

PHOSPHORUS DYNAMICS
IN THE NETLEY-LIBAU MARSH

BY

JOE ACKERMAN

A thesis submitted to the Faculty of Graduate Studies in partial fulfillment of
the requirements of the degree of

MASTERS OF SCIENCE

Department of Biosystems Engineering
University of Manitoba,
Winnipeg, Manitoba

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ABSTRACT

Release of phosphorus (P) into the environment has led to severe problems of eutrophication in water bodies around the world. Accumulation and storage of P in natural and constructed wetlands as a means to limit eutrophication of water bodies is uncertain because sediment processes are poorly understood. Emergent macrophyte P accumulation may remove P from the water column or the sediment, and harvest of macrophytes may reduce surface water P or sediment P. This thesis is comprised of two papers: a critical review of wetland P processes responsible for P retention and release, and an investigation into the source of biomass P in a natural cattail (*Typha* spp.) wetland. The review finds evidence of organic P accumulation in wetlands but few measurements of complex inorganic P compounds of iron and calcium that would verify storage of P in these forms. The second paper documents the use of sequential fractionation of sediment and compares labile and recalcitrant P forms between vegetated and open water sites over a six month growing season. Temporal changes were found in some labile fractions, with a seven fold decrease in the OH P fraction countered by increases in HCO_3^- , HCO_3^- organic, and pore P. The paper concludes that the majority of plant biomass related P originated from sediment sources but no soil P fraction declined in response to the $\sim 5 \text{ g/m}^2$ biomass uptake of P during *Typha* growth.

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TABLE OF CONTENTS

	Page
ABSTRACT.....	ii
APPROVAL AND COPYRIGHT FORM.....	iv
ACKNOWLEDGEMENTS.....	v
TABLE OF CONTENTS.....	vi
List of Tables.....	viii
List of Figures.....	ix
CHAPTER 1 INTRODUCTION.....	1
1.0.1 Navigating this Thesis.....	1
1.1 Importance of P in the Biosphere.....	2
1.2 P in Deep Water and Wetlands.....	3
1.3 P Storage and Harvest in Wetlands.....	4
CHAPTER 2 PROCESSES OF PHOSPHORUS RETENTION AND RELEASE IN FRESHWATER WETLAND SEDIMENTS.....	6
2.1 Introduction.....	6
2.2 Separation and Analysis.....	8
2.3 Processes of P Retention.....	10
2.3.1 Settling.....	10
2.3.2 Adsorption.....	10
2.3.3 Precipitation.....	12
2.3.4 Algal driven precipitation.....	12
2.3.5 Organic P.....	13
2.3.6 Poly P.....	14
2.3.7 Organic P adsorption.....	15
2.3.8 Sorption capacity.....	16
2.4 Processes of Release.....	16
2.4.1 Flux.....	17
2.4.2 Anaerobic conditions.....	17
2.4.3 Shifts in pH.....	20
2.4.4 Organic P hydrolysis and enzyme attack.....	21
2.5 Buffers to Retention and Release.....	22
2.5.1 Oxidized layer.....	22
2.5.2 Microbial role.....	23
2.6 Accumulation.....	26
2.6.1 Inorganic P.....	27
2.6.2 Organic P.....	28
2.7 Discussion.....	32
2.8 Future Research.....	34

CHAPTER 3 CHANGES IN SEDIMENT PHOSPHORUS FORMS IN A TYPHA MARSH IN SOUTH CENTRAL CANADA.....	36
3.1 Introduction.....	36
3.2 Methods.....	40
3.2.1 Study site.....	40
3.2.2 Sampling.....	41
3.2.3 Laboratory analysis.....	42
3.2.4 Analytical observations.....	45
3.3 Results.....	46
3.3.1 Surface water.....	46
3.3.2 Physical features.....	48
3.3.3 Spatial variation.....	49
3.3.4 P extracts: differences between sites.....	49
3.3.5 P extracts: seasonal changes.....	52
3.4 Discussion.....	54
3.4.1 Site differences.....	56
3.4.2 Seasonal change.....	57
3.5 Conclusions.....	59
 CHAPTER 4 GENERAL DISCUSSION AND CONCLUSIONS	
5.1 Processes.....	61
5.2 <i>Typha</i>	61
5.3 Extraction Methods.....	63
5.4 Recommendations for Future Students.....	65
5.5 Engineering Significance.....	66
 REFERENCES.....	68
 APPENDIX	
A Site location.....	78
B Enclosure construction.....	79
C Sediment corer construction.....	80
D Spatial variation.....	81
E Resin strip preparation.....	82
F Special analytical procedures and results	83
Type II Geometric mean calculation.....	83
HCl ICP error.....	84
Alternate NaOH extract method.....	87
Blanks and spikes.....	93
Compaction.....	94
Iron, aluminium, magnesium and calcium.....	96
Soil texture.....	97
Presentation of P extract data.....	99

List of Tables

Table 2.1 Total phosphorus.....	29
Table 2.2 Quantification of several organic P forms.....	31
Table 2.3 Peat and P accretion.....	31
Table 3.1 Physical characteristics of each sediment horizon.....	49
Table 3.2 Comparison of total phosphorus and the coefficient of variation	55
Appendix	
Table 1 Comparison between standard method of OH extract SRP.....	89
Table 2 Comparison between reporting data as ppm and wt/vol.....	89
Table 3 Aluminium (Al), iron (Fe) and calcium (Ca) found in sediment.....	92
Table 4 Relative percentage of minerals found in intact soil cores.....	92
Table 5 Blanks processes with each extraction process.....	94
Table 6 Percent recovery from standards processed with solvents.....	94
Table 7 Sampling compaction data.....	95
Table .8 Soil texture as a percentage of ashed sediment.....	98
Table .9 Total P in sediment profiles in the enclosure and open water.....	100
Table 10 Correlation between percent water and P extracts.....	100

List of Figures

Figure 3.1 Total mass of unfiltered reactive phosphorus in the water column.....	47
Figure 3.2 Sediment P extracts from enclosure (EN) and open water (OW).....	51
Figure 3.3 All P extracts from the enclosure (EN) and open water (OW).....	53
Figure 3.4 Labile P extracts from the enclosure and open water.....	54
Appendix	
Figure 1 Location of Netley-Libau Marsh.....	78
Figure 2 Construction of the enclosure.....	79
Figure 3 Removal of the 40 cm sediment core.....	80
Figure 4 Bulk density of sediment horizons in enclosure.....	81
Figure 5 Bulk density of sediment horizons in open water.....	82
Figure 6 Regression curve showing relationship between sediment water.....	83
Figure 7 Analysis error found in ICP results with HCl extracts.....	85
Figure 8 Analysis error between certified standards sent for ICP	86
Figure 9 Enclosure (EN) and open water (OW) OH extract constituents.....	90
Figure 10 OH extract components as a percentage of TP for September.....	91
Figure 11 Sediment concentration of Fe, Al Mg and Ca in enclosure.....	96
Figure 12 Sediment concentration of Fe, Al Mg and Ca in open water.....	97
Figure 13 Soil texture in grams/m ² of horizon depth in enclosure	98
Figure 14 Soil texture in grams/m ² of horizon depth in open water	99

Chapter 1 INTRODUCTION

In recent decades, increased phosphorus (P) use by farmers and urban centres has led to eutrophic conditions in rivers and lakes (Baldwin et al. 2002; Turner 2005), the most notable in Manitoba being the highly eutrophic condition of Lake Winnipeg (Board 2006). In search of remedies to the problem of P abundance in lakes, two of many avenues of research emerge: 1) storage of P from natural processes inherent in the landscape, and 2) removal of P in an economically viable way.

1.0.1 Navigating this Thesis

This thesis addresses the areas of P processes affecting storage in wetlands and wetland biomass harvest for P reclamation. Two journal papers are included here that have been written over the course of a Master's degree in Biosystems Engineering at the University of Manitoba. The first paper (Chapter 2) is a critical review of the processes responsible for P retention and release in wetland ecosystems. Natural biogeochemical mechanisms are described and their importance evaluated by evidence of P accumulation in wetland sediment. The second paper (Chapter 3) reports an experiment in Netley-Libau Marsh, at the south end of Lake Winnipeg, designed to measure the changes in P forms over a growing season and determine P dynamics. The experiment was part of a larger project that assessed the viability of harvesting cattail biomass for biofuel and P removal. The two papers compliment each other because anomalies found in experimental data are explained by scientific literature in the first paper. Applied wetland management for P retention or removal depends on good understanding of P cycling mechanisms at work in a wetland. Important background to the project and aspects of wetland P cycling are

included in this introduction and not in the journal papers. Chapter 4 offers general discussions of both papers and recommendations resulting from the research.

1.1 Importance of P in the Biosphere

Phosphorus is a very important element: not only is it essential for life for cellular construction and energy transfer, but it is also very scarce in the biosphere, in view of its considerable demand by living organisms. Relative to its concentration in biota, and aside from carbon and nitrogen that can partition from the atmosphere, phosphorus is the least abundant nutrient in aquatic environments, requiring significant concentration in living organisms to meet their needs. P can be thought of as the most valuable nutrient, one that organisms hold onto and actively recycle. For this reason P is sometimes referred to as “life’s bottleneck” and explains the efficiency of biological scavenging for the nutrient (Asimov 1974). It also explains the evolutionary norm that life has adapted to: one of low P availability. The introduction of large amounts of sedimentary P back into the ecosystem (mined calcium phosphates converted to phosphate fertilizer, leading to soil P saturation and subsequent runoff), have very large impacts on ecosystems that have developed in nutrient poor conditions. For this reason, small shifts in P concentration (e.g., 20 $\mu\text{g/l}$ to 50 $\mu\text{g/l}$ in Lake Winnipeg) result in major ecosystem changes (Downing and McCauley 1992), because the system has a species composition for an oligotrophic environment and increased P concentrations cannot be quickly removed by natural processes.

1.2 P in Deep Water and Wetlands

Perhaps the best model of P cycling in aquatic systems can be found in the ocean, as it shows the cycling dynamics on a large scale (Tyrrell 1999). In the photic zone, dissolved P in the water column are actively scavenged by algae and bacteria, which is consumed by microbe grazers and they in turn, by predators. Wastes and organic material (all containing P) make their way through various organisms until gravity takes it into deep water, out of the reach of sunlight. Once out of the photic zone, algae can no longer make use of abundant P (Tyrrell 1999), and it makes its way to the bottom (sorbed to particles or incorporated into organic matter). From the ocean bottom the only way P can then return to the surface is by deep water up-welling, but for the most part it can be considered removed from the ecosystem.

In lakes, the process operates as described above, except the up-welling events are spring and fall turnover, and the photic zone is proportionally larger than the ocean. As the lake depth decreases, the photic zone increases relative to depth and a larger proportion of the water column is available for primary production and P recycling. When the depth of the water body is less than two metres, the photic zone extends to the bottom and thus P is potentially present for biological incorporation throughout the water column. Wetlands are characterized by their shallow depth and intense nutrient cycling, resulting in the high primary productivity found in many of them (Mitsch and Gosselink 1996).

1.3 P Storage and Harvest in Wetlands

Wetlands often serve to concentrate P in the landscape and they are ideal places to retain P from possible downstream transfer, either from sequestration in the sediment or from the harvest and removal of biomass. This function in a delta coastal marsh is important in retaining river borne P from entering a lake, such is the case in the Netley-Libau Marsh at the south end of Lake Winnipeg and the site of the experiment in Chapter 3. The fate of P in a wetland is dependent on which natural biogeochemical processes are dominant in a particular site. If processes of sequestration are more active (the process is described in detail in Chapter 2), then the wetland will cause net reduction in exiting stream flow P. If processes of mobilization are dominant, a net increase in bioavailable P results, and labile P will exit the wetland. Predicting and promoting sequestration necessitates a full understanding of the chemical and biological mechanisms as well as the conditions that promote these processes. Understanding these processes is key to determining P fate and wetland managers can then decide if alteration of conditions will improve P retention or if P recapture is a better alternative.

On the surface, harvesting of emergent *Typha* biomass from a wetland appears to be a simple equation: removal of P that plants have collected over the growing season lowers the total amount of P in the wetland, making more room for deposition. The real question, however is the source of P for *Typha* growth. Of the myriad of P forms present in a marsh, which ones are used by plants during the growing season? If P for *Typha* growth is sourced from surface water, harvest will have an immediate effect on surface water quality. If *Typha* biomass P is derived from sequestered P in the sediment, little short term effect would be expected from annual harvest, and harvest could be seen as counter productive by taking recalcitrant P from storage and potentially bringing it back

into the ecosystem, after harvested *Typha* is processed. Our hypothesis is that if *Typha* removes surface water P then P concentration should diminish and that if labile P in sediment are removed, those concentrations should be reduced by the end of the growing season. Removal of stored P may have an effect on the deposition of incoming P or on the viability of *Typha* growth. These questions have not been addressed in the literature and this thesis takes preliminary steps towards answering them.

Chapter 2 Processes of phosphorus retention and release in freshwater wetland sediments

2.1 Introduction

Eutrophication of lakes and rivers has been linked to nutrient increase, with phosphorus (P) commonly implicated as the limiting nutrient. Research has indicated that wetlands can offer an opportunity for P removal from stream flow (Mitsch and Gosselink 1996; Reddy et al. 1999), and treatment wetlands are being constructed to improve water quality—often with P reduction as a primary goal (Herskowitz 1986). In addition, natural wetlands are being used as tertiary treatment for human and animal wastewater with the expectation of low cost “permanent” P removal from streamflow (DeBusk et al. 2001).

The problem with this remedy is the poor predictability of a wetland’s ability to remove P. Examples of wetlands as either a source or a sink for P can be found. The ability of a wetland to remove P has been measured by inflow/outflow studies and results vary depending on loading history and wetland type (Richardson and Qiun 1999; DeBusk et al. 2001). Removal rates as high as 100% have been reported along with studies indicating a net release of P from the wetland (Herskowitz 1986; Maine et al. 2005).

While some reviews point out that net P reduction is the norm for many wetlands for most of the year (Reddy et al. 1999), other studies have found high outflow concentrations in newly constructed wetlands (Newman and Pietro 2001), diminishing P retention over a period of years (DeBusk et al. 2001; Mitsch et al. 2005), or not at all (Herskowitz 1986; Johnston 1991). Uncertainties may be governed by a gradual increase in P saturation of the wetland as a function of high loading (Richardson and Qiun 1999), or by abrupt

changes in sediment chemistry (e.g., anaerobic conditions). For example, some studies suggest that anaerobic conditions cause high P levels to flux from sediment to the water column (Shenker et al. 2005; Surridge et al. 2005), while other studies find no such release (Schindler 1976), or link P dynamics to algal production and decomposition (Gomez et al. 1998). Suggested mechanisms often cannot be applied successfully under different temporal conditions or to different wetlands. This leaves wetland scientists unable to make meaningful comparisons and encourages “black box” generalizations of P dynamics.

Phosphorus in surface water bodies has been reviewed by many authors, with topics covering bioavailability (Reynolds and Davies 2001), retention in wetlands and streams (Reddy et al. 1999), effects of macrophytes (DeBusk et al. 2001), and processes linked to P movement (Bostrom et al. 1988; Gachter and Meyer 1993). These reviews give excellent background and exploration of specific aspects of the current situation of surface water P contamination. However, conditions such as changes in hydraulics, sediment deposition, plant species, aeration, and biomass accumulation can greatly impact wetland P storage. Further, the fate of organically bound P, which often comprises the majority of wetland total P, has not been fully elucidated.

This review attempts to address important literature on the processes governing P storage and release for the purpose of clarifying future research priorities. These processes include: 1) the forms produced and retained in wetlands, 2) the chemical and biological mechanisms by which P is released to surface water, 3) buffering mechanisms affecting P retention and release, and 4) the evidence of accumulation of various P forms. In addition, a brief introduction describes the methods of chemical separation and analysis commonly used in sediment P studies.

2.2 Separation and Analysis

There are many techniques for the separation and analysis of the many P forms found in sediment. Analysis begins with separation from the soil matrix, usually performed by sequential fractionation, using successively stronger solvents. Labile P is removed first, followed by extractions of Fe, Al, Ca, Mg and C associated P, leaving residual, insoluble forms. Extracts are analyzed for soluble reactive phosphate (SRP) and total P, enabling inference of organic as non reactive P. Since the first methods were developed in the 1950s, many variations of solvents, concentrations, and procedures have been used; each designed to better define a particular P form. Many methods now exist, each producing operationally defined extracts with differences in solvent, molar strength, or sequence. Differences can affect the forms and quantity of P extracted. Good reviews exist of these procedures (Kleinman et al. 2001; Turner et al. 2005), including critical evaluation of specific techniques (Levy and Schlesinger 1999). In this review, P forms are discussed according to the associated form, because more than one solvent is used to remove a P form. (e.g., to remove Fe~P some studies use NaOH and others use dithionite). These differences in extraction methods reduce inter-study comparability, a problem made worse by relatively few studies.

A problem with sequential extraction is that the P removed with one solvent sorbs to another before the supernatant can be separated from the soil matrix, thereby incorrectly adding mass to the next extract (Moore Jr. and Reddy 1994; de Vicente et al. 2003; Benzing and Richardson 2005). Remedies for this, such as separate extraction solvents for Fe (dithionite), Al (oxalate) and organic P (e.g., hot NaOH) have created new problems: extraction techniques become more elaborate, lowering reproducibility. A test of four methods by 15 laboratories found standard deviations of up to 186% for some

steps (Ruban et al. 1999), leading to recommendations for the use of simpler, but more reproducible methods (Ruban et al. 2001).

When using the same extraction procedures, differences in wetland sediment make comparison of results difficult. For example, NaOH is a common fractionation solvent that extracts P associated with Fe and Al compounds, but in peaty sediments, it also dissolves humic and fulvic acids, removing P associated with them (Paludan and Jensen 1995; Reitzel et al. 2006). NaOH extracts of highly organic sediments have been found to hold little Fe and Al yet are rich in P (Reddy et al. 1998), thus confusing the assumption of the P forms present in NaOH extract.

