detection limit,*Calculated by original authors

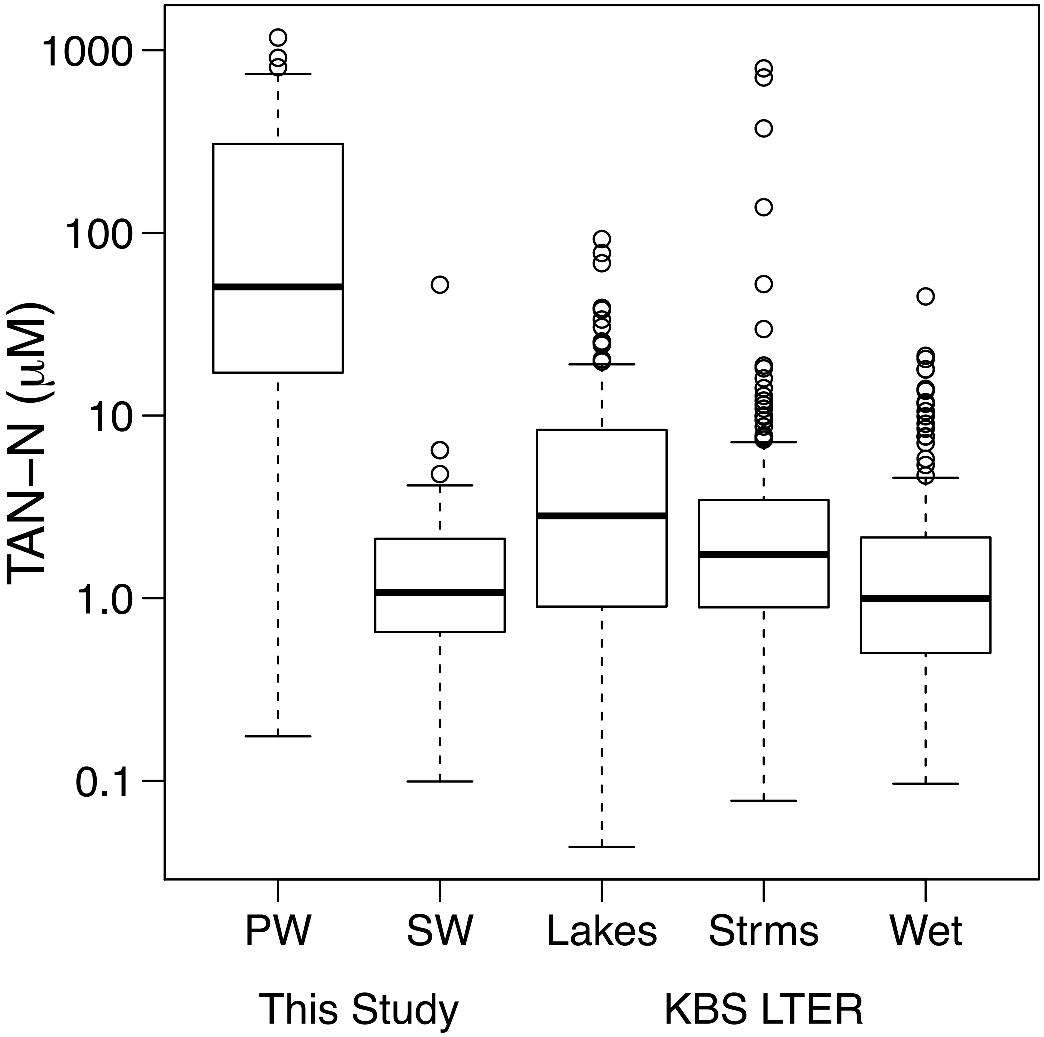

Figure 4.9 Box-and whisker plots of total ammonium nitrogen (TAN-N) concentrations measured in surface waters of southwestern Michigan freshwater lakes, streams (Strms), and wetlands (Wet) (Hamilton, unpublished data) compared with TAN-N concentrations measured in pore (PW) and surface waters (SW) in 24 freshwater ecosystems for this study.

rivers system in which sediment toxicity to invertebrates (*Chironomus tentans, Lumbriculus variegatus, and Ceriodaphnia dubia*) and fathead minnows (*Pimephales promelas*) was attributed to ΣNH_4^+ (Ankley et al. 1990, Schubauer-Berigan and Ankley 1991).