Defining the organic P composition removed with an extract poses an additional challenge. It is estimated that 40- 50% of soil organic P compounds have not been extracted, isolated or identified (Celi and Barberis 2005), probably due to their low concentration, structural complexity, and sensitivity to analytic procedures (Turner et al. 2005; Worsfold et al. 2005). Organic P can be extracted by several methods, such as single-step qualitative procedures aimed at speciation (Turner et al. 2005), or sequential extraction used to quantify a broad spectrum of organic compounds. Recent techniques used in qualitative organic P determination have expanded the possibilities of P analysis (Turner et al. 2005; Worsfold et al. 2005). These include column separation (Pant et al. 2002), NMR (Reitzel et al. 2006; Turner et al. 2006), gel permeation (Gilbin et al. 2000), mass spectrometry (Cooper et al. 2005), and ion chromatography (Suzumura and Kamatani 1995). Additionally, photodegradation of P-C bonds or enzyme activity assays (Pant et al. 2002) are capable of measuring specific families of organic P.

2.3 Processes of P Retention

Phosphorus transported into a wetland can become retained by the wetland through many mechanisms. These processes include: 1) the physical settling of silt and particulates containing adsorbed non reactive and reactive P to particle surfaces, 2) formation of P associated precipitants in the water column, and 3) the incorporation into biota, forming organic P (Bostrom et al. 1988).

2.3.1 Settling

Silt associated P, which often makes up the highest portion of river P load (Chacko et al. 1981; Reynolds and Davies 2001), constitutes the majority of retained P in many wetlands (Fennessy et al. 1994; Reddy et al. 1999). Stream flow transports colloids and fine clay (both with large surface areas), providing extensive P adsorption sites per gram. Particulate matter is deposited in wetlands as velocity slows due to presence of vegetation (increased tortuosity) (Reddy et al. 1999). When tracking P movement through a watershed, studies in France (Garnier et al. 1999) and Canada (Bourne et al. 2002) found up to 60% lower total P concentrations after a river has passed through an impoundment reservoir.

2.3.2 Adsorption

Adsorption describes the physical association of P with a surface, ranging from loosely to tightly held. The tetrahedral shape of phosphate exhibits greater reactivity at its edges (oxygen) than from its centre (phosphorus), creating a stable molecule that easily forms associations (Harris 2002). This reactivity is responsible for change in protonation due to pH of the aquatic environment (e.g., HPO_4^{-2} and $H_2PO_4^{-1}$) and attraction to positive sites on mineral and colloidal surfaces. Aqueous orthophosphate (PO_4^{-3}) exits the water

column by its tendency to adsorb to particle surfaces and bond with cations. "Sorption" is a relative term dependent on variables that yield a dynamic flux of adsorption/de-sorption as a function of adsorption site availability, strength of attraction, P abundance, and pH. Depending on the sorption substrate and these variables, P may become more tightly bonded over time (Brady and Weil 1996).

Association of P to surfaces can be due to charge attraction or chemisorption: a ligand exchange reaction with phosphate substitution for OH^- or H_2O , resulting in a covalent bond (Pierzynski et al. 2005). Charge attraction uses little free energy and is reversible. Chemisorption is only partly reversible because net energy loss is greater, and the rate of desorption is slower, decreasing with time (Baldwin et al. 2002; Pierzynski et al. 2005). When P is covalently bonded to a mineral surface, particle surface charge is affected and the energy required for additional sorption (and desorption) decreases. This was shown as ratios of Fe:P in a $\text{FeOOH}\sim\text{P}$ complex decreased from 50 to 12, the energy required for sorption dropped from 32 to 3.4 J per mol (Miltenburg and Golterman 1988). These observations suggest that less additional P is likely to adsorb as P saturation of a particle increases: an example of "loosely bonded" P.

Low P saturation on a substrate with many binding sites promotes P chemisorption, beginning with charge association and resulting in ligand exchange: first monodentate, then bidentate, then occlusion. Common minerals likely to adsorb P in this way are Fe and Al (as oxides and oxyhydroxides common in acidic soils), and Ca and Mg (as carbonates, primarily in calcareous soils). Competition for sites is controlled by how tightly H^+ and OH^- ions are attracted; thus, pH is important in sorption. The number of sorption sites occupied by P and other anions influences particle charge: the greater the number of anions on a particle, the weaker the charge attraction, due to lower overall

charge which also favours de-sorption back into solution. As a particle approaches saturation, further P association more closely follows standard chemical equilibrium dynamics.

2.3.3 Precipitation

There have been suggestions that sorption and precipitation form a continuum so it is difficult to verify if P is retained out of solution via strong adsorption (Harris 2002), or precipitation. Phosphate precipitation is the bonding of soluble ionic species to form a lattice structure (Baldwin et al. 2002), taking PO_4 and available cations (Fe, Al, Ca or Mg) out of solution. Once precipitates form they are generally more resistant to dissolution than monodenate chemisorption. P adsorbed to metal oxides or carbonates may become more occluded, forming a metal phosphate in time, such as a six-fold coordination with Fe and Al cations or eight-fold coordination with Ca and Mg (Harris 2002).

2.3.4 Algal driven precipitation

Algal production in lakes plays a role in P co-precipitation with Ca and the same process may occur in wetlands. This is a biologically driven process where algae consume dissolved CO_2 (as carbonic acid) faster than it can be replaced by atmospheric partitioning, driving up the pH. At high pH (e.g., pH 8.3) the saturation point is reached for calcium carbonate (CaCO_3) and its formation provides a sorption substrate for P. Laboratory conditions that replicated this process found 80% of P had exited the water column along with most of the Ca and all of the algae (Hartley et al. 1997). This observation agrees with Bostrom's assertion (1988) that CaCO_3 precipitation is self-flocculating and removes P, bacteria and algae. Penn *et al.* (1997) found no increase in

CaCO₃ precipitation at 18 meters and at the bottom of a eutrophic, calcareous New York lake, showing carbonate precipitates originated at the surface. This also indicates dissolution did not occur even in deep water where pH would presumably be lower than in the photic zone. Seston was fractionated and labile P, refractory P (Fe and Ca~P), and chlorophyll were correlated, confirming algal production reduced soluble P in the water column. Surface water SRP in eutrophic shallow wetlands is sometimes below detection limits (Gomez et al. 1998; Noe et al. 2003), probably a combined effect of surface sorption dynamics, microbial scavenging, and algal co-precipitation with Ca.

2.3.4 Organic P

High levels of primary production in wetlands cycle available inorganic P into organic P forms, often comprising the majority of total P. Plants and microbes primarily absorb P as simple inorganic phosphate, but inside the cell it is converted to cellular constituents, such as polyphosphate, nucleic acids (DNA and RNA), phospholipids, adenosine triphosphate (ATP), and others. These complex organic P compounds can be excreted during normal cell activity or released after cell death. Few studies separate and analyze wetland sediment into classes of organic P, yet retention in a wetland may be largely dependent on the chemical structure. Identifying persistent forms of organic P would help in determining P retention processes.

Forms of organic P are classified by the bonding of the P functional group (PO₄) with carbon. Common forms are monoesters; a phosphate end-group attached to a carbon, such as sugar phosphates, ATP or inositol phosphates (a ring carbon with phosphate one or more C); and diesters, where the phosphate bridges two C components, such as DNA

or phospholipids. Identification of these bonds is helpful in explaining reactivity with sorption substrates and enzymes.

Organic P can also be retained in a wetland by the slow accumulation of recalcitrant organic matter (humic acids and peat). Large amounts of P are associated with organics but it has yet to be resolved whether P is intrinsically incorporated into the humic structure, lyophilically absorbed, chelated with multi-valent metal cations and humic substances, or simply sorbed to positively charged portions of organic matter (Johnston and Tombacz 2002).

2.3.5 Poly P

Poly phosphates are chain phosphates, also called condensed phosphates, due to the missing oxygen between the P groups. Short chain P of two (pyrophosphate) and three (triphosphate) groups have been reported in nature and are probably the remnants of ADP and ATP that have been cleaved from the C portion. Tripoly P is also a product of the detergent industry (Wazer 1958; Baldwin et al. 2005) and exists in various lengths as a byproduct of phosphate fertilizer (Tisdale et al. 1985). There is also evidence that condensed phosphates are produced by some bacteria species in wastewater treatment plants where excess P allows for luxury uptake (Marais et al. 1983). Under aerobic conditions excess P is stored by certain bacterial species as poly P and can then be used for respiration during anaerobic conditions. While this has been theoretically proposed for freshwater sediment (Davelaar 1993), the process is questioned by some and has not been proven to date (Golterman et al. 1998).

2.3.6 Organic P adsorption

Investigation of retention mechanisms in terrestrial soils have found a direct relationship between P end-group availability and persistence in the soil (Celi and Barberis 2005). Authors found monoesters with many P groups, such as Myo-inositol hexakisphosphate (IP_6), dominate organic P soil composition, constituting 80% of total organic P (Turner et al. 2006), even though these compounds are produced in small amounts by plants. IP_6 is thought to attach to iron oxide at 4 sites and to calcium minerals, such as illite and kaolinite at three sites (Celi and Barberis 2005). Multi site bonding inhibits release to soil solution because several anions are needed simultaneously to replace all bonding sites (Celi and Barberis 2005). Thus, in both acid ($FeOOH$ and $AlOOH$ dominated) and calcareous terrestrial soils, IP_6 is usually the dominant form of organic P. The same trends occur in aqueous solutions where surface and colloidal sorption increases with compounds with more P end groups, such that $PO_4 <$ pyrophosphates $<$ polyphosphates (Wazer 1958).

Fewer bonding sites on a molecule result in weaker sorption to surfaces, as is the case with phosphate diesters, which have a single sterically available bonding site from the conjugated PO_4 (Baldwin et al. 2002). Sorption to particles removes them from soil solution and protects them from attack by enzymes or acids, enabling persistence in soil. Indeed, these compounds are found in low concentrations in terrestrial soil: phosphate diesters (DNA and phospholipids) make up 70-80% of organic P contained in *E. coli* and fungi but make up only 7% of organic P in terrestrial soils (Celi and Barberis 2005). Monoesters, however, make up 20-25% of microbe organic P but constitute 50% of soil residual organic P. This trend confirms theoretical sorption dynamics in which phosphate end groups (i.e., monoesters) can sorb more tightly to clay surfaces than diesters. These

processes of sorption, precipitation and organic P formation can be reversed depending on factors influencing their dissolution and bioavailability.

2.3.7 Sorption capacity

Considerable work has been done on P sorption capacity of wetland sediments (Reddy et al. 1999; Richardson and Qiun 1999; Reddy et al. 2005), broadly concluding that loading history and substrate composition effectively determine the sorption potential in wetlands. In an oligotrophic system with unsaturated Fe and Al oxides, a poorly nourished bacterial population can potentially hold large amounts of P (Schindler 1976). On the other hand, a wetland with a history of high P input is likely fully saturated and release may equal or exceed new inputs (Johnston 1991; Richardson and Qiun 1999). Wastewater additions to wetlands are comprised of labile forms of organic and dissolved P but include virtually no clay or silt as new potential sorption surfaces. P retention of these additions then depends on existing particle surfaces, which may already be fully saturated. A maximum loading rate of $1 \text{ g P m}^{-2} \text{ yr}^{-1}$ has been proposed (Richardson and Qiun 1999), above which P export and risk of habitat deterioration increase.

2.4 Processes of Release

Phosphorus release from a wetland can be defined as the conversion of stationary P to dissolved, bio-available P that can enter the food chain or exit the wetland. Depending on the form, different processes act upon it. From a mechanism-driven perspective, factors in P release have been listed as: concentration gradient, bioturbation, O_2 consumption, CO_2 addition, acid and enzyme release, and microbial activity (Golterman

1995). Sediment erosion from increased hydraulic flow and P removal from biota transport (e.g., fish migration) are additional factors not reviewed here.

2.4.1 Flux

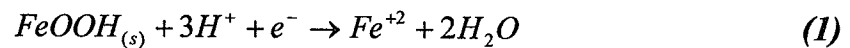
Flux of P from sediment to surface water roughly follows equilibrium dynamics, where P moves from high to low concentration. This gradient can occur by exposing high P sediment to low P water, either from fresh water exchange caused by wind, rainfall, snowmelt, or by mechanical mixing of the sediment by benthic organisms (Schindler 1976), carp or other benthivores. It has been noted that flux correlates with high sediment P concentrations (Fisher and Reddy 2001), where sediment may be fully saturated. High flux is also seen concurrent with high pore water concentration (Theis and McCabe 1978), indicating movement and exchange via pore water to floodwater.

2.4.2 Anaerobic conditions

Anaerobic conditions often cause the release of P from sediment as recorded in lakes (Theis and McCabe 1978), constructed wetlands (Herskowitz 1986; Maine et al. 2005), and natural wetlands (Newman and Pietro 2001). Several factors can produce this oxygen-depleted environment but biomass accumulation is the most common. After periods of high productivity, biomass collects on the sediment where decomposition by micro organisms consume oxygen from the ambient water and sediment. Microbial activity slows, and populations shift to those able to use other electron acceptors for their respiration, creating a reducing environment (low redox conditions). In a homogenous anaerobic system, micro organisms sequentially reduce oxidized forms of O, N, Mn, Fe, S, and finally, C (Mitsch and Gosselink 1996; Essington 2004). Relative abundance of

alternate electron acceptors (NO₃ and Mn) and microbial species composition may buffer P release from Fe oxides (FeOOH).

Observations of increased P solubility under reducing conditions began with Mortimer (Mortimer 1942), who concluded P release was driven by microbial activity using Fe⁺³ as an electron receptor. In the process of changing the oxidation state of Fe⁺³ to Fe⁺², the FeOOH compound becomes soluble, releasing the P sorbed to its surface. The reduction reaction is as follows:



Most studies find negative correlations between soluble Fe⁺² in the water column and anaerobic conditions (Eh) (e.g., $r = -0.55$, $p \leq .001$ (Sallade and Simms 1996)) or positive correlations between reactive P and aqueous Fe ($r^2 = 0.96$, $p \leq 0.01$ (SurrIDGE et al. 2005)). P flux into solution from flooded calcareous peaty soils in Israel was monitored for 120 days and a 1:1 molar ratio of Fe⁺² to PO₄ was found at first, increasing over time to 15 - 60:1 (Shenker et al. 2005). This was attributed to surface-sorbed P dissolving at the onset, and less available P from the inner layers of the matrix dissolving later in the experiment. Others have also found non-linear behaviour in reducing conditions. Soils released P under anaerobic conditions but, if P was added to floodwater, they also absorbed more P under similar aerobic conditions (Patrick and Khalid 1974), due structural changes of the iron oxyhydroxides. When 14 soils were flooded for 60 days, a three-day trend reversal was observed in pore water and floodwater P after 22 days in the majority of soils (Young and Ross 2001). Bacteria fed on various C sources and then exposed to low Eh did not provide evidence of Fe⁺³ reduction, Fe-S precipitation or Fe-P re-adsorption during anaerobic core incubations (Mitchell et al. 2005). Instead, they found

Fe⁺² release concurrent with P uptake, in direct contrast to the findings of Mortimer and many others. Numerous similar observations can be found, perhaps due to differences in sediment composition, underscoring the gap in understanding of P behaviour.

Environmental conditions can also produce P behaviour contrary to the accepted norm. Gomez (1998) sampled a shallow Mediterranean lagoon monthly for a year and found no evidence of redox-influenced P flux. Instead, P moved to and from the sediment due to decomposition and algal demand. Lower redox conditions corresponded with lower temperature, lower bacterial activity and yet an increase in sediment Ca and Fe associated P.

In a review of sediment P release, Bostrom (1988) questions Fe chemistry as the primary mechanism responsible for P flux from sediments. He suggests other reactions compete for P, listing nitrate abundance as an alternative electron acceptor, microbial uptake and storage of P, H₂S reactivity with Fe to produce FeS, and CaCO₃ as a sorption substrate for P released from FeOOH dissolution. Golterman (2005) augments these ideas with a simple division of hard water and soft water sediment chemistry: soft water is Ca poor and thus Fe is the driving force in P release, while hard waters tend to be Ca rich. He points out that Mortimer worked in soft water sediment where Fe chemistry would be dominant. Other authors have stated P dynamics in particular sediment can be Ca or Fe governed (Moore Jr. and Reddy 1994). There has been no quantification of what Ca and Fe concentrations or ratios are needed to dominate these dynamics.

Humic acids in sediments may play an important role in reduction of Fe acting as a medium between the microbe and the Fe⁺³ (Lovley et al. 1996). Humic acids may act as temporary electron acceptors due to the abundance of their charged sites (Johnston and Tombacz 2002).

Aluminium functions as a precipitate oxide in P retention and is not reduced by bacteria. Al has been credited as acting as a buffer, re-adsorbing P released under low redox conditions (Ann et al. 2000; Koski-Vahala et al. 2001). Others found buffering of flux from pore water (at low redox potential) correlated strongly with the oxalate Al extractable fraction (Young and Ross 2001). In a comparison of peat wetlands for P retention, Richardson (1985) found amorphous Al to be a better predictor of the P adsorption index than Fe ($r=0.93$ and 0.62 , respectively).

2.4.3 Shifts in pH

Solution pH is a main driver in the dissolution equilibria of many soil phosphate compounds (Johnston and Tombacz 2002; Essington 2004), as well as oxides and carbonates holding sorbed P. Carbonates of Ca and Mg are more soluble below pH 7, and solubility increases for oxides of Fe and Al above 6 (Havlin et al. 2005). In illustration of pH and P release, Everglades wetland sediment cores were incubated at various redox conditions and pH levels (Moore Jr. and Reddy 1994). Lower pH (5.5) resulted in much higher exchangeable Fe^{+2} and P release to solution than higher pH levels. At higher pH, P released from FeOOH was re-adsorbed by CaCO_3 , but the lower pH caused dissolution of this and other Ca~P compounds. A study of lake sediments in China found P release (at high pH) from sediments with a high Fe~P:Ca~P ratios and at low release for those with low ratios (Huang et al. 2005). This agrees with studies describing Ca~P fractions sensitive to low pH (Gomez et al. 1998; Penn et al. 1999), and FeOOH ~P fractions to high pH. Therefore the potential buffering capacity provided by Ca and Fe presence in a soil may be eliminated by significant shifts in pH allowing P release.

Changes in soil solution pH can be driven by a number of known factors, such as lower redox conditions and microbial activity. Under extended low redox conditions acidic soils becoming more basic (due in part to reaction 1), and carbonate soils become more acidic. An endpoint of pH 7 is theorized for each soil under extended anaerobic conditions (Essington 2004). Faster rate of organic matter decomposition increases organic acid production and CO₂, producing carbonic acid and lowering pH (Essington 2004). These effects have been seen with the flooding of terrestrial soils and decreased pH at greater depths of lake sediment (Penn and Auer 1997), where CO₂ diffusion is slower. There may be additional causes of pH shift such as inundation of acidic snowmelt waters or chemical reactions within sediment, but these areas appear to be unstudied.

2.4.4 Organic P hydrolysis and enzyme attack

Organic P in wetlands can become bio-available or labile as PO₄ through two mechanisms. Whether it is present in soil solution or sorbed to surfaces, cellular acid excretion causes desorption of organic P from its substrate (Havlin et al. 2005), enabling hydrolysis or enzymatic attack. Cellular release of phosphatase enzymes cleave PO₄ from organic compounds, allowing cellular absorption (Quiquampoix and Mousain 2005).

Enzymes can act specifically or generally depending on the enzyme type and the environment it enters. Some enzymes target specific PO₄ cleavage sites and others are generalists. A review determined enzyme extracts from different plant roots and bacteria have optimum P release activities at differing pH (Quiquampoix and Mousain 2005). The presence of some metal cations can inhibit or promote enzyme function. If organic P classes accumulate, it could be because enzymes particular to that family are not present or active (Pant et al. 2002). In the Everglades, wetlands phosphatase activity was

undetectable in areas of high P loading while it was significant in areas of lower loading (Richardson and Qiun 1999).

2.5 Buffers to Retention and Release

There are several mechanisms that act as buffers to the transfer of P from sediment to floodwater. Depending on sediment chemistry and environmental conditions, these mechanisms may play a significant role in P transfer or retention. Three primary buffers have been noted in literature: the oxidized sediment layer, microbial activity, and alternate electron acceptors. High levels of alternate electron acceptors to Fe under anaerobic conditions (e.g., nitrate) could buffer P release from FeOOH (Bostrom et al. 1988; Golterman 1995), and several experiments have been conducted to test effects of these compounds on P flux (McLatchey and Reddy 1997; Noe et al. 2003), none producing definitive results. This review will focus on oxidized layers and microbial buffering effects.

2.5.1 Oxidized layer

In some lakes with high Fe content, the zone between aerobic and anaerobic conditions can be seen as a reddish layer of oxidized Fe just under the sediment-water interface. This layer of FeOOH acts as a buffer binding soluble P as it is released from the lower anaerobic sediments (Penn et al. 1999). Studies of incubated cores revealed that a visible layer developed after 2-5 days of anaerobic conditions (Penn et al. 1999) and in the majority of 14 incubated flooded soils (Young and Ross 2001). The authors measured P release into pore and floodwater but found increases did not correspond to each other, indicating a buffering effect of the layer. Penn *et al.* (1999) ascribed high P flux from

eutrophic lake sediments to low levels of sediment Fe (one third of the average of several similar lakes) and concluded this oxidized layer lacked enough Fe to hold all the P being released. Some authors suggest high P concentrations in surface sediment are the result of soluble P diffusion into this layer where it sorbs to FeOOH, increasing the P concentration disproportionately (Moore Jr. and Reddy 1994). If true, high surface sediment P ascribed to sedimentation or anthropogenic origin may be from precipitation in the oxidized layer. This issue could be resolved by determining the redox potential in these layers as well as the forms of P present there.

The source of oxygen in this layer can be ambient aerobic waters or benthic algae. Lake sediment algal diel oxygen production has been shown to negatively correspond with P release (Carlton and Wetzel 1988) and selective herbicide treatment of wetland benthic algae induced nutrient release (Goldsborough and Robinson 1985).

2.5.2 Microbial role

Microbial activity in sediment is responsible for nutrient cycling and O₂ consumption, making it pivotal in affecting P retention, release and buffering. Increases in bacterial populations create a demand for P that can remove P from ambient water or recalcitrant forms. However, direct experiments on buffering capacity of bacteria are relatively uncommon.

Research on the role of microbial activity in P release from sediment makes comparisons of P behaviour between normal and sterilized samples. In a highly calcareous (50% dry wt) lake sediment, microbial activity buffered P exchange between surface water and pore water. P flux increased four fold in sediment without bacteria compared with controls (Eckert et al. 1997). This result is explained by P diffusion from

high concentration to low (pore water to surface water), moving freely when no bacteria mediated its exchange. In another experiment, different C forms (glucose, acetate) were added to Australian wetland sediment samples and P uptake was measured (Khoshmanesh et al. 1999). Sediment with live bacteria removed more P than treatments without bacteria, and C-fed bacteria reduced P to zero. Subsequent anaerobic conditions (ten days) caused no release from any treatments except those fed acetate, which released large amounts of P. The authors proposed this P came from poly P, produced and stored by bacteria during aerobic conditions.

Both of these experiments used sediment of roughly the same total P concentration (1000 ppm) and both incubated soils for similar time frames. The results are conflicting, however, and may be due to differences in experimental design (measurement of native P flux directly from sediment in one case, versus measurement of added P to sediment and then flux from it). In the first experiment (Eckert et al. 1997), the causes of P release in the absence of bacteria was not elucidated, which leaves in question if P release would have taken place in aerobic conditions.

A review of bacterial involvement on the fixation and mobilization of P from sediments concluded that retention or release depends on bacterial nutritional needs and seasonal changes of bacterial biomass (Gachter and Meyer 1993). This conclusion appears reasonable; however, authors noted direct measurement of bacteria and their buffering effect has not been found in the literature.

In some studies, P release and low redox conditions are explained by microbial activity and Fe reduction, whereas in others different causes are suggested. For example, in dried English floodplain soil, horizons were flooded and incubated for 28 days (Surridge et al. 2005). P release was below detection limit in all horizons for the first 24

hours, after which high release was measured. This suggests establishment of bacterial colonies that then facilitated P release. Authors measured concurrent Fe^{+2} and P flux, giving support to the bacterial mediated release mechanism. In contrast to this study, re-flooded Georgia floodplain clay sediments were fractionated monthly for half a year to determine the form of P released to surface water (Wright et al. 2001). No change was found in the Fe fraction (NaOH extract) and all P release was attributed to microbial death and stored poly P release, but was not a measured parameter. Taken together, results suggest significance of Fe on P release in one case (even though the sediment was mostly organic matter) and not in the other.

Algae can also play a role in buffering P release from sediment. In a large, newly reclaimed wetland in Florida, N and P flux from sediment was measured in the months after flooding. The flux increased for three months, decreasing when diel oxygen cycles appeared. The oxygen came from algal mats on the sediment surface that buffered high pore water P concentrations to the low P floodwater (Newman and Pietro 2001). The exact process involved algal buffering of P release has not been defined (Goldsborough and Robinson 1985; Carlton and Wetzel 1988).

Buffers can be a combination of mechanisms in nutrient deficient systems, such as Canadian Shield lakes, where bacterial nourishment and unsaturated sediment can receive large amounts of P. Phosphorus was added to an oligotrophic lake for six years, producing algal blooms and subsequent organic matter deposition on the bottom (Schindler 1976). Contrary to expectations, this did not result in a thin layer of concentrated P on the sediment surface. Instead, P was measured in slightly elevated concentrations (compared to pretreatment) within the top 6 cm of sediment (this thickness represented 60 years of deposition). Annual P deposition had been incorporated to a

surprising depth either by microbial processes or bioturbation by benthic worms. Nutrient additions to the lake were mediated by these buffers, protecting the surface water from nutrient flux under anaerobic conditions (this was tested and no flux was found). Others have also concluded bacteria controls sediment P release in oligotrophic lakes whereas redox controls it in eutrophic lakes (Gachter and Meyer 1993).

2.6 Accumulation

Measured accumulation of P forms in sediment support theoretical retention and release processes at work in a particular wetland. However, comparison of accumulated forms is frustrated by the lack of good data on the subject, as few reports record rates of accumulation or define compounds beyond their (often differing) separation methods. Most sediment accumulation of inorganic P is in the Fe~P and Ca~P forms, with Al and Mg rarely mentioned. More data has been found on organic P, enabling wetland comparisons.

Silt deposition represents the largest P retention mechanism. Work with sediment traps in wetlands has shown great variation in deposition patterns both spatially and temporally (Fennessy et al. 1994). Accurate data requires constant sampling of defined outflow channels for inflow/outflow silt measurements (Mitsch et al. 2005). Measuring accumulation by ^{137}Cs and ^{210}Pb dating of wetland cores has shown hydraulic influences to be the major factors in silt deposition, indicating that accretion is site specific (Kadlec and Robbins 1984; Graham et al. 2005).

2.6.1 Inorganic P

Accumulation of Fe and Ca bonded P has been measured in lake sediment and much less so in wetlands. Evidence of precipitation/sorption is found in both systems, as correlations ($r^2=0.64$, >0.001) between total P and total Fe in lake sediment of 116 Danish lakes (Jensen et al. 1992), suggest co-deposition of P and Fe. However, stream input to these lakes was not measured, so the mechanism could also be silt deposition. In some cases, similar proportions of Fe~P and Ca~P present in stream flow has also been found in wetland (de Vicente et al. 2003) and delta sediment (Mayer et al. 2000). This suggests inorganic P accumulation is via silt deposition rather than from precipitation and sorption within the wetland.

Moore and Reddy (1994) state Ca dominated marshes are not subject to P re-suspension by changing redox where as Fe dominated marshes are. Seventy five percent of total P in an Everglades wetland sediment was Ca and Mg bonded and the authors concluded P dynamics in that wetland are governed by these cations (Reddy et al. 1998).

Ca~P sequestration in wetland sediments may operate in the same way as in terrestrial soils, with increasing stability over time. As occlusion increases over time, more complex forms of Ca~ P compounds form and solubility of these decreases with complexity (Pierzynski et al. 2005). For example, dicalcium phosphate, octacalcium phosphate and hydroxyapatite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, $\text{Ca}_4\text{H}(\text{PO}_4)_3 \cdot 2.5\text{H}_2\text{O}$, $\text{Ca}_5(\text{PO}_4)_3\text{OH}$) have increasing complexity and decreasing solubility (Olila and Reddy 1997; Havlin et al. 2005). These compounds may form in sediment as they do in terrestrial soils (Brady and Weil 1996), but measurements of abundances within wetlands are rare. Data are available from lake sediment where increasing concentrations of apatite P (Ca~P) was found at greater depth in both Lake Winnipeg (Mayer et al. 2000), and in a British Columbia lake

(Slaymaker 2003). Al and Fe bound P were in greater concentrations at the surface (Mayer et al. 2000). These studies, as with most, did not identify the species of phosphate present. Olila and Reddy (1997) used x-ray spectrophotometry but did not identify hydroxyapatite in Everglades sediment, either because it was amorphous or too scarce.

Aluminum oxides are not frequently mentioned in wetland sediment studies but some authors have attributed re-adsorption of released P to Al (Ann et al. 2000; Koski-Vahala et al. 2001), or found pore water P flux buffering capacity (at low redox) correlated strongly with the oxalate Al fraction (Young and Ross 2001). Though Ca and Mg appear in tandem as sorption and bonding partners for P, no specific measurement of Mg~P is found in the literature.

Long-term storage of inorganic P in wetland sediment would presumably consist of compounds involving Fe, Al, Ca and Mg. The determining factors are the abundance of available cations and their stability within the system. If conditions change significantly from those favouring deposition, these forms will dissolve and be released back into the water column.

2.6.2 Organic P

Organic P is often present as the majority of total P in wetlands (Table 2.1), although systems contain sediment of different character. Highly organic sediment, short hydroperiods, and different levels of primary productivity may affect organic P

Table 2.1 Total phosphorus (TP) and two organic phosphorus fractions from sediment of different wetland types.

Reference	Wetland type	TP, ppm	Organic P as % of TP	Humic P as % of TP
Graham et al. (2005)	Natural marsh	880	*	38
	Restored marsh	954	*	28
Gilbin et al. (2000)	Marine lagoon	725	17	3
Paludan and Jensen (1995)	Danish lake sediment	98	81	30
SurrIDGE et al. (2005)	River floodplain	600-1400	75-95	NA
Reddy et al. (1998)	Everglades	686-1050	56-70	18-25
Wright et al. (2001)	River Floodplain	375	33	NA

* Differences in extraction procedure gave no comparable TP values.

production and buildup. Even within a wetland type, significant variation in organic and humic P is found. For instance, natural lake marshes in Oregon were compared with recently restored marshes and higher humic P was found in natural marshes (Graham et al. 2005). This was attributed to new sedimentation in the restored wetlands that changed the relative proportions. Reddy *et al.* (Reddy et al. 1998) sampled multiple sites along a transect from a high P inflow to an Everglades marsh, and found decreased P with distance, reflecting the effect of human impact on the marsh. Two studies examined river floodplains, with SurrIDGE *et al.* (2005), looking at a richly organic substrate in England and Wright *et al.* (2001), at experimentally flooded clay sediment in Georgia, USA. Both of these studies show that organic P can be a major portion of total P and that humic acid P is a principal component.

Studies use various techniques to determine whether P is sorbed, chelated or structurally incorporated with organic matter. Using the same extraction procedures on different sediment one study found high Al levels in humic extract and concluded Al

bonding between P and humic acids (Paludan and Jensen 1995). In another study, Al and PO₄ were added to sediment and no increase of P in the humic extract was detected (Jensen et al. 2005). This author eliminated sorbtion and chelation as binding mechanisms, concluding that virtually all humic P was incorporated in the structure. This finding contrasts with Giblin *et al.* (2000), who used gel permeation chromatography to separate extracts and found large molecule humic acids (>50,000 Da) did not contain P, but that P was associated with lower molecular weight species, similar in size to fulvic acids. Different conclusions may arise from different analytical procedures (Giblin used hot 2M NaOH to extract humic and fulvic acids whereas Jensen and Paludan used 0.1M NaOH) or from inherently different sediment composition.

Few researchers have published quantification of specific classes of organic P from wetlands. Only three studies have been found giving a broad spectrum of organic P classes (Table 2.2). Diester P in wetland sediment was unexpectedly high when compared to terrestrial soil extracts (Turner and Newman 2005), where they are under 10% of total organic P (Celi and Barberis 2005). Also surprising was the lack of IP₆ in two studies; this compound usually makes up the majority of terrestrial soil organic P. Poly P was found in lake sediment affected by human impact and not in more natural waters, suggesting detergent and fertilizer sources (Reitzel et al. 2006).

Table 2.2 Quantification of several organic P forms found in wetland sediments and constructed wetlands (CW). Specific P class columns are given as a percentage of OH extract P.

Reference	Wetland type	OH~P % of TP	Monoester P	Diester P	Pyro P	Poly P	Humic P	IP ₆ (Phytate)
Golterman (1995)	Marine lagoon	18	NA	NA	NA	NA	3	5
Reitzel et al. (2006)	Lake sediment	27- 41	15-30	5-17	3-11	0-15	4-14%	NA
	Humic fraction*	NA	48-59	13-31	0	0-9	NA	NA
Turner and Newman (2005)	Everglades CW floc	24-67	13-62%	16-53%	3-22%	NA	NA	NA

* Humic P from the row above was further qualified

The rate of accumulation of peat is perhaps the most important measure of organic P increase in wetlands, as this constitutes a large proportion of wetland P. Three studies of peat accretion used radioisotope dating and found surprisingly similar values (Table 2.3) in Everglades and Washington state wetlands. Peat and P accretion correlate ($r=0.90$) in Reddy's data, but P build-up also correlates with cations (Ca, $r=0.98$, Fe, $r=0.86$, Al, $r=0.84$), suggesting precipitation or silt deposition.

Table 2.3 Peat and P accretion. Radioisotope dating gives approximate accretion rates in 3 American wetlands since 1964. Note different units in peat accretion of second Everglades study.

Reference	Wetland type	Peat Accretion	P accretion
Reddy et al. (1993)	Everglades	39 mm/yr (mean)	0.11-1.14 g /m ² /yr
Craft and Richardson (1993)	Everglades	94-161 g /m ² /yr	0.46 g /m ² /yr
Graham et al. (2005)	Lakeshore peatland Oregon	37 mm/yr (mean)	0.45 g /m ² /yr

Both Craft and Reddy found increased peat accumulation in areas of higher nutrient input, but P burial as a percentage of P inputs was greater in unenriched wetlands

compared to enriched wetlands (100-133%, 87%) (Craft and Richardson 1993).

Considering only 13-20% of annual biomass production is incorporated into peat (Craft and Richardson 1993) this represents a high assimilation.

2.7 Discussion

Predicting P behaviour in a wetland is complicated by the interdependence of the processes described so far. Abiotic influence on biological processes and vice versa can create unexpected P behaviour. In addition, retention mechanisms understood in terrestrial soil may not hold true in wetland sediment.

Detailed mechanisms of P release under redox conditions have not been fully explained by research to date. Low redox conditions can liberate P from FeOOH and this may be retained by adsorption to carbonates. If pH gets low enough (reaction 1), carbonates dissolve, releasing all sorbed and occluded P. (Moore Jr. and Reddy 1994) Al has sometimes acted as a buffer and adsorbed freed P (Sallade and Simms 1996; Young and Ross 2001), but the mechanism can only be considered permanent where sediment experiences no increase of pH. Some authors have noted the importance of Al in P storage (Richardson 1985), but this may not pertain to all wetlands at all times. With acidic and calcareous soils experiencing opposite pH change under reducing conditions, relative abundance of Fe, Al, Ca and organic matter may determine what forms of P can exist. *In situ* Eh and pH measurements during anaerobic periods, in conjunction with detailed sediment analysis, would elucidate many of these questions.

Organic P sorption dynamics as understood in terrestrial soils appear to be fundamentally different in wetland sediment, judging from the limited data available.

Proportional differences in diester P abundances in wetland sediment and terrestrial soils may mean enzymatic activity is different in the two environments. Some metal cations (Quiquampoix and Mousain 2005) are known to affect enzyme activity and perhaps anaerobic conditions produce oxidation states in these metals inhibiting or promoting specific enzymes, thus affecting hydrolysis of particular families of organic P. Greater P availability in wetlands may also provide microbes with adequate P supply without the energy investment in enzyme release. This has been noted in laboratory studies (Quiquampoix and Mousain 2005) and field work (Richardson and Qiun 1999), where enzyme activity was only induced by P deficiency. On the other hand, the monoester IP_6 is normally persistent in terrestrials soil but was not found in three of four studies analyzing wetland sediment (Suzumura and Kamatani 1995; Reitzel et al. 2006; Turner et al. 2006), suggesting fast breakdown in wetlands. Are these anomalies due to a tiny sample size and poor separation and analysis techniques, or do they point to fundamentally different mechanisms at work in wetland sediments? Enzymatic breakdown of P may be the largest single activity releasing stored P from wetlands, yet it is virtually unstudied in that setting. More extensive collaborations between wetland scientists, biochemists, and soil scientists may lead to an improved understanding of the enzymatic processes in wetlands.