Many of the ecosystems we sampled contained pore water dissolved Fe(II) concentrations that were within published ranges, although there were some that were on the extreme high end of published values (Table 4.6). Our maximum pore water Fe(II) concentrations (1440 µM) are similar to those measured in pore waters of an agricultural ditch draining high-iron acid sulfate soils (Burton et al. 2006), and much higher than published values for many relatively unimpacted freshwater wetlands in the literature (Table 4.6). Surface water concentrations of dissolved Fe(II) measured in a restored wetland in winter and one date in summer under low oxygen conditions and floating duckweed cover (July) were within the range of published values of total iron for ecosystems draining high-iron areas impacted by mining, agriculture, and drainage of acid sulfate soils (Table 4.6).

What controls concentrations of natural stressors in our sampled ecosystems?

Sulfide

We would predict that dissolved ΣH_2S would accumulate in anoxic waters that receive a supply of SO_4^{2-} to fuel SO_4^{2-} reduction, but do not contain enough iron to sequester the sulfide as iron sulfides. However, we did not detect relationships between measured pore water ΣH_2S concentrations and any of our measured indicators of SO_4^{2-}

Table 4.6 Published values of reduced (Fe(II)), oxidized (Fe(III)), and/or total (Fe(II)+Fe(III)) iron in pore and/or surface waters of aqueous systems, including pore sizes used for measurements of dissolved constituents in filtered samples.

Ecosystem Type	Filter pore size (mm)	Fe Form Detected	Fe conc. (mM)	Source
Pore Water				
Restored wetland, California	0.2	Fe(II), Fe(III)	1.1-100, 1.1-18	Maynard et al. 2011
Eutrophic Peel-Harvey Estuarine System, Australia	0.45	Fe(II)+Fe(III)	~5-170	Morgan et al. 2012
Freshwater peaty lowlands, Netherlands (n=75)	Ceramic Sampler	Fe(II)+Fe(III)	1.1-555	Smolders and Roelofs 1996
Chesapeake Bay estuaries	0.45	Fe(II)+Fe(III)	0-~700	Hartzell and Jordan 2010
Southwest MI wetlands	**0.45**	**Fe(II)**	**0-1440**	**This Study**
Agricultural drain, Australia, acid sulfate soils	0.45	Fe(II), Fe(III)	1468, <1.79	Burton et al. 2006
Surface Water				
Eutrophic Peel-Harvey Estuarine System, Australia	0.45	Fe(II)+Fe(III)	3.7-8.2	Morgan et al. 2012
Agricultural drain, Australia, acid sulfate soils	0.45	Fe(II), Fe(III)	<1.79, 27	Burton et al. 2006
Restored MI wetland	**0.45**	**Fe(II)**	**1-196**	**This Study**
River Vidaa, Denmark, drains mining and agriculture	0.45	Fe(II)+Fe(III)	2.1-569	Rasmussen and Lindegaard 1988
	Unfiltered	Fe(II)+Fe(III)	14-569	

reduction or iron binding, including pore water and surface water SO_4^{2-}, pore water and sediment Fe, sediment AVS, and surface water indices of groundwater input, particularly Mg^{2+}. In fact, we were unable to detect strong relationships between ΣH_2S and any other measured variables at our sampling resolution. It is likely that integrating measurements over multiple depths, locations, and through time within a single location is required to determine what controls H_2S concentrations, which are highly heterogeneous in space and time (Jorgensen 1977, DeVries and Wang 2003).

Solid iron mono-sulfides (assumed to be the predominant component measured as AVS in our samples) are comparatively less reactive than ΣH_2S in anoxic sediments. In most of the sediments we sampled, the quantity of sulfur as sediment AVS was several orders of magnitude greater than pore water dissolved ΣH_2S. Significant relationships between AVS and indicators of groundwater influence (Ca^{2+}, Mg^{2+}, SO_4^{2-}, and conductivity), iron content (sediment TFe, Ox-Fe, and pore water Fe(II)), and oxygen demand (OM) are not unexpected. A positive relationship between sediment AVS and the amount of groundwater influence could be explained if groundwater provides a sulfur source by delivering SO_4^{2-}. The availability of iron provides the sediment with the ability to sequester reduced S as insoluble iron sulfides, and the amount of sediment organic matter could correlate with the organic sulfur loading as well as the activity of iron- and sulfate-reducing bacteria. Further evidence that AVS is high in sediments experiencing high rates of organic matter mineralization is provided by the positive correlations with SRP and ΣNH_4^+, which tend to accumulate under anoxic conditions, and the negative correlation with NO_3^-, which tends to be consumed under anoxic conditions. Although sulfur bound in AVS is not directly toxic to organisms, in influences sediment toxicity by binding and detoxifying heavy metals. In addition, AVS may provide a more useful indicator of a sediment's potential to contain toxic sulfide because it is a less transient indicator of organic sulfur mineralization and SO_4^{2-} reduction than ΣH_2S.