The literature shows a healthy macrophyte community producing peat may be equal in importance to silt deposition for P retention. Greater biomass production leads to more peat, but initially can lead to higher P in the bioavailable pool, which may also exit the wetland. For example, an invasive *Typha* species increased biomass litter 14-fold over native emergent species but also produced 10 times more soluble P in surrounding sediment (Angeloni et al. 2006). The increase of bioavailable P may have come from the

water column via pore water infiltration or from stored P in the sediment via root cell scavenging activity. The former source would improve water quality (until the biomass decomposed), and the latter would release sedimentary P, contributing to total bioavailable P. Harvesting of above ground biomass would remove P regardless of its source, but this is an untested procedure on a large scale (Cicek et al. 2006).

2.8 Future Research

There are many areas in need of further research to shine light into the black box of P dynamics in wetlands. Some of them are as follows:

- Developing techniques of P form separation and analysis to encourage standard techniques and data comparison between studies. Each aspect of analysis needs improvement, ranging from sampling conformity, fractionation methodology and analysis procedures. Methods need to be developed in the use of phosphate standards during each extraction and analysis procedure.
- Investigating factors affecting peat accumulation in different types of wetlands.
- Measuring organic P forms under varying conditions over the year.
- Determining effects of environmental conditions on P retention and release processes, including seasonal change, pH change, influence of ice cover, spring flush, flood events, drying and anaerobic conditions.
- Development of enzyme analysis that conforms to conditions found in wetland sediment; that is, functionality under reducing conditions, high ion densities, activity under differing levels of P concentration.

- The use of analytical techniques that can identify Fe and Ca phosphates so their abundance can be monitored over time.

Chapter 3 CHANGES IN SEDIMENT PHOSPHORUS FORMS IN A *TYPHA* MARSH IN SOUTH CENTRAL CANADA

3.1 Introduction

Increased human use of phosphorus (P) has led to higher P concentrations in aquatic systems, leading to eutrophication of river, lake and marine ecosystems.

Wetlands normally accumulate incoming P in biota and sediment (Johnston 1991; Reddy et al. 1999; Reddy et al. 2005), providing a point of concentration on the landscape and establishing conditions of high productivity and biomass accumulation. This is also true for constructed wetlands (CW), leading to their increased use for water quality improvement (DeBusk et al. 2001). Continued P inputs often lead to saturation of a wetland's P retention capacity, resulting in occasional or steady release of P to downstream locations, effectively negating P storage (Johnston 1991; Reddy et al. 1998; Richardson and Qiun 1999). This situation highlights the need to remove excess P from wetlands and a proposed remedy is the harvest of wetland biomass for removal of P stored in plant tissues (Cicek et al. 2006). Biomass collection can serve a dual purpose of providing a renewable energy source for heating, methane generation, or hydrogen production, as well as the removal of significant quantities of P from the landscape.

Typha spp. are a good focus species for P accumulation and collection due to high natural predominance in wetlands throughout North America and high biomass production. *Typha* appears to prefer nutrient rich locations and has been documented invading wetlands, becoming the dominant species (Grosshans et al. 2004; Angeloni et al. 2006; Cicek et al. 2006). For example, a CW planted with eight emergent

macrophytes and three floating species were reduced to only *Typha* within one year (Maine et al. 2005). As producers of biomass and collectors of P, *Typha* is very efficient. Stands of the hybrid *Typha* × *glauca* have produced 14 times the biomass litter compared with native species in the same marsh (Angeloni et al. 2006). *Typha* normally produce 1 to 2 kg/m² of aboveground biomass (Prentki et al. 1978; Mitsch and Gosselink 1996; Maine et al. 2005), and contain a higher percentage of P compared to other marsh species (Mitsch and Gosselink 1996; Angeloni et al. 2006; Cicek et al. 2006). P content is reported as 0.32% of dry weight (Cicek et al. 2006) for the mix of *Typha* from the marsh in this study. In terms of removal potential, annual *Typha* harvest represents removal of 3 to 6 grams of P/m²/yr or 30-60 kg/ha/yr.

Plant P demand in early summer is initially satisfied by translocation of P stored in rhizomes and then replaced from root scavenging over the following months (Prentki et al. 1978). For replacement and further P requirements, plant roots absorb P via interstitial pore water and then translocate it into actively growing plant tissue (Prentki et al. 1978), causing a deficit in pore water P concentrations. It is unclear, however, from where pore water P is replaced. Three possible alternatives exist: 1) Hydraulic diffusion from surface water may bring new P into the root zone but the circuitous route through microbial, mineral and organic layers offer many potential adsorption sites and microbial scavenging opportunities for labile P (Baldwin et al. 2002; Harris 2002). 2) Equilibrium dynamics suggest replacement of pore water P by dissolution of less labile forms present in the sediment: this is P adsorbed and bonded to the many mineral precipitates (e.g., CaCO₃, FeOOH, AlOOH) and solid mineral particles (e.g., CaHPO₄·2H₂O, Ca₄H(PO₄)₃·2.5H₂O, Ca₅(PO₄)₃OH)(Harris 2002). 3) Conversion of complex organic P

and mineral P forms to labile P by root and microbe release of phosphatase enzymes and organic acids (Celi and Barberis 2005; Havlin et al. 2005). The first mechanism would reduce surface water P concentrations, improving water quality. The latter two alternatives involve the conversion of stored, non-bioavailable forms of P to labile (mobile), bioavailable forms, reducing the total stored P in sediment. Measuring abundances of these P forms over a growing season may indicate from which of these pools P is sourced by *Typha*.

Forms of P in sediment can be viewed several ways, in terms of bioavailability, adsorption and bonding chemistry, or as labile and non labile (mobile). Generally speaking, labile forms are considered to be bioavailable (e.g., PO_4 is soluble or weakly adsorbed, to a surface (Baldwin et al. 2002)). As bonding and structural complexity increases, P becomes less labile and less bioavailable (Harris 2002; Havlin et al. 2005). Wetland sediment has a complex P cycle, and exchanges between forms is part of the balance between biological needs bringing P out of non-available forms and the inherent reactivity of P, increasing its bonding complexity in association with cations and particles (Baldwin et al. 2002; Havlin et al. 2005).

Analysis of these forms of P requires removing them sequentially from the soil matrix. Sequential extraction methods wash a small amount of soil with solvents of increasing strength and differing pH. In this study, water removed soluble P (as PO_4 and dissolved organic P); a dilute bicarbonate solution (NaHCO_3) removed loosely sorbed P and organic P; NaOH dissolved ferric and aluminium oxides, along with P sorbed and occluded to those surfaces and organic humic acid P; and HCl dissolved Ca and Mg compounds and P associated with them.

Temporal changes in P fractions can result from several known processes and previous studies have found changes corresponding with biological activity (Gomez et al. 1998; de Vicente et al. 2003) and anaerobic conditions (Wright et al. 2001; Maine et al. 2005). Plant growth and microbial requirements can be supplied by phosphatase release, that attacks specific organic P compounds (Cross and Schlesinger 1995; Celi and Barberis 2005). These compounds are not generally identified or isolated in fractionation schemes. Reduction of this fraction may, however, be countered by slow accumulation of more recalcitrant organic P forms (e.g., humic acid related P) as organic matter builds up in sediment (Craft and Richardson 1993). Anaerobic conditions can release the P fraction sorbed to FeOOH (Mortimer 1942; Richardson 1985), by increasing the solubility of FeOOH and releasing sorbed P. The OH fraction is related to Fe and Al bonded P and during anaerobic conditions bacteria use Fe^{+3} as an electron acceptor, changing Fe^{+3} (as $\text{FeOOH}_{(s)}$) to $\text{Fe}^{+2}_{(aq)}$, releasing P sorbed and/or bonded to the solid surface (Mortimer 1942; Baldwin et al. 2002). Incubated wetland soils under reducing conditions have been found to release P (Moore Jr. and Reddy 1994; Shenker et al. 2005), as well as aqueous Fe (Jensen et al. 1992; Huang et al. 2005; SurrIDGE et al. 2005). Studies using incubated cores have shown OH P extract decreased with lower oxidation reduction potential (ORP) (Moore Jr. and Reddy 1994) and P flux to surface water was concurrent. Change in P form abundance may come from plant, microbe or chemically driven processes.

This study examined the change in P forms in sediment over a growing season in order to establish which processes are dominant in replenishment of P removed by plant growth. The study hypothesis was that if P came from surface water, surface water concentrations would decrease over the growing season. If P was sourced from sediment,

labile P forms (adsorbed and loosely bonded to precipitates and surfaces) would decline in months of *Typha* growth and less labile forms would be gradually drawn down as these forms replace depleted ones.

3.2 Methods

3.2.1 Study site

The study area was located on the east side of the Netley-Libau Marsh, a large (25,000 ha) coastal marsh at the mouth of the Red River on the southern end of Lake Winnipeg in central Canada (N 50°21', W96°45', see Appendix A for site map). The Red River contributes 60% of Lake Winnipeg's annual P input (Bourne et al. 2002). The site was a large shallow pond (1.5 ha, 1 m max depth) with dense *Typha* stands extending into all but the deepest portions. The pond was hydraulically connected to the nearby marsh (100m) during north wind seiche events that drove nearby lakewater into the marsh, causing water level fluctuations of up to 1 m.

In order to prevent P from accumulating in the vegetated site from water borne silt, a floating enclosure was constructed (4 × 4 m square) and anchored to poles, allowing vertical movement during water fluctuations. A plastic curtain was fastened to the inside of the enclosure and secured into the sediment, limiting potential incoming P in the form of silt deposition (for a more complete description see Appendix B). Some water exchange was allowed by overlapping, not sealing the curtain, in case water fluctuations caused pressure-induced water exchange. The enclosure was situated to contain a dense *Typha* stand (a mix of *Typha* × *glauca*, *T. latifolia* and *T. angustifolia*) with open water (without emergent vegetation) immediately adjacent. The open water

section (4 × 4 m) was defined by an open-frame wooden perimeter, allowing free water flow. These two defined areas made up the study sites and are described in this paper as “EN” (within the enclosure, and vegetated with *Typha*) and “OW” (open water, outside the enclosure and with no vegetation). In an effort to minimize contact between newly exposed lower sediments and surface water (so potential P exchange would not be encouraged by our activities), great effort was taken to not disturb the sediment bottom of either sample area throughout the sampling season. Core samples were taken by bridging across the perimeter of either site with a 16 foot (5 meter) aluminium ladder enabling access to the central areas of the site. Only one core was taken each month from each site.

3.2.2 Sampling

Water depth was recorded three times daily with a stationary meter (Ecotone, Remote Data Systems, Whiteville, N.C.) located just outside the EN. *Typha* biomass was collected from vegetated sites similar and proximal to the EN every 2 weeks, dried and weighed. Sediment cores were taken monthly from May to October from randomly selected locations within each site (six from each site, no replicates). Coring was performed with a custom built, 2” (5.08 cm) internal diameter, thin-walled metal pipe, driven into the sediment with a sledge hammer and pulled by hand (see Appendix C). Spatial variability could not be incorporated with replicates (see above), so it was measured in November at the end of the sampling season (see Appendix D). Surface water samples were collected monthly and measured (Stainton et al. 1977) as unfiltered total reactive phosphorus (TRP) in µg/l and pH was recorded with a hand held meter (Hach Sension2).

3.2.3 Laboratory analysis

Extruded cores were immediately sectioned with a knife into 10 cm horizons, bagged, and transported to the lab in a cooler for analysis the same day. Fresh core horizons were hand sorted to separate detritus (distinct plant matter), *Typha* rhizomes (large woody structures) and live roots (white fleshy roots < 2 mm in diameter). The horizons were hand homogenized in plastic bags for 5 minutes and a portion centrifuged at 3100 RPM (g-force of 1611) to extract pore water. This was then filtered through a 0.45 μm membrane filter and tested for soluble reactive phosphorus (SRP) using the molybdate ascorbic acid method (Clesceri et al. 1998). Triplicate sub-samples of sediment were dried at 105° C overnight to determine dry wt. equivalency of fresh sediment. These samples were sent to a contract lab (Dept. of Geological Sciences, University of Manitoba) for perchloric acid digestion and analyzed with inductively coupled plasma electron spectroscopy (ICP-ES) for total elemental P (TP). *Typha* biomass P was derived from biomass values collected and P concentration from previous *Typha* analysis from the same marsh (Cicek et al. 2006).

Forms of P were extracted from fresh sediment samples in a process modified from Hedley and Stewart (1982), that sequentially extracts P with solvents of increasing strength. Bioavailable forms are removed with water, a mild bicarbonate solution, and then NaOH. Recalcitrant forms are removed with mild acid (1 M HCl) and finally perchloric acid for TP.

The most labile P form was isolated first by placing replicate (n=6) two-gram portions of homogenized fresh soil were placed in screw top plastic 50 ml falcon tubes with 20 ml of distilled water and a 2x8 cm strip of prepared anion exchange resin membrane (GE Ionics, Watertown, MA), pre treated with 0.5 M HCO₃ (for a complete

description, see Appendix E). This was followed by 16 hours of horizontal shaking @ 120 oscillations/min. The strip was removed and shaken in 20 ml of 0.5M HCl for 2 hours, which brings P back into solution and yielding “Resin P” for SRP analysis. Microbe P was determined by splitting above replicates into two streams, one stream was treated with chloroform (Hedley and Stewart 1982) and both streams were then dried in a fume hood overnight. The next day 20 ml of 0.5 M HCO₃ (pH 8.5) was added, shaken (16 hrs), and centrifuged and filtered as above. The SRP difference between chloroformed sample supernatant and the paired sample was recorded as microbe P. This test also produced the HCO₃ P extract.

The soil residue from the above chloroformed stream was then used to find Fe and Al bound P (20 ml 0.1 M NaOH, with 16 hrs of shaking, filtration and analysis as above) and the residue was then extracted for calcium (Ca) and magnesium (Mg) bound P (20 ml of 0.5 M HCl and the described process). The residual sediment remaining after all extractions was digested and analyzed for TP by ICP-ES. In addition, all extracts were split and analyzed for TP, the difference was assumed to be organic P removed by that solvent. Due to high organic matter content in sediment (20-30%), HCO₃ and OH extracts were darkly colored, possibly creating interference with spectrophotometric P analysis. To account for this, a blank of each extract was made up with molybdate reagent minus ascorbic acid.

Root zone depth of *Typha* in the marsh was found to extend to 20 cm, indicating *Typha* P requirements would be extracted from this zone. This depth is similar with that of other emergent macrophytes (*Spartina maritime*) and has directed other researchers to focus P extraction to the top 20 cm (Coelho et al. 2004). On the basis of this observation,

P extract results from the top two horizons were combined, and unless otherwise stated, all extract values and correlations are presented for the top two horizons.

Measuring P in wetland soils give very different results when expressed as wt/wt (ppm) and as wt/vol, due to the difference in bulk density in the soil horizon (Johnstone 1991). Although most sediment studies report P as ppm, my study required P mass/marsh area and thus wt/vol. It became evident that sediment bulk density changed from month to month and it was necessary to extrapolate measured bulk density to the months when this parameter was not collected (May-July). Bulk density was recorded from August to October by filling a 16 cm³ vial, drying it for 48 hrs at 105° C and dividing the dry soil wt into the volume. A strong relationship was found between bulk density and percent water ($y = -1.0073 \ln(x) + 4.6373$ $r^2 = 0.994$). Due to scatter on the x axis, a Type II predictive regression was used (Geometric mean regression, see Appendix F) to correct the curve (Ricker 1975). Recorded May-July percent water data were extrapolated with this regression curve to create a "calculated" bulk density for those months. This bulk density data was then used to calculate P extracts in each horizon for all months by multiplying ppm of P extract by bulk density and then by the volume of horizon in 1 m².

Sediment was dried at 105° C for 24 hrs to determine water content and then fired at 550°C for 4 hrs to find organic carbon content by loss on ignition (L.O.I.). Further heating to 900° C for 1 hr released CO₂ from carbonates (McKeague 1978). This loss was multiplied by a factor of 1.36 to account for the difference in molar wt between L.O.I. loss (CO₂ only) and carbonate. Soil texture was determined using the pipette method (McKeague 1978) and the ashed soil (550° C).

Since a single core sample was taken each month (i.e. no replicates), site homogeneity was measured (spatial variation) from seven cores systematically sampled in November (see Appendix D).

3.2.4 Analytical observations

To find organic P of an extract, extract SRP was subtracted from TP (measured by ICP-ES), but consistently low HCl ICP results often led to negative organic P values. By sending commercial P standards in 1 M HCl for ICP analysis, it was determined the readings were consistently 16% below true values. Correcting for this reduced, but did not eliminate negative values in all cases, so HCl P is reported here only as corrected HCl ICP.

Sum of all extracts is different from TP due to over-recovery during the sequential extraction process. Some of this is from error related to sediment mass loss from solvent extraction. The mass of residual sediment was not recorded after each extraction and solvent dissolution of organic matter and CaCO_3 undoubtedly occurred. Residual sediment was weighed in September and October and mass loss was averaged and used to reduce the calculated residual P values for all other months. The inherent error in this procedure appeared in the difference between sum of extracts and TP. For more separation and analytical details, please see Appendix F.

Statistical analysis was performed using the software package Jump In 5.1 and all correlation were Spearman's Rho.

3.3 Results

3.3.1 Surface water

Over the course of the sampling season, surface water P concentration decreased by 0.5 mg/l and wind-induced water fluctuations (10-20 cm) occurred weekly in June and early July and then not again until October. Drought conditions and hot weather decreased mean surface water levels from ~70 cm in May and June to zero for August, September and October. Concentrations of P in surface water (Fig. 3.1) were higher in the EN compared with OW section and a substantial decrease was measured over the season. Surface water pH fluctuated in a narrow range between 6.4 and 7.5, with little difference between inside and outside the enclosure. Sediment pH recorded from extracted pore water ranged from 6.9 to 8.1, increasing with depth but showing no change over the season.

In early September when water levels dropped to zero, abundant adventitious roots were found on the base of *Typha* stalks just under the detritus layer in the EN. They were not seen before this date.

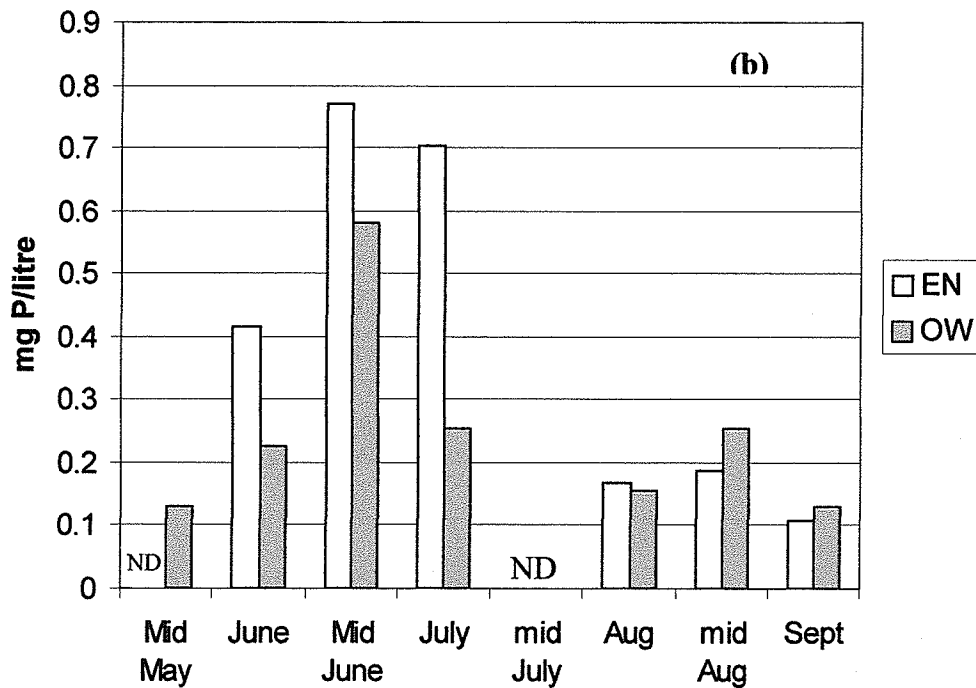
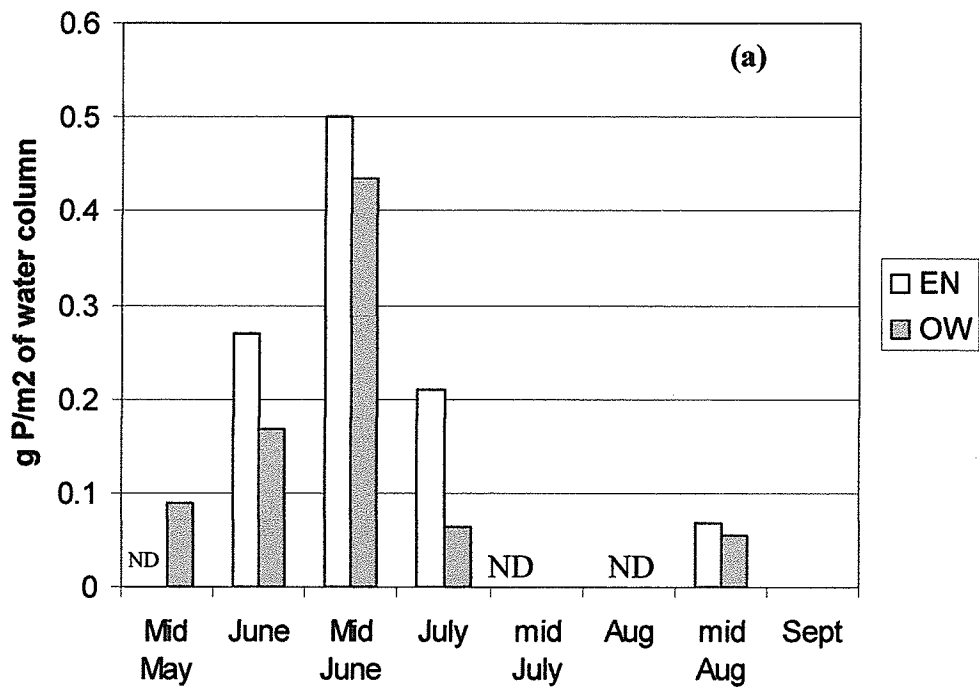


Figure 3.1 Total mass of unfiltered reactive phosphorus in the water column (a) and phosphorus concentration (b), within the enclosure (EN) and open water area (OW). No data were available from mid July to mid August and in Sept the water level was at zero.

3.3.2 Physical features

Physical features within the EN site showed differences from the adjacent OW site (Table 3.1). Sediment was fine textured and similarly high in clay (15-20% of ashed dry soil) and silt (35-70%) in both sites. The OW site displayed a shorter distance (~ 25 cm) from sediment surface to visible clay layer than the EN (> 40 cm), indicating a thinner layer of highly organic sediment layer in the OW and largely inorganic deeper sediment (clay). Dominance of clay and carbonates in the sediment was reflected in pH readings (pH 7.6 EN, 7.7 OW), consistent with calcareous soils (Essington 2004). The EN was more homogeneous in the distribution of roots and detritus while the OW site was more homogeneous in organic matter content. The EN contained 87% of all live roots and live root predominance (88%) in the top 20 cm suggested *Typha* would source P for growth from this depth. The EN site displayed lighter bulk density (mean of 3, 40 cm cores: 0.71 EN vs 0.90 g/cm³ OW) and higher percent water (mean of 6, 40 cm cores: 56% EN vs 48% OW).

Above ground *Typha* biomass increased from 0g/m² in May to a maximum of 1543 g/m² in August, equivalent to 4.94g P/m².

Table 3.1 Physical characteristics of each sediment horizon reported as means of monthly samples in grams of dry material. Months sampled (N) differ between parameters. Organic matter was found by L.O.I. @ 550° C and carbonates by L.O.I. at 900° C (multiplied by a factor of 1.36).

Horizon depth	Rhizomes (g dry wt)	Live roots (g dry wt)	Detritus (g dry wt)	Organic Matter % of sediment dry wt	Carbonate % of dry wt	Bulk Density g/cm ³	Pore water pH
	N = 6	N = 6	N = 6	N = 4	N = 3	N = 3	N=3
EN 0-10cm	2.032 ±1.711	0.130 ±0.122	2.252 ±1.284	27.3 ±7.7	3.7 ±0.5	0.25 ±0.06	7.5 ± 0.6
10-20cm	0.880 ±1.805	0.042 ±0.014	0.724 ±0.894	20.3 ±6.3	2.9 ±0.3	0.45 ±0.12	7.6 ±0.5
20-30cm	0.000	0.018 ±0.013	0.215 ±0.001	13.2 ±8.9	2.7 ±1.1	0.83 ±0.25	7.7 ±0.4
30-40cm	0.000	0.005 ±0.013	0 ±5.3	9.0 ±5.3	3.3 ±1.1	1.01 ±0.19	-
OW 0-10cm	0.000	0.024 ±0.038	2.771 ±3.833	25.5 ±1.9	2.2 ±0.4	0.30 ±0.06	7.8 ±0.1
10-20cm	0.000	0.001	0.002 ±0.010	17.3 ±5.5	2.6 ±0.8	0.62 ±0.23	7.8 ±0.2
20-30cm	0.000	0.000	0.001 ±0.005	5.5 ±1.1	5.4 ±4.8	1.07 ±0.12	7.7 ±0
30-40cm	0.000	0.000	0.000	4.3 ±1.0	8.9 ±2.5	1.19 ±0.09	-

3.3.3 Spatial variation

Spatial variation tested in the enclosure in November and the coefficient of variance (CV) was found to be within the range of temporal variation for bulk density, TP and HCO₃ extracts, indicating the site was homogeneous. Means of six monthly cores were 508 ppm P for TP (CV 24.8%) versus TP of 714 ppm for spatial variation (CV 6.5%). Similarly, 6 month HCO₃ P extract was 16.1 ppm versus 24.1 ppm (CV of 40.3 and 16% respectively) and bulk density was 0.439 vs 0.349 g/cm³ (CV of 27% and 14% respectively).

3.3.4 P extracts: difference between sites

Phosphorus extracts from the top two horizons showed differences between sites: recalcitrant forms (Organic OH, HCl, and Residual extracts) were higher in the open water section and labile forms (Pore, Resin, HCO₃ and organic HCO₃) were higher in the

enclosure (Fig. 3.2). The sum of six month means for each extract in the OW vs EN were higher for residual P (26.4 vs. 19.2 g/m²), and HCl P (19.1 vs. 15.2 g/m²), but lower for Pore (0.018 vs. 0.036 g/m²), Resin (0.344 vs. 0.654 g/m²), Microbe (0.48 vs. 0.68 g/m²), and HCO₃ organic (0.80 vs. 1.22 g/m²). HCO₃, OH and OH organic were approximately the same in both sites, measuring 1.28 vs 1.40 g/m², 1.37 vs 1.62 g/m², and 10.6 vs 8.93 g/m² for OW and EN of respective extracts.

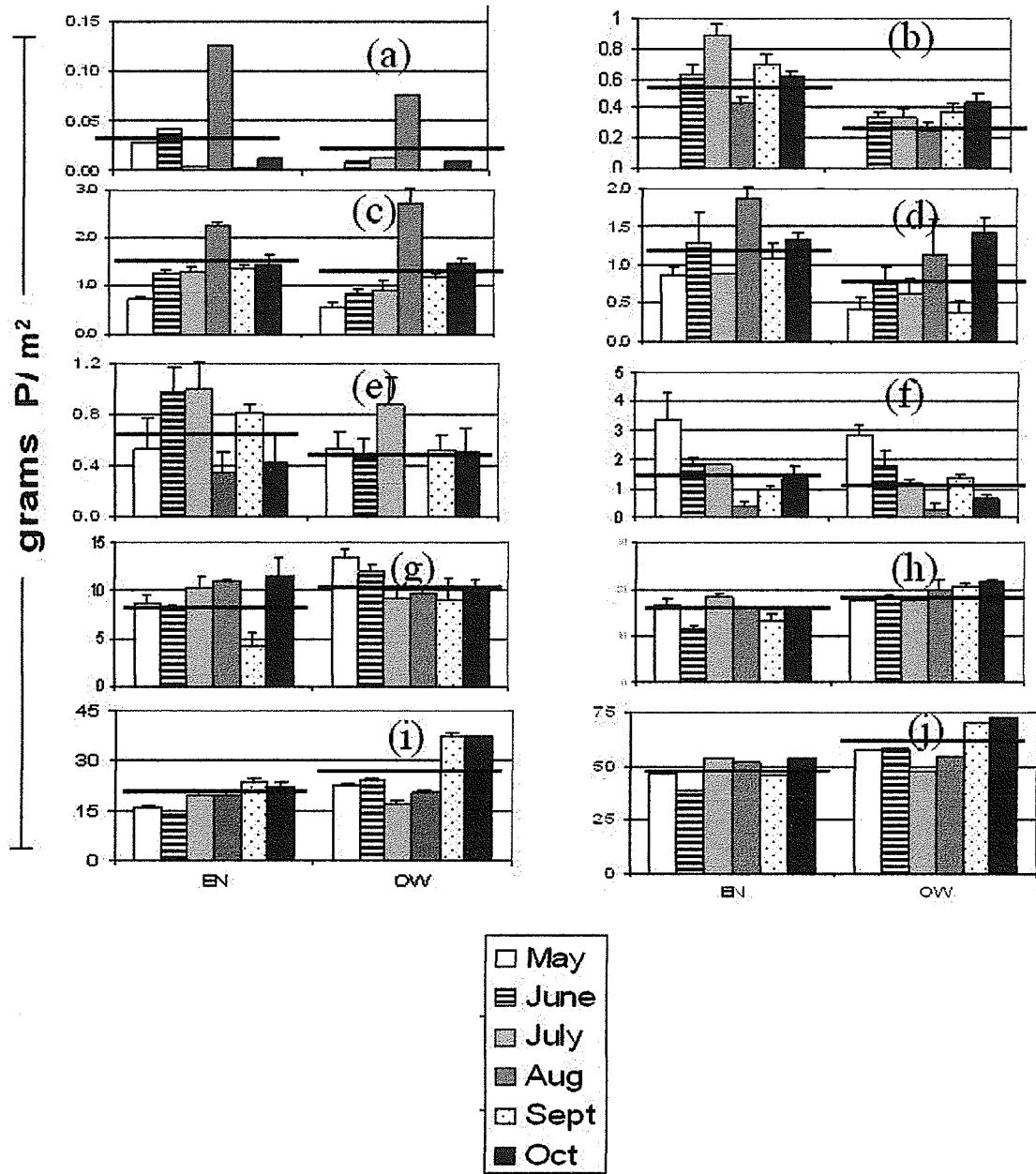


Figure 3.2 Sediment P extracts from enclosure (EN) and open water sections (OW) expressed in g P/m² of sediment to 20 cm depth. Error bars indicate standard deviation of replicate (3) sub sample determination of single monthly cores, and bars approximate means over the 6 months. The extracts are pore water (a), resin (b), HCO₃ (c), organic HCO₃ (d), microbe (e), OH (f), organic OH (g), HCl via ICP (h), residual (i), and sum of extracts (j). Of these, (b), (d) and (h) were significantly different ($p < 0.05$). Pore water was sampled without replicates and no resin P was collected in May.

In addition to TP, the sum of all extracts (Microbe P is independent of this sum), was calculated to evaluate recovery. Sum of extracts showed higher P in the OW section but this was largely on account of Residual P. When Residual P was not included, the sum of extracts was almost equal (30.4 g/m² EN versus 34.4 g/m² OW). Depth (using data from all four horizons) was correlated with TP (EN 0.80 p<.0001, OW 0.67 p<.0003), and Residual P (EN 0.89 p<.0001, OW 0.76 p<.0001). Extracts increasing with depth were: (mean of 6 months for 0-10 cm and 30-40 cm EN horizons) TP, 14.6 g/m² for 0-10 cm, increasing to 36.7 g/m² 30-40 cm horizon; Residual, 7.3 to 25.4 g/m²; HCl, 6.3 to 15.3 g/m², and Resin, 0.20 to 0.34 g/m². Organic OH and organic HCO₃ fractions decreased with depth by a factor of 3 to 6 (i.e. 4.2 to 2.7 g/m² and 0.7 to 0.1 g/m² respectively in the EN. Trends were similar in both sites. Correlations between extracts was negative for labile P forms (OH with Resin, HCO₃, and HCO₃ organic) and positive with recalcitrant forms (TP with HCl (0.85 p<.0001) and residual (0.89 p<.0001). These relationships changed somewhat between sites with stronger correlations for recalcitrant forms in the EN and stronger correlations between labile forms in the OW. Microbe P was almost identical in both sites (mean of 0.5 (EN) and 0.4 (OW) g/m²), decreasing dramatically with depth (0.50 to 0.00 g/m² EN) and increased from May to July (0.49 to 0.69 g/m²).

3.3.5 P extracts: seasonal changes

Changes in P extract abundance over the season was found for several labile extracts. From May to August OH P declined seven and eight fold in the vegetated and open water areas, and then increased somewhat in the fall (Fig. 3.2f). However, HCO₃ P (Fig. 3.2c) shows an opposite trend, increasing from May to August four-and six-fold in

the EN and OW areas. Increases were also found in Pore P in both sites. Viewing the extracts together in a stacked bar chart (Fig. 3.3), no definite trends can be seen, but an approximate net mass balance was found when the labile extracts were combined (Fig. 3.4). No corresponding reduction from any P pool was found when P from *Typha* biomass was included in P extracts. Since some roots were found within the OW section, it was reasoned that nutrients may be collected there by *Typha* in the EN. Combining each extract from both EN and OW did not identify a diminished P form.

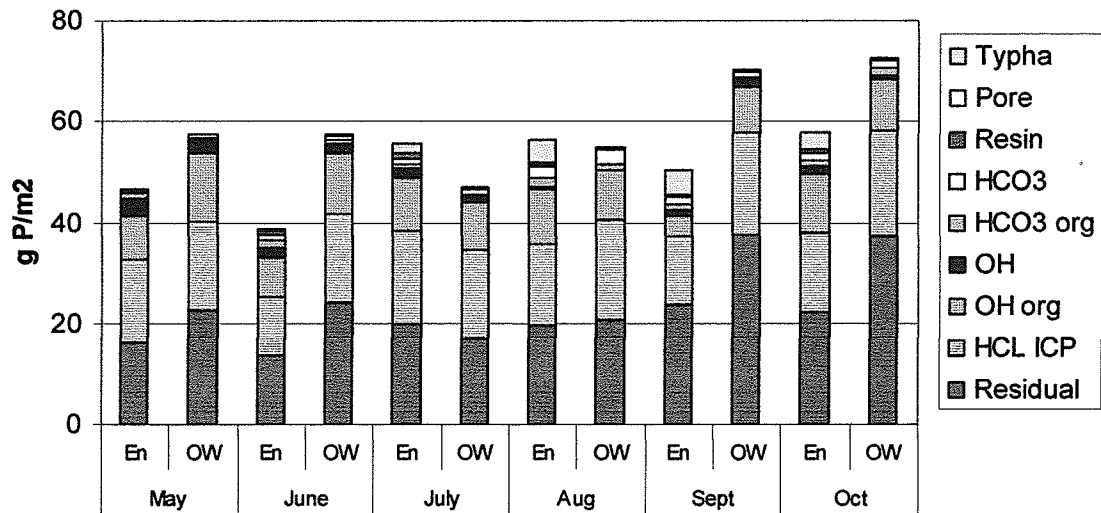


Figure 3.3 All P extracts from the enclosure (EN) and open water (OW) sites. Above ground *Typha* biomass P was included for the enclosure.

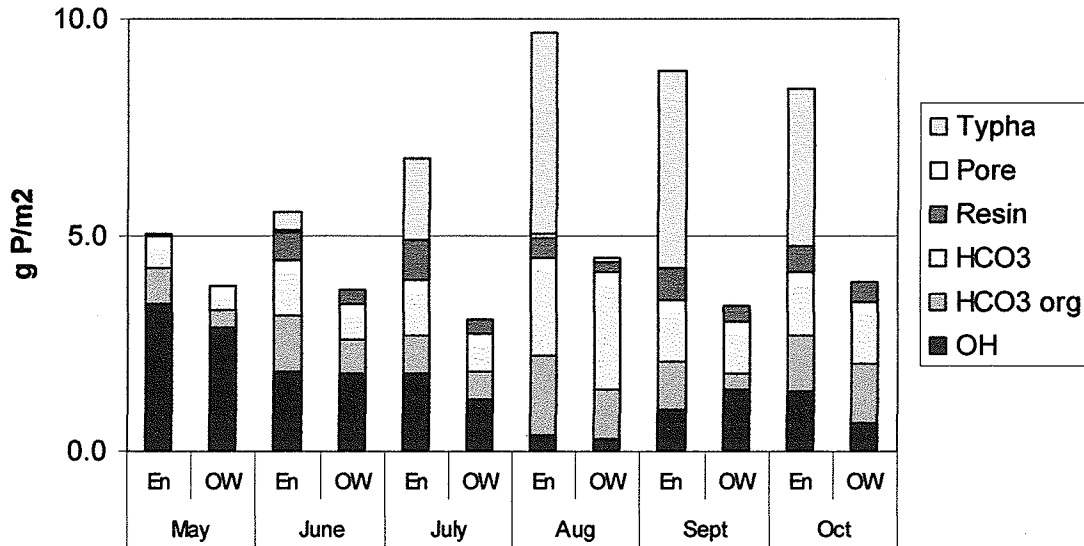


Figure 3.4 Labile P extracts from the enclosure (EN) and open water (OW) sites. Above ground *Typha* biomass P was included for the enclosure. Resin P was not measured in May.