Ammonium

Ammonium in sediments is mainly a product of decomposition, so we expect concentrations to be highest in anoxic, highly organic sediments. At our research sites, ΣNH_4^+ was positively related to sediment OM and also to pore water SRP and Fe(II), and sedTP. The correlations among ΣNH_4^+, dissolved Fe(II), and SRP may reflect their propensity to co-occur in sediments experiencing high organic matter remineralization rates. High ΣNH_4^+ also correlated negatively with surface water dissolved oxygen and positively with pore water SRP, both of which are also indicative of high mineralization rates. Pore water ΣNH_4^+ concentrations correlated positively with dissolved Fe(II), making the potential for toxic effects higher as high concentrations of both toxicants tend to co-occur (ΣNH_4^+ and Fe(II) concentrations both exceeded EPA criteria in 18 of our 54 pore water samples).

In our repeatedly sampled restored wetland, ΣNH_4^+ concentrations in surface and pore waters varied seasonally, changing in roughly opposite directions through the year. In pore waters, concentrations were highest in summer and lower in winter, similar to other published data on temporal patterns in pore water ΣNH_4^+ concentrations (Frazier et al. 1996). In contrast, surface water ΣNH_4^+ concentrations were highest in winter and lower in summer. These pattern is likely because plant, algal, and microbial uptake draws ΣNH_4^+ down in surface waters during summer, while it is continually produced in pore waters during warm weather, whereas in winter, mineralization rates slow, slowing rates of accumulation in pore waters, but uptake rates also slow, leading to higher measured concentrations in surface waters. These patterns suggest that benthic invertebrates are most likely to encounter toxic levels of NH_3 from pore water exposure in warm summer months.

Iron

We would expect dissolved Fe(II) to be highest in ecosystems with a supply of poorly crystalline, easily reducible iron oxides and with insufficient production of sulfide to bind and sequester reduced Fe(II) (Azzoni et al. 2001). In most of our sample sites, sediment TFe, as well as the fraction of sediment iron in amorphous, poorly crystalline forms, was in excess of sulfur on a molar basis. The significant positive correlation between pore water Fe(II) concentrations and sediment AVS is indicative of the importance of iron sulfide precipitates as a sink for Fe(II) as well as ΣH_2S. We measured poorly crystalline iron oxyhydroxides as oxalate-extractable iron (Ox-Fe), and the positive correlation between Ox-Fe and dissolved Fe(II) concentrations reflects the strong control of iron mineralogy over dissolved Fe(II) concentrations in pore waters. Pore water Fe(II) was also positively related with nutrients, possibly reflecting the intensity of organic matter remineralization. The negative relationships with surface water pH and dissolved oxygen may reflect redox phase partitioning (Fe(II) is more stable at low pH and oxygen), although the degree to which surface water conditions influence conditions in pore waters may be limited.

Freshwater Sediment Toxicity Potential: Caveats & Considerations

The most common stressor to exceed EPA criteria for aquatic life in pore waters sampled in this study was ΣH_2S, although Fe(II) and ΣNH_4^+ were frequently measured at potentially toxic levels as well. We deemed it useful to assess ecosystem toxicity using US EPA criteria, even though they are variably well supported, and may or may not be relevant to actual ecosystems. Defined as protecting “aquatic life,” all three criteria are based on toxicity to animals, particularly invertebrates and freshwater fish, and none include assessments of toxicity to plants (U.S. Environmental Protection Agency 1976, 1986, 2009), which are often the focal organisms of ecosystem restoration. The H_2S and Fe criteria were established in 1986 and 1976, respectively, and have not been updated since their establishment. Both the criteria for H_2S and Fe are Criterion Continuous Concentrations (CCCs), which are an “estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed indefinitely without resulting in an unacceptable effect” (U.S. Environmental Protection Agency 2012). Considering that these are values for chronic, not acute, toxicity, they may represent conservative benchmarks with which to compare our single-point-in-time measurements.

The EPA criterion for H_2S of “2 µg L^{-1} (0.06 µM) undissociated H_2S for fish and other aquatic life, fresh and marine water” is based entirely on toxicological studies with aquatic animals, not plants, and mostly on studies of fish at different life stages with some evidence from studies of aquatic invertebrates eaten by fish (U.S. Environmental Protection Agency 1986). The EPA criterion for Fe is very poorly supported (Thurston 1979), largely because of large gaps in the toxicological literature: few organisms have been tested, toxicological tests are at times contradictory, and tests are often conducted under environmentally irrelevant conditions (i.e., lower-than-typical pH) (Vuori 1995). The current EPA Fe criterion is based largely on a single study of fish survival in an Fe-contaminated Colorado river (U.S. Environmental Protection Agency 1976, Thurston 1979). In sharp contrast to criteria for H_2S and Fe, ΣNH_4^+ criteria are well-supported for benthic invertebrates, especially unionid mussels, and are in the process of being updated based on new literature (Augspurger et al. 2003, U.S. Environmental Protection Agency 2009). However, toxicity tests to some non-unionid taxa remain limited, and toxicity to wetland plants is particularly unknown.