3.4 Discussion

This study was conducted to determine whether the source of *Typha* biomass P was primarily from surface water or from sediment sources. During the course of this study it became evident that resolution of sediment P abundance in both vegetated EN and non-vegetated OW sites sediment was highly variable. This variability came primarily from seasonal variability. This is shown by low spatial variation CV data of seven cores collected on the same day, yet much higher CV for data from six cores of the same horizon collected at monthly intervals. We recorded P extracts as wt/vol and variability in bulk density directly affects these measurements in more than one way. Firstly, bulk density increases due to lower water content, so compaction of the soil profile due to drought or sampling methods may have an influence on soil horizons P measurements. Secondly, P mass increased with depth (TP correlation with depth: EN 0.808 $p < .0001$, OW .673 $p < .0003$), as did high yielding extracts, such as HCl P (0.77

p<.0001) and Residual P (0.81 p<.0001). A high bulk density soil horizon collected 10 cm from the surface would include more P rich material. Without a second reference point (other than the soil surface), horizons are not including the same objective mass of material each month: a core is deeper in months of high bulk density and more shallow in months of low bulk density. To give an example of this, my study found higher residual P extract in months when marsh water levels dropped to zero (September and October) because this extract increases with depth. This may indicate a contraction of the sediment profile in these months core samples including lower depth material.

Monthly variability of bulk density may be from water inundation, wind activity, evapo-transpiration of plants, sampling technique or spatial variability. Other authors have also noted reduced bulk density after flooding (Newman and Pietro 2001) and high variability in bulk density at some sites (0.42 ± 0.1 , n=7 (Reddy et al. 1998)). In comparison with other literature on temporal changes in sediment P, the variance in TP observed in this study falls within the ranges reported (Table 3.2).

Table 3.2 Comparison of total phosphorus (TP) and the coefficient of variation (CV) from studies of sediment. ND indicates no data were provided from the study.

Reference	Water body	Depth cm	TP ppm	CV	N	No. of mixed cores
This study	marsh	EN 0-10	600	21	6	1
		OW 0-10	617	25	6	1
deVincet et al. (2003)	lagoon	Site 1 0-5	705	14	14	ND
		Site 2 0-5	399	31	14	ND
Fabre (1996)	floodplain	0-10	697	14	13	8
Gomez et al. (1998)	lagoon	0-5	741	15	5	ND

3.4.1 Site differences

Physical differences in sediment were found between the two adjacent sites, some of which can be attributed to the long-term effects of plant growth and the buildup of organic matter in the soil. The enclosure sediment had lower bulk density, higher organic matter content and lower carbonates than the open water section. Higher sediment heterogeneity found in the enclosure (higher CV of bulk density, for all horizons) may be due to the clumping effect of *Typha* growth, leading to areas of high biomass accumulation, higher organic matter and more water retention. Open water is subject to more wave and wind effects, creating more homogeneous conditions.

Higher surface water P concentrations in the EN in June and July may have been from increased internal cycling of P compared with the OW section. An abundance of decaying organic matter and a more protected environment (compared to the OW section) may have encouraged microbial activity releasing P stored in dead biomass.

Total P (~600 ppm in top 10 cm) for both sites were within the range expected for other wetland marshes, being lower than those with direct nutrient enrichment (e.g. ~700 ppm for some Everglades marshes (Reddy et al. 1998) and peatlands (e.g., ~ 900 ppm for Oregon peatlands (Graham et al. 2005)), but above other coastal marshes in the region (e.g., ~500 ppm for Delta Marsh on Lake Manitoba (Bourne 2000)).

Measurements of labile P extracts (Pore, Resin, HCO₃ organic, HCO₃, and OH) were higher in EN than OW, consistent with expected increase in biological activity, decomposition of biota, and breakdown of recalcitrant P for biological use. Less labile forms (OH organic, HCL and Residual P) were higher in the open water section and increased with depth. These trends can be explained by soil composition and are exaggerated in the OW section due to lower organic matter and higher relative silt and

clay deposits there. Organic P in extracts of OH and HCO₃ decreased with depth, confirming trends reported by others (Reddy et al. 1998).

3.4.2 Seasonal change

The dramatic rise in HCO₃ P from May to August was not expected, as the levels of this labile bioavailable form (Cross and Schlesinger 1995) during rapid *Typha* growth would be expected to diminish in the soil. *Typha* growth did not appear to affect the temporal abundance of this extract as nearly identical seasonal trends were measured in both sites. Plant effects can also be ruled out for the decrease in OH P extract over the same time frame because of decrease in both sites. Removal or addition of these forms by hydraulic activity can be ruled out because of little surface water exchange with the lake over this period, as measured by little wind-driven surface water fluctuations. The fact that OH P and other labile P extracts showed similar trends maintained over the season in both sites indicates a causal agent other than emergent macrophytes.

Mid summer anaerobic conditions are the probable cause for the trends observed in OH and HCO₃ extracts. Although this study did not successfully measure ORP, other authors have reported a distinct decline in the OH P extract when conditions became anaerobic (Moore Jr. and Reddy 1994; Olila and Reddy 1997). Additional support for this process is found in my study with the increase of HCO₃ during midsummer. If P released from Fe to soil pore water was adsorbed by soil particles, increased levels of labile P (Pore, Resin and HCO₃ extracts) would be expected for those months. This is indeed what was observed (Fig. 3.4), with temporal exchange between P forms, yet maintaining a balance of labile P over the season. Similar observations were reported by other researchers, who suggested that the decline in OH P at lower ORP is countered by a

simultaneous rise in $\text{NH}_4\text{Cl P}$ (similar to $\text{HCO}_3\text{ P}$) and P release to surface water (Olila and Reddy 1997). Pore water P was found to be highest in August in a 13 month study of coastal lagoon sediment in Spain (de Vicente et al. 2003), which is what was found in our study also. Some studies of seasonal changes in P forms have not found reductions in OH P, and attribute all P released from flooded sediment to reduction and release of P stored by microbes (Wright et al. 2001). In different wetlands, different processes may be responsible for the conversion and release of P forms.

The central question of this study is whether *Typha* takes P from the sediment or the surface water: this study infers that it comes primarily from sources other than surface water. Above ground *Typha* biomass P totalled approximately 5 g of P/m² at its peak in mid August. Contribution to this mass by surface water P is possible given evidence of the decrease in P concentrations from June to August; however, there was a simultaneous decrease in OW surface water P over the same period. Even if water levels were reduced by *Typha* water uptake and all SRP within that water was incorporated into biomass, surface water P can only account for 5% of above ground biomass P. This calculation was done using estimations of evapo-transpiration rates of wetland surface water decrease of 5 mm/day (Kadlec & Knight 1996); measured surface water P concentrations; and measured P biomass over from May to August (but not allowing for selective P removal from water).

The development of adventitious roots on the *Typha* stalks suggest a greater part of the nutrient requirement could have been met by surface water P, because these roots are in the water column and can selectively absorb nutrients disproportionately to water requirements. Internal nutrient cycling is complicated in wetlands and may be better

explored through alternate methods, such as radio labelled nutrient studies (Noe et al. 2003). Noe et al.(2003) found more than 60% of ^{32}P added to the water column had entered the floc and soil compartments within 18 days, but that less than 1% had been taken up by emergent macrophytes. In any case, plant requirements exceed the P available in the water column, so the majority of P must have been extracted from other places, either translocated from *Typha* rhizome storage and/or from depletion of sediment P pools.

If *Typha* biomass P came from sediment pools, it is unclear from which ones, as none showed direct loss concurrent with aboveground P increase. Six month mean of total P m^2 in sediment to 20 cm depth was measured as 48g (CV of 12.7%, EN) and 60g (CV 16.5%, OW), indicating a seasonal removal by plants of 5 g P/ m^2 should be measurable. As variance of sample extracts was high, it was difficult to determine which extract, (if any) was depleted over the growing season. It is evident, however, that the most labile forms of P were not diminished by *Typha* P requirements, suggesting more recalcitrant forms may have been removed by plant activities. The processes responsible for this, either equilibrium dynamics or plant mediated hydrolysis of P compounds cannot be deduced from the data collected.

3.5 Conclusions

My initial hypothesis has not been supported by the data collected. Surface water P concentrations did decrease over the season but equally in both sites, eliminating emergent macrophytes as causal to the decrease. Similarly, labile P extracts were not diminished in the vegetated site to a greater degree than the open water site. More

recalcitrant P forms did not decrease in proportion to P accumulation in *Typha* aboveground biomass. This study did, however, identify that sufficient P for *Typha* growth is not present in surface water and infers recalcitrant sediment P is the likely source. The study also found fluctuations in P forms over the season and that these are likely driven by anaerobic conditions.

The particular P form sourced by *Typha* has implications for wetland management, especially when considering harvest of emergent macrophytes to reduce P levels. Macrophyte growth is credited (Craft and Richardson 1993; Reddy et al. 1993) to storing P in organic forms (represented as extracts of OH organic P, HCO₃ organic P and possibly residual P in this study) but if these forms are an intrinsic part of active P cycling, harvest may be bringing stored P back into the ecosystem. Annual *Typha* harvest would remove 5 g P/m², completely depleting the top 20 cm in 5 to 10 years, this may increase retention of surface water P, but could also potentially impact *Typha* viability. A controlled study of *Typha* harvest and the effect on surface water P conducted in a flow-through flume or hydraulically managed enclosure would serve to further address these questions. Repeated annual harvest of a plot with simultaneous sediment P form abundance would enable definitive conclusions as to *Typha* P sources.

Chapter 4 General Discussion and Conclusions

The critical review of wetland P processes (Chapter 2) compliments the experiment conducted in Netley Libau marsh (Chapter 3) and together they increase the scope of this thesis. Relevant connections between these two papers are: 1) processes at work in the marsh, 2), storage/release role of emergent macrophytes, and 3) ambiguity of current extraction methods.

4.1 Processes

The critical review of P release and retention process identified which processes are possibly at work in Netley-Libau Marsh (see conceptual model in Fig.4.1), but with no long term data on P form accumulation, it is not possible to determine the dominant process of retention, or even if net retention is taking place. Although ORP was not measured, the study potentially identified anaerobic conditions in August of 2006, which appeared to affect abundance of labile P sediment pools as OH P decreased and HCO₃ P increased. This is in agreement with literature studying anaerobic P release (Moore Jr. and Reddy 1994), although just where this released P resided is not known, as it could be sorbed to organic matter or CaCO₃ precipitate.

4.2 *Typha*

The addition of a healthy emergent macrophyte community complicates P release and retention processes, as the role of macrohytes on these processes is not known. Others have found evidence of P accumulation in emergent macrophyte wetlands (see Chapter 2), but this study brought into question the source of the P accumulating in macrophyte biomass. The study did not identify from which pool biomass P was sourced

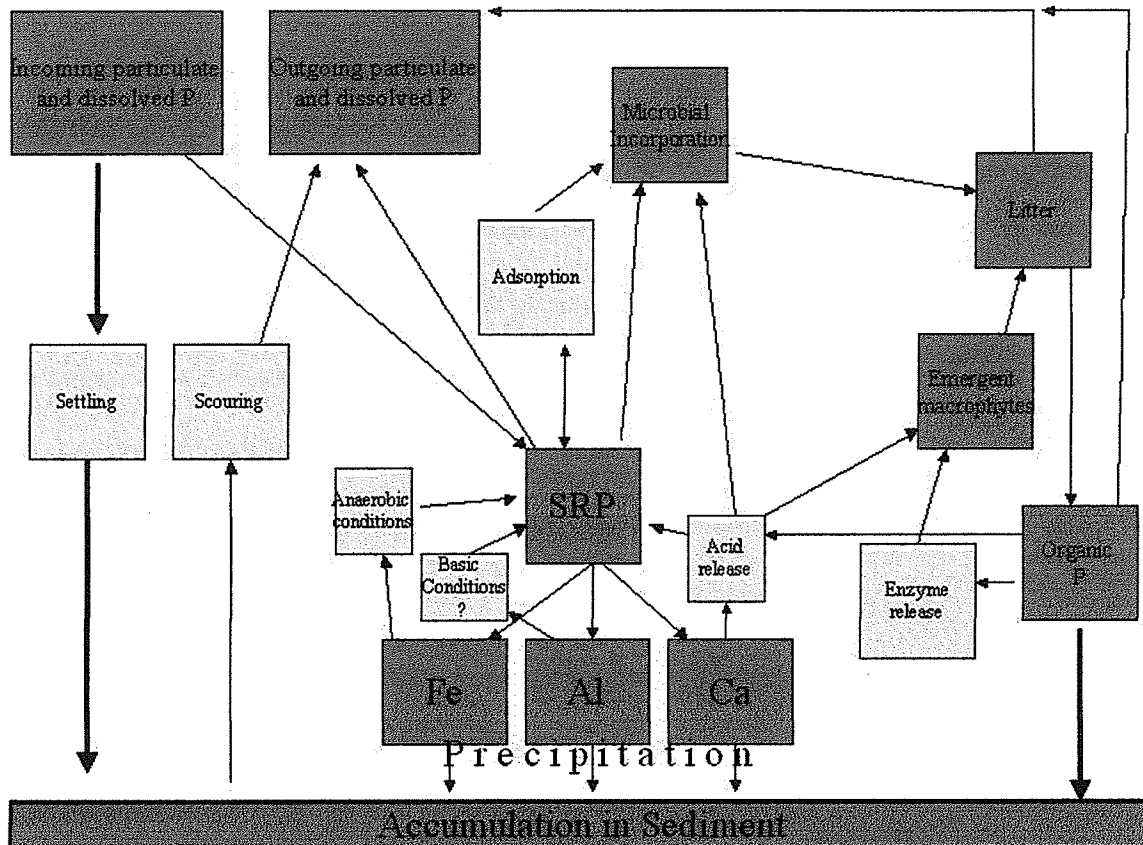


Figure 4.1 A conceptual model of the processes of P retention and release at work in wetlands. The model is based on mechanisms and processes reviewed in Chapter 2.

and cast some doubt on the likelihood of it coming from surface water or the labile sediment pools, although this was the primary hypothesis of the marsh experiment. Reduction in the larger recalcitrant P pools was not measurable due to their size and variability (spatial variability of bulk density was 14%) and this variability obscured the comparatively small biomass P (5g over several months). Gradual reduction of the large HCl P pool (~15g/m² to 20 cm depth) or organic pool (~11g/m² to 20 cm depth) was difficult to verify or refute. A further complicating factor was the probable accumulation of both of these pools over time, due to algal-driven CaCO₃ precipitation and build-up of organic P from biological processes. With these possibilities in mind, a new hypothesis is

warranted, that the source of annual *Typha* P is recalcitrant P forms in the sediment. The idea that these recalcitrant forms may be reduced by *Typha* growth when more labile forms are available is unconventional, but plants and microbes have the ability to break down these forms through phosphatase and acid release. If the types of phosphatase released by *Typha* were known, then organic P forms broken down by this enzyme could be targeted for measurement or phosphatase activity could be measured.

A long term study of the effects of annual harvest on the abundances of the various P pools and the changes in P cycling dynamics could add data vital to answering these questions. *Typha* may react to lower P sediment levels by greater surface water P removal, a deeper root system, increased enzyme release (Pant et al. 2002; Celi and Barberis 2005) or reduced viability. Changes in incoming stream flow P deposition may also occur, as flux from surface water may increase from changes in equilibrium dynamics of a more nutrient poor sediment surface.

4.3 Extraction methods

Determining dominant retention and release processes and the role of plants in P dynamics rests on the ability to monitor changes in P pool abundance and to correctly infer processes from the results. The relatively new science of wetland P pool extraction and analysis has many problems to solve in this area.

The Hedley method of sequential extraction was chosen due to expertise with this method in the Dept. of Soil Science. This technique required three weeks of lab work for each monthly pair of cores and resulted in data that was in many ways too general to positively identify processes at work in the marsh. The major problems with the technique are as follows:

- The role of Fe and Al oxides are not well answered by the method used, other methods use oxalate (Young and Ross 2001) or dithionite (Jensen et al. 1992; Golterman 1998), and may be better suited.
- The OH extract is fundamentally different from agricultural soils (for which the Hedley method was developed (Hedley et al. 1982)) and from wetland sediment, due to the high levels of humic and fulvic acids in the latter. This led to uncertainty in analysis as identified in Chapter 4. Also, OH extract combines Fe and Al related P with alkali-soluble organic P and poly P.
- CaCO_3 related P is poorly defined in the method. It is assumed to be the HCl extract but no verification of this was found in the literature and complaints were found of this fraction being too high due to transfer from the preceding solvent (Villar et al. 1999; Benzing and Richardson 2005).
- Using fresh soil (rather than dried and ground) led to high sub-sample replicate variation and a single monthly core cast some doubt on measured temporal trends. Even without these ambiguities in the P extract data, sequential P extraction gives only operationally defined results, making comparisons with other studies that vary the process, difficult or impossible.
- The alternative OH P analysis method (Chapter 4) suggested much of the OH P was associated with humic acids. If the process were tailored to organic P extraction, much stronger OH molarity would have been used and likely more humic P would be found (e.g., 2 M NaOH (Worsfold et

al. 2005), rather than 0.1 M Na OH). Unextracted organic P stays in the soil residue and is counted as residual P, thus confusing it with residual mineral P (e.g., calcium phosphates). This ambiguity is also within the scientific literature, as some studies group residual P as organic P (Reddy et al. 1998), and others count it as undefined mineral P (Mayer et al. 2000).

These problems illustrate a dilemma of sediment P research: adapt an extraction process to isolate species of interest, or adhere to a general scheme that gives low definition but at least is comparable with other sediments and studies.

4.4 Recommendations for Future Students

If this project were to be repeated, there are several recommendations of changes that would improve sampling and analysis outcomes. These are mentioned here to benefit future work with wetland sediment analysis.

- Under take a large-scope study to quantify P form accumulation and the conditions promoting inorganic precipitation (all forms of Ca~P as well as Fe and Al~P), organic P formation (mono and diester P or at least measure humic and fulvic P as determined from Method 2 in Appendix F).
- Include litter and detritus as a potential P source for *Typha* biomass
- Monitor changes in bulk density with other environmental conditions to determine causal relationships
- In situ sampling of pH and ORP would greatly aid in detecting and explaining anaerobic changes in sediment and changes in P form abundance (i.e., is pH or ORP the driver of P release)

- Try to solve the problem of single reference (sediment surface) core depth measurement in light of changing bulk density.
- A smaller diameter of corer pipe (one inch) would enable more cores to be taken per month with no greater impact on the site than one two inch core. Three or five cores could be sectioned into horizons and mixed in the field before analysis, improving statistical strength of data without increasing laboratory time.
- Try Fe and Al specific separation analysis as these forms have different release mechanisms and current methods do not differentiate forms
- Try alternate residual P methods (ashed at 550° C and washed with HCl, peroxide digestion) and resolve the composition of this fraction (i.e., is it organic or mineral).
- Try UV organic P digestion of OH extract for poly-P analysis to gain information on the abundance and sources of poly P forms
- A replicate core analyzed in a N₂ glove box would enable oxidization changes of Fe during analysis to be quantified. This could be done in tandem with a normal extraction to find relative differences in extracts.

4.5 Engineering Significance

From an engineering perspective, this study has added part of the information required to determine the feasibility of biomass harvest for P reduction in Netley-Libau Marsh. I successfully quantified forms of P present in the top 40 cm of sediment and identified which forms changed in abundance over the growing season. The study also determined *Typha* biomass P is a relatively large portion (~10%) of the total P within the root zone of *Typha*, and suggests continued harvest over several years would have a

marked impact on sediment TP. Effects on *Typha* viability and sediment deposition are not known and cannot be predicted without future study.

The study did not conclude from which P pools annual P for *Typha* growth is taken, just that surface water could not account for all P accumulated in biomass, and that labile forms did not decrease in tandem with biomass accumulation. A controlled harvest over several years, matched with occasional sediment P fractionation would determine from what pools biomass P is being derived. This is important because removal of organic P (e.g., humic P) may be counter productive to the aims of cattail harvest if such removal takes stored P rather than surface water P. This study did not consider the general hydrology of the eastern Netley-Libau Marsh but observations indicated the classic “flow through” marsh delta system was not in effect and that the source water inundation (and likely P deposition) was from wind induced seiches from nearby Lake Winnipeg. This project has made a start in elucidating the complex P cycling processes taking place within a *Typha* dominated wetland.

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Appendix

These appendices include all data and methodological information of the wetland sediment experiment not found in the preceding journal papers.

Appendix A Site location

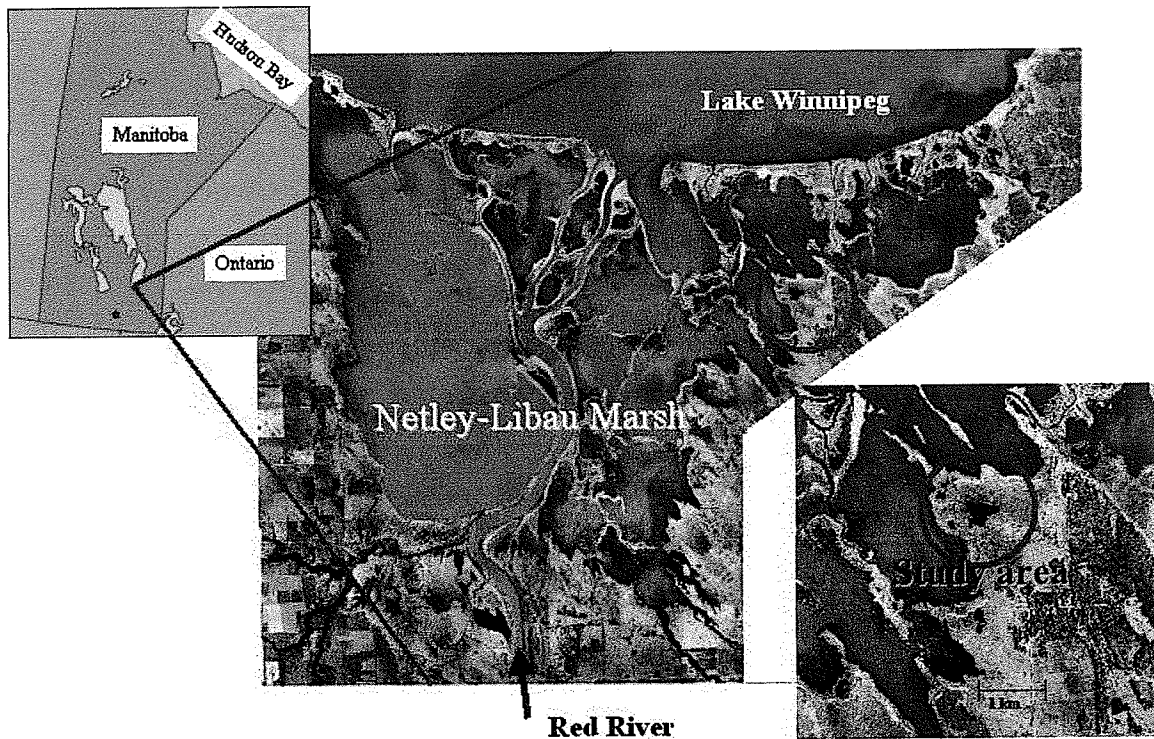


Figure 1 Location of Netley-Libau Marsh site in Central Canada. The Red River flows into Lake Winnipeg through the marsh from the south. The study area was a 1.5 ha pond about 2 km from the closest road. (Satellite map from Grosshans et al. 2004. *Changes in the emergent plant community of Netley-Libau Marsh between 1979 and 2001* used by permission 2007.

Appendix B Enclosure construction

The enclosure was built from wood frame construction, 18 inches by 16 feet long, decked with ½ inch plywood and Styrofoam cut to fill the cavity on the bottom side (Styrofoam 10" deep). Four of these sections were transported to the marsh and assembled making a floating square (14 feet inside dimension), with an 18 inch boardwalk around the perimeter. This was chained to two long metal poles driven into the sediment serving to anchor lateral movement but allowing perpendicular movement to adjust to changing water levels. Inside the structure, a 6 foot curtain (6 mil woven poly, Curry Enterprises, Winnipeg MB.) wrapped the interior and was secured into the sediment by cutting the *Typha* roots with a sharp shovel and pushing the weighted curtain (a sewn pocket with rebar inside) into the sediment. As the water was normally only 2-3 feet deep and the curtain 6 feet, approximately 3-4 feet of water increase could be accommodated without lifting the curtain out of the sediment. Rather than sealing the curtain, incremental water exchange was allowed for by a 3 foot overlap.



Figure 2 Construction of the enclosure and assembly in the marsh.

Appendix C Sediment corer construction

My objective was to take sediment cores with the least disturbance to the vegetation and sediment of our sites. Conventional coring techniques can be very destructive to the marsh, often removing cores by digging them out (Reinhardt et al. 2000). I used a 6 foot long 2 inch in diameter thin walled metal pipe (made for electrical conduit). This was sharpened at the end to cut through *Typha* stalks and rhizomes and two metal handles fastened to the sides with muffler hanger “U” bolts. After the corer was hammered in to the sediment, a temporary plumbing plug (rubber gasket tightened by a wing nut) was used to create a vacuum, preventing loss of the core on extraction, but it was found this was not needed as clay at lower depths plugged the other end. The core was pushed out of the pipe with a 1 inch pipe and tight fitting plunger made up of plastic plumbing fittings.



Figure 3 Removal of the 40 cm sediment core from corer with a plunger.

Appendix D Spatial variation

In November spatial variation was measured by taking 7 cores from the EN and individually tested for bulk density, percent water, total P, and HCO_3P using triplicate sub-samples. These data are presented here for comparison with measured bulk density for August, September and October (Fig. 4 and 5).

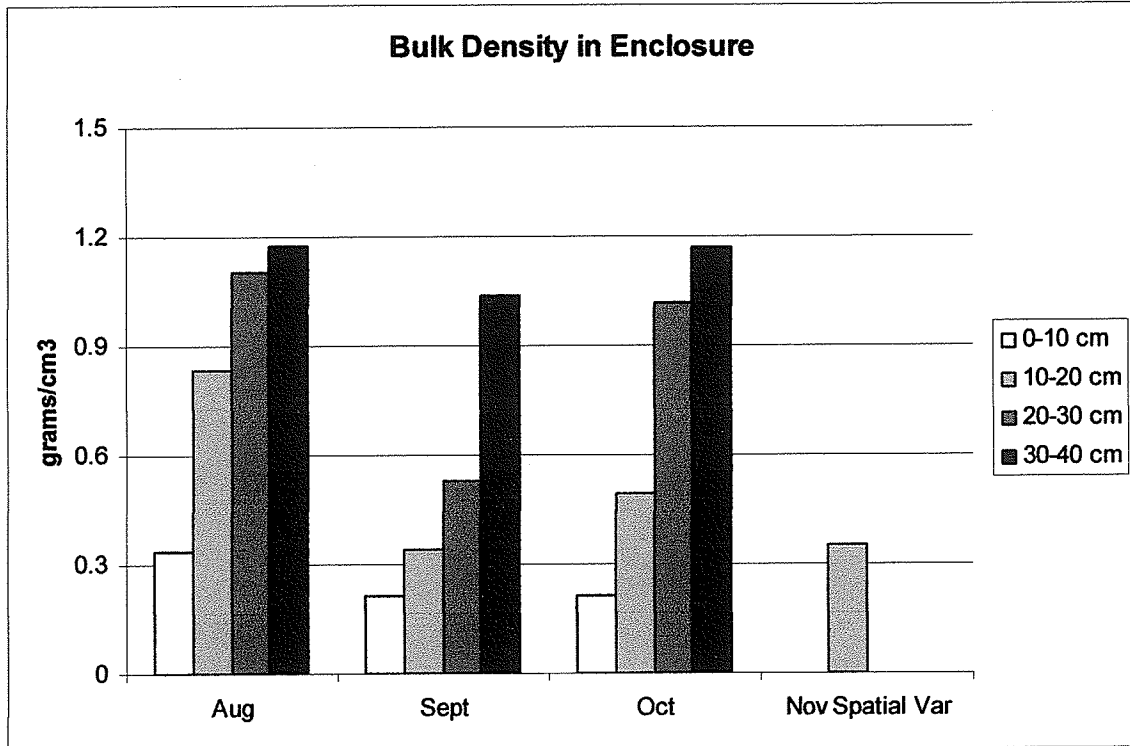


Figure 4 Bulk density of sediment horizons in enclosure with November spatial variation test of 10-20cm horizon.

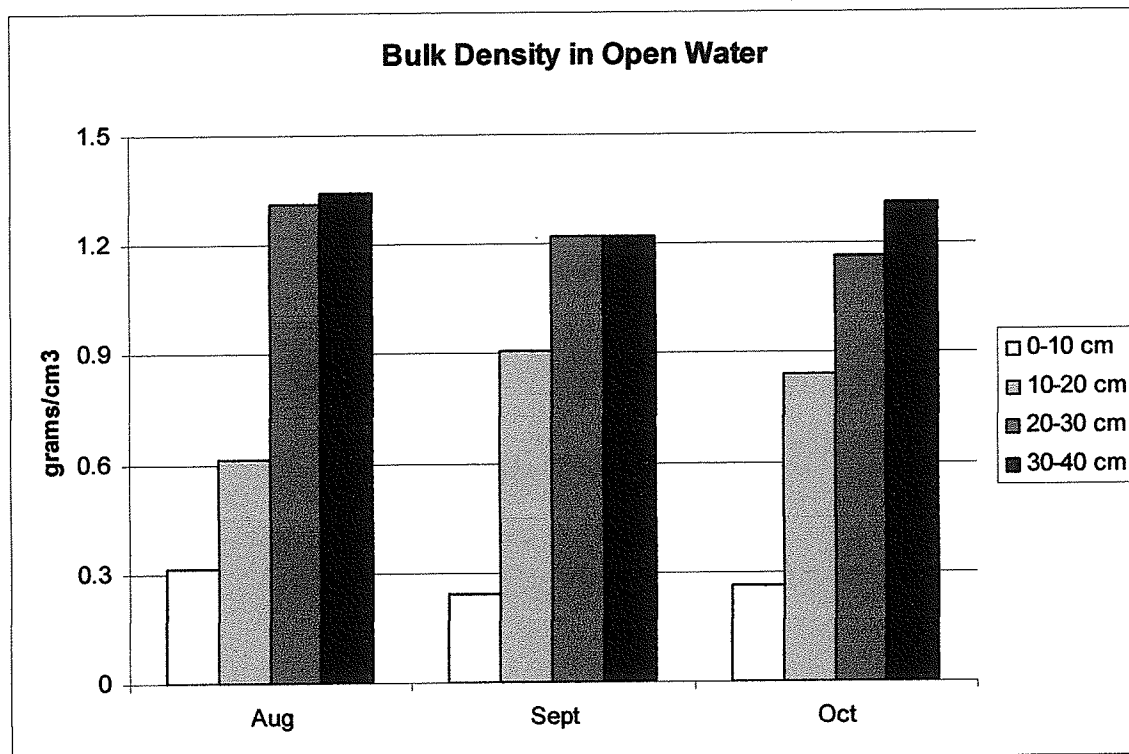


Figure 5 Bulk density of sediment horizons in open water with November spatial variation test of 10-20cm horizon.

Appendix E Resin strip preparation

Resin sheets from GE Ionics (0.5 mm thick, 2.4 meq/dry gram resin, Watertown, MA.

Cat. # AR204) were cut into 2 x 8 cm strips and prepared with bicarbonate (Bissani et al, 2002, Kuono 1995). This involved soaking the strips in 0.5 M HCl for 1 hr, rinsing well with D.I. water and then agitating in 0.5M NaHCO₃ (pH adjusted to 8.5) for 2 hrs, changing the NaHCO₃ solution four times. The prepared strips were then kept in the refrigerator in D.I. water until used. The preparation process was repeated after each resin P extraction.

Appendix F Special Analytical Procedures and Results

Type II Geometric mean Calculation

Conversion of sediment percent water data to a calculated bulk density required the use of the regression curve $y = -1.0073 \ln(x) + 4.6373$ $r^2 = 0.9944$, see Fig. 6. The use of a regression curve for predictive values depends on little variation on the x axis. If, however, the x axis has some variation, it is recommended that a Type II Geometric Mean (GM) regression be performed. The formula is as follows:

For the standard regression $y = a + b \cdot x$

$$GMslope = \frac{b}{r}$$

$$GM \text{ intercept} = Y - (GMslope \cdot X)$$

Where r = the regression, Y = mean y , X = mean x .

Using this equation the GM regression curve becomes: $y = -1.01013 \cdot \ln(x) + 4.64792$

The use of the GM regression for the calculated bulk density had a marginal difference, increasing the final extracts by about 1%.

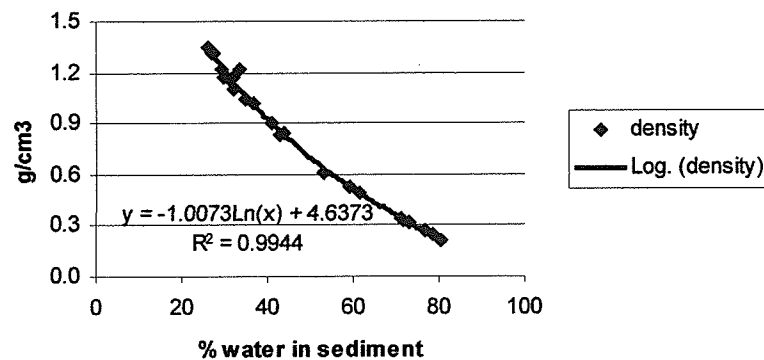


Figure 6 Regression curve showing relationship between sediment water content and bulk density. This curve was used in creating a calculated bulk density for the months it was not measured.

HCl ICP error

It had been noted by several students and staff in the Soil Science Dept. that sometimes HCl P extracts sent for total elemental P analysis (using ICP-ES contract services in the Dept. of Geology, University of Manitoba), returned lower values than the molybdate ascorbic acid method performed by students testing only for SRP. This discrepancy yielded negative organic P values and the error was attributed to “matrix effects” in the molybdate method and the ICP results were given more veracity. We experienced similar problems in our analysis, so we sent lab standards in water and 1M HCl for ICP determination after testing them for SRP on the spectrophotometer (Fig. 7). Finding consistent error we then sent commercial standards to ICP for comparison with SRP (Fig. 8). The results showed consistently lower ICP P values at all concentrations when in a 1 M HCl solution. This led to an upward adjustment of all HCl ICP results by 15% to account for the error. The adjustment eliminated most negative HCl organic P values in the data set. Since all were not eliminated, the adjusted ICP values are reported in this thesis.

Conversations with the technician conducting the ICP analysis yielded few clues as to this anomaly. He runs his standards in water, not HCl and theorized that perhaps the difference in density may slow the elution through the instrument, giving reduced values.