EPA water quality criteria are defined as being for surface water exposure, and no criteria exist specific to pore water concentrations. We extend these surface water EPA criteria to pore water, which we feel justified in doing for several reasons, while being mindful of the differences between surface and pore water that have implications for the potential toxicity of high pore water concentrations. Although many organisms will avoid the threat of toxic reduced species by virtue of living in the higher-oxygen surface water environment, which is typically devoid of dissolved Fe(II) and ΣH_2S, and lower in ΣNH_4^+ than pore waters, pore waters are a frequent source of toxicant exposure, especially to benthic organisms and rooted plants. Studies often show that the toxicity of sediment contaminants to benthic macroinvertebrates is directly related to concentrations of those contaminants in pore waters (Ankley et al. 1990, Whiteman et al. 1996). Many organisms spend all parts of their life cycles in the benthos, either at the sediment-water interface or burrowing in the top layers of the sediments themselves. In addition, surface waters are, at times, poorly mixed due to stagnation and chemical or thermal stratification. During these times, dissolved toxicants in sediment pore waters can enter surface waters unchanged, threatening organisms with variable abilities to move to higher oxygen areas. Furthermore, fluctuating water levels can drive pore waters into surface waters, creating ephemeral pulses of toxicant exposure. If nothing else, the levels of these stressors will determine the suitability of habitat for various species, and may be an important filter for benthic species composition.

A broader challenge to investigating effects of chemical stressors on ecosystems may be attempting to relate highly controlled laboratory toxicological tests to environmental conditions. Lab-controlled studies allow for meaningful comparisons among studies that use standard techniques and measure standard endpoints, but may not reflect actual response of organisms, communities, and function to similar concentrations under *in situ* conditions. Environmental factors and organism behaviors *in situ* may either mitigate or exacerbate the hazards of these toxicants in comparison to effects in controlled lab studies. For example, some *in situ* studies have shown freshwater bivalves to be tolerant of environmental ΣNH_4^+ concentrations that have caused negative effects in the lab (Frazier et al. 1996, Bartsch et al. 2003). In contrast, a study in New York streams discovered a relationship between mussel recruitment failure and sites with $NH_3 > 0.014$ μM, a concentration much lower than EPA-established criteria (Strayer and Malcom 2012), suggesting mussels may be even more sensitive than lab tests suggest. Ideally, studies would provide both highly controlled, laboratory components and studies of response and exposure in the field. However, this is seldom the case, and environmental effects are often assumed from laboratory responses.

Our own sampling design, which sampled a large number of ecosystem components but over a limited spatial and temporal scale, may also limit our ability to assess the potential for chronic toxicity of natural chemical stressors. Pore water ΣNH_4^+, ΣH_2S, and Fe(II) concentrations are highly spatially and temporally heterogeneous (e.g., Frazier et al. 1996, DeVries and Wang 2003), especially in vegetated wetland sediments (Lamers et al. 2012). While our data provide a snapshot of the potential for toxic levels of these substances, more comprehensive sampling over space and time would be required to truly assess the toxic potential of a given sediment to organisms of interest, which also vary seasonally their behavior and life cycles.

General Implications

We have demonstrated that the sediments and sometimes the water columns of many natural, non-industrially contaminated freshwater ecosystems can be naturally stressful places for organisms to live due to naturally high concentrations of ΣNH_4^+, ΣH_2S, and/or Fe(II). Our sampling sites have no history of industrial contamination, although diffuse loading of nitrogen and suflur in their watersheds, largely from agriculture and atmospheric deposition, may play a role in producing elevated concentrations in the sediments. When developing bioassessment tools and restoring ecosystems to support biodiversity, it is important to remember that these naturally inhospitable sediment conditions constrain ecological communities, even in the absence of anthropogenic stressors. When natural stressors do co-occur with anthropogenic contaminants, the presence of toxic levels of ΣNH_4^+, ΣH_2S, and/or Fe(II) may lead to false attribution of toxic affects and misappropriation of resources to remediation. The natural biogeochemistry of aquatic ecosystems may constrain the success of restoration efforts, as sediments high in ΣNH_4^+, ΣH_2S, and/or Fe(II) may only be able to support limited biodiversity and ecological function.

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