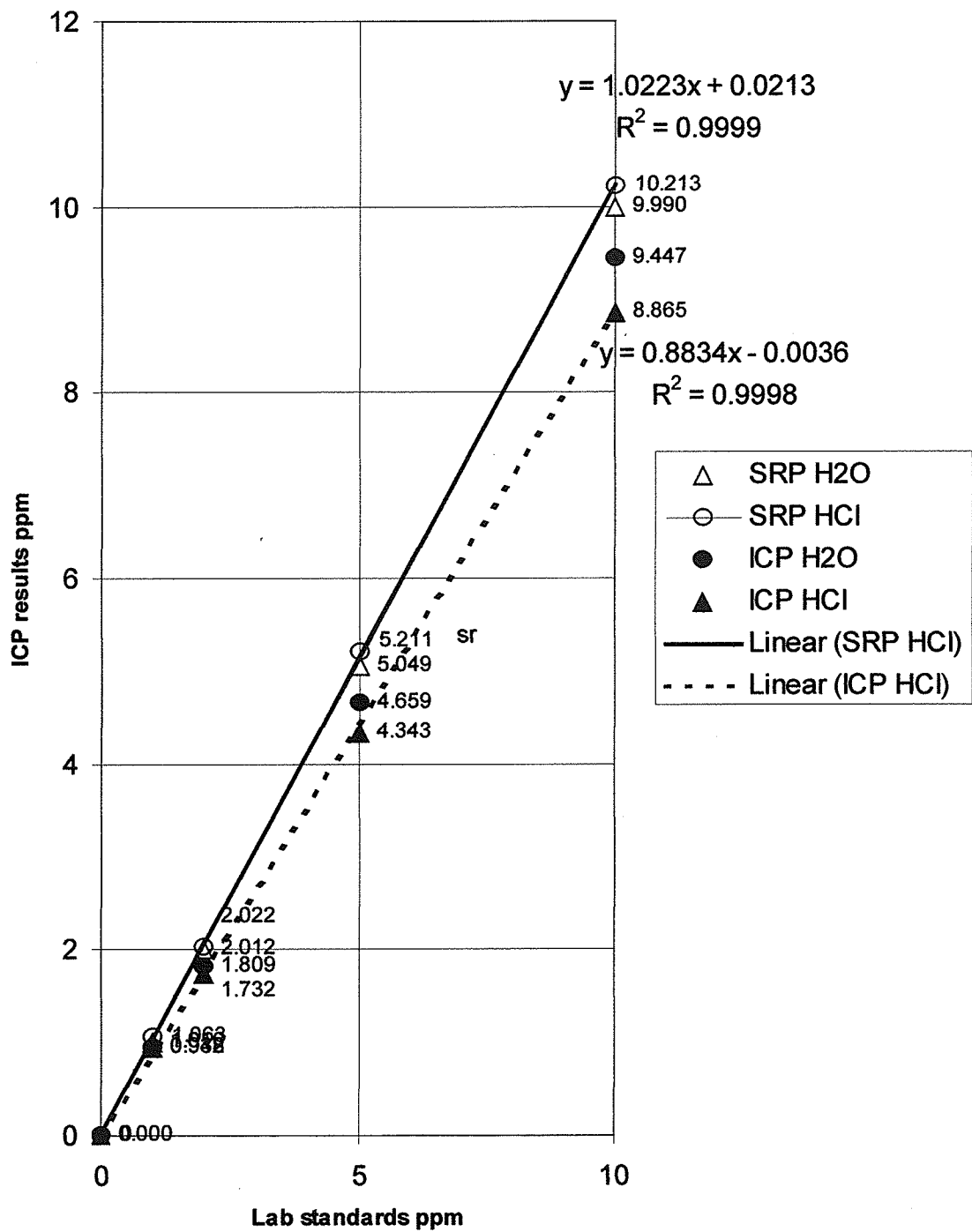


Figure 7 Analysis error found in ICP results with HCl extracts. Lab standards in 1M HCl and water were tested for soluble reactive phosphorus (SRP HCl and SRP H2O). The same samples were sent for ICP-ES analysis (ICP HCl and ICP H2O).

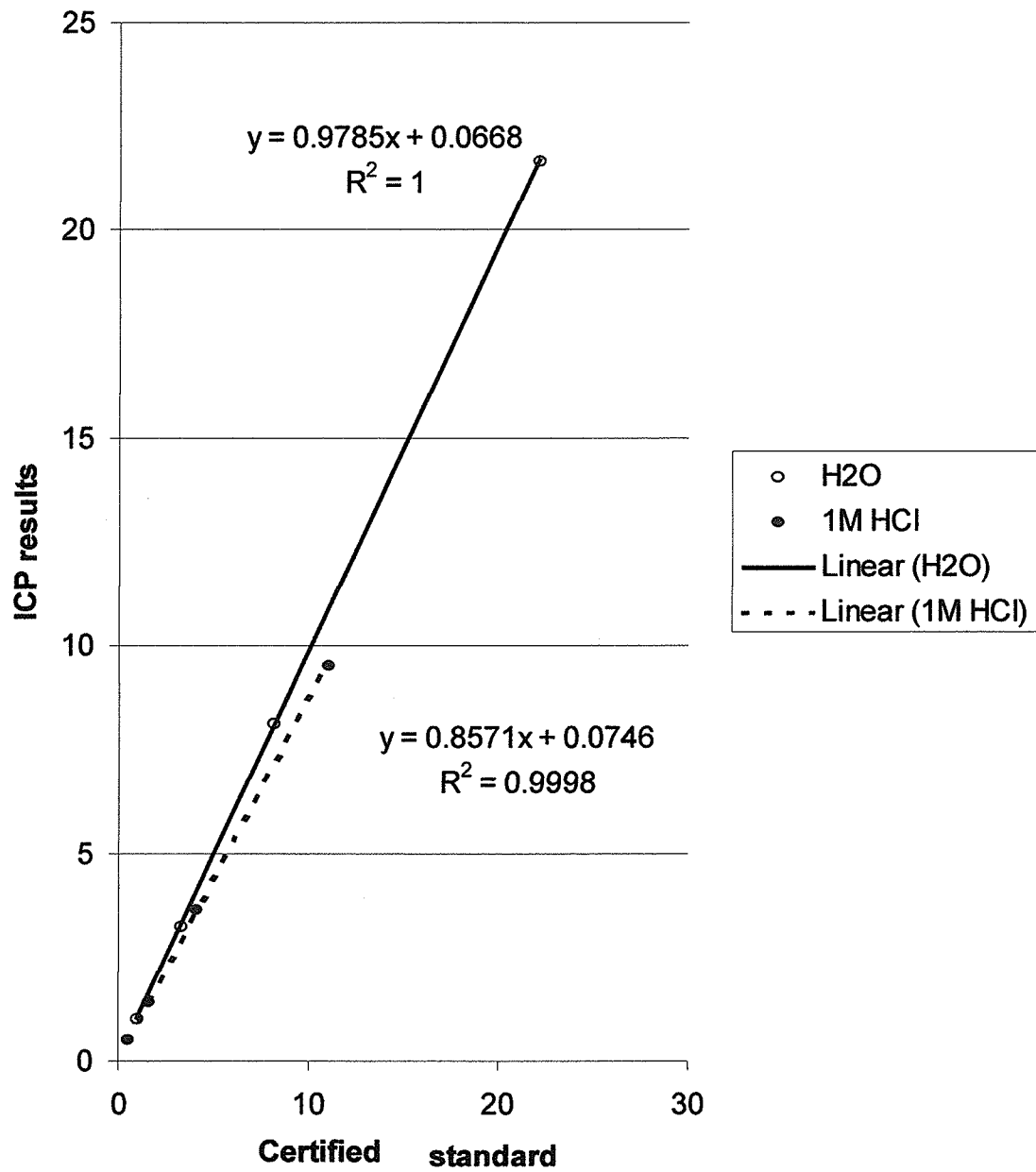


Figure 8 Analysis error between certified standards sent for ICP analysis in H₂O (H₂O) and in 1M HCl (HCl).

Alternate NaOH extract method

The sequential extraction method used to isolate P forms from sediment includes addition of 20 ml of 0.1 M NaOH as outlined in the project paper. This solvent dissolves Al and Fe oxides and releases the P sorbed or occluded in those structures. This P is detected in SRP analysis of the OH extract. Also soluble in this step are organic compounds, and some horizons from our sites produced very black supernatant, evidence of the abundant organic component. Normal SRP procedure (labelled Method 1 in this section, and used for all OH extracts collected in Chapter 3) involves adding 1-3 ml of supernatant, 4 ml of reagent and D.I. water to 25 ml. In some extracts the very acidic reagent caused precipitation to occur in the volumetric flask, possibly interfering with spectrophotometric P determination.

No mention of this problem occurred in scientific literature until several papers were found mid-way through sampling season (Paludan and Jensen 1995; Jensen et al. 2005; Reitzel et al. 2006). These authors used an alternate method (Method 2) in which they acidified the supernatant and collected the precipitate before SRP analysis. The precipitate was identified as humic acids that are soluble in alkali and precipitate at pH 2. This was filtered, dried and analysed for TP, Al and Fe. The new supernatant was identified as containing fulvic acid P compounds and any SRP. This was then analysed for SRP, TP and metals. Proceeding this way allowed division of the OH extract P into three distinct fractions: humic acid related, fulvic acid related and SRP. Determination of Fe and Al in each fraction helped these researchers to determine the role of Fe and Al in binding P to organic compounds. In theory, Fe and Al found with the fulvic acid supernatant are dissolved Fe and Al oxides (e.g., aqueous Fe^{+2}), and the metals in the

precipitate are incorporated or chelated into the humic acid structure. We used this method concurrent with the standard method to verify SRP results. In addition, the information of fulvic acid P and humic acid P shed some light onto the nature of P association in OH extracts, although results should be viewed as preliminary data because the method was used only twice (on Sept and Oct cores) with the regular 2 grams of wet sediment, yielding minimal precipitate (0.028g) from some horizons.

The results show very similar SRP data from each method, with a few outliers (Table 1). A paired t-test reveals improved technique in October (Oct t critical=0.162 and Sept = 0.221). When displayed as g/m^2 a different picture of SRP in the soil horizon emerges (Table 2) including a change in t-test results. This is because bulk density has a strong effect on g/m^2 data. (For more comparative data see section "Presentation of P extract data"). Relative abundances of each P fraction are compared with the parent OH extract (Fig. 9) and with soil TP (Fig. 10). Total recovery of precipitate was emphasized in these experiments and no replicates were available for verification, thus values should be taken as approximations, or an average of both months used. The humic P fraction forms a majority of the OH extract and is makes up a surprisingly high proportion of TP.

Table 1 The comparison between the standard method of OH extract SRP (Method 1) and with acid precipitation (Method 2) for two selected months. Better quantitative results were achieved in October. The figures are in $\mu\text{g/g}$ (ppm) of dry soil wt. A paired 2-tailed t-test was performed.

<i>horizon</i>	<i>September</i>		<i>October</i>	
	Method 1 Sept	Method 2 Sept	Method 1 Oct	Method 2 Oct
EN 0-10	24.4	25.2	30.5	32.6
10-20 cm	22.0	24.3	18.2	22.5
20-30	5.1	15.2	8.4	8.5
30-40	12.2	10.9	9.3	9.3
OW 0-10	21.3	43.5	9.9	32.3
10-20 cm	9.9	10.3	4.6	7.1
20-30	6.6	5.9	5.8	6.8
30-40	6.9	4.6	4.4	5.2
t-test	0.221		0.162	

Table 2 Comparison between reporting data in ppm and as wt/vol. Identical data as in Table1, but data is presented as g/m^2 of sediment depth (10 cm for each horizon). Note difference in t-test due to added variable of bulk density.

<i>g/m²</i>	<i>September</i>		<i>October</i>	
	Method 1 Sept	Method 2 Sept	Method 1 Oct	Method 2 Oct
EN 0-10	0.52	0.53	0.71	0.75
10-20 cm	0.72	0.79	0.84	1.03
20-30	0.27	0.79	0.84	0.86
30-40	1.22	1.09	1.05	1.05
OW 0-10	0.51	1.03	0.28	0.91
10-20 cm	0.92	0.95	0.38	0.59
20-30	0.77	0.70	0.68	0.79
30-40	0.81	0.54	0.57	0.68
t-test	0.426		0.056	

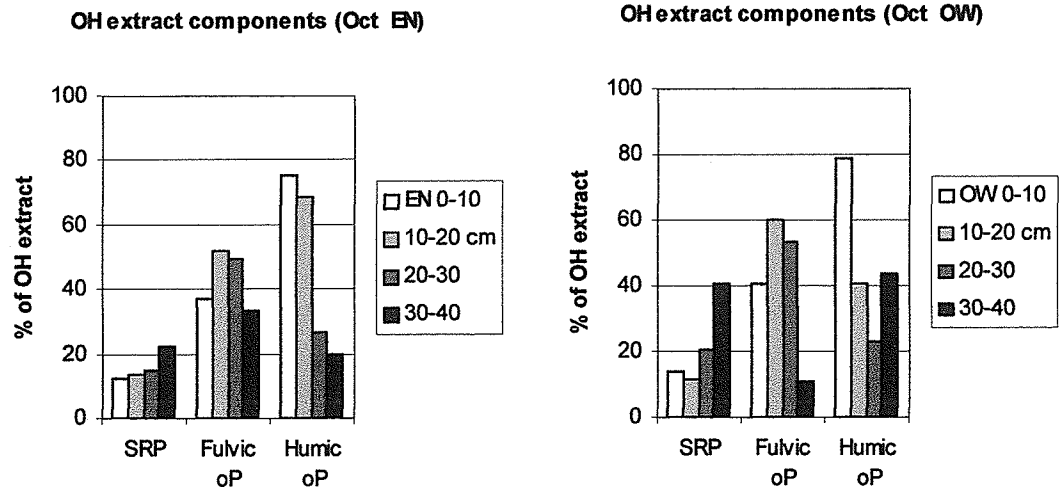


Figure 9 Enclosure (EN) and open water (OW) OH extract constituents as identified using Method 2 (acid precipitation). It is suspected over-recovery of Humic P accounts for the poor overall balance (i.e., > 100%).

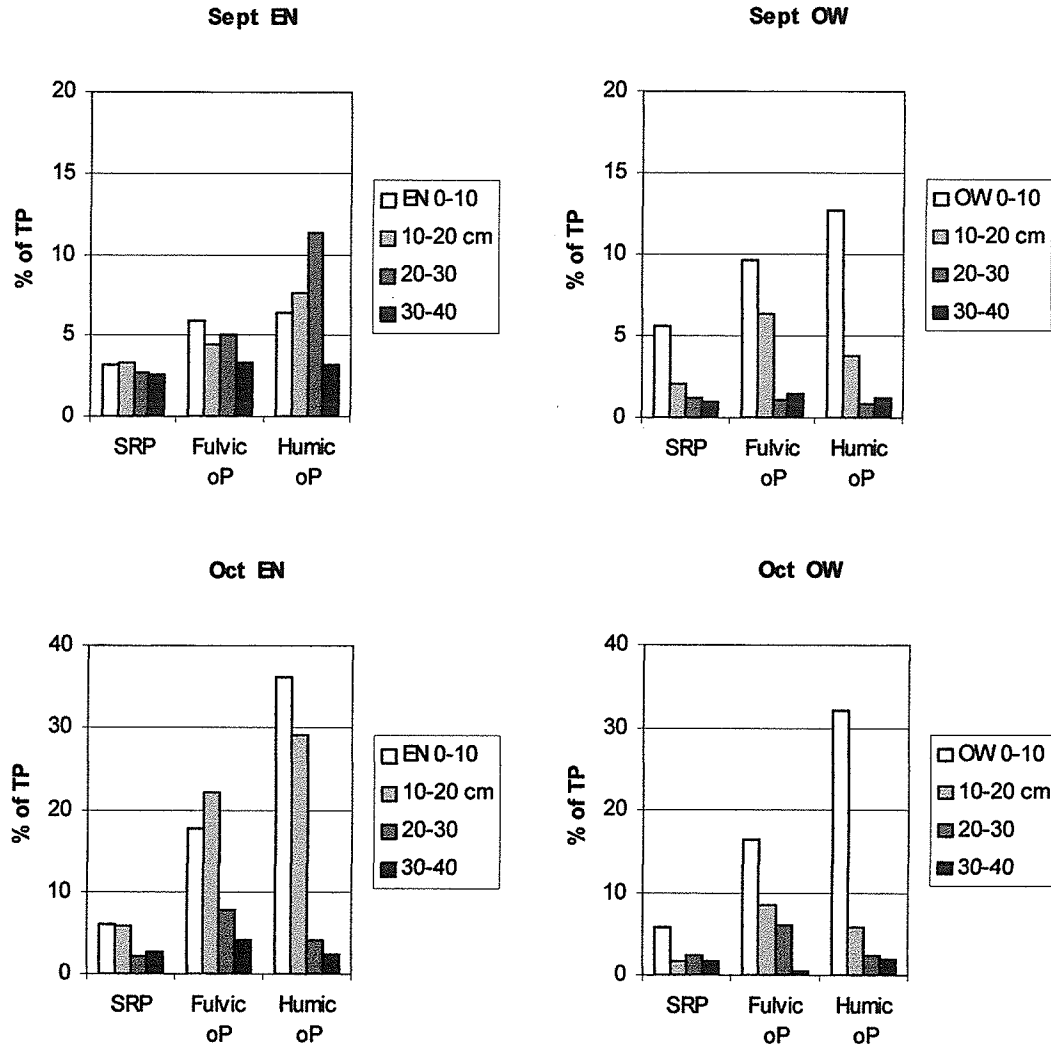


Figure 10 OH extract components as a percentage of TP for September and October from the enclosure (EN) and open water (OW) sections.

Additional to the confirmed SRP data and the different forms of organic P recovered by Method 2, analysis of precipitate and filtrate for Al and Fe gave some interesting results. These results may be meaningful in finding the relationship between Al, Fe, P and organic matter and in resolving the question of sorbtion, chelation or incorporation into the fulvic and humic acids. Presentation of Fe and Al in OH extract constituents is only relevant when compared with total soil Fe and Al (Table 3). A higher

relative percentage of each metal is related with the humic acid precipitate than the fulvic acid supernatant that contains dissolved Fe and Al oxides. Even though less Fe is found in enclosure sediment, more of what is found there is associated with the humic acid precipitate. For simple comparison, these data are presented again on a relative basis with total Fe and Al from intact soil (Table 4).

Table 3 Aluminium, (Al), iron (Fe), and calcium (Ca) found in sediment, OH extract acidified filtrate and precipitate. Values are means of Sept and Oct $\mu\text{g/g}$ data for the enclosure (EN) and open water (OW).

Horizon	Intact Soil			Method 2 OH filtrate		Method 2 OH precipitate		
	Al	Fe	Ca	Al	Fe	Al	Fe	Ca
EN 0-10 cm	40630	6589	12777	281	357	1861	933	62
10-20 cm	25838	3861	17133	306	290	2552	1212	51
20-30 cm	59704	9772	9338	323	225	2090	1006	15
30-40 cm	56272	9454	14073	153	52	528	312	3
OW 0-10 cm	38900	5459	12328	316	461	1854	925	18
10-20 cm	50178	7094	10110	204	68	687	369	8
20-30 cm	73043	12161	9563	86	4	96	49	5
30-40 cm	76184	13356	25947	105	4	45	24	0

Table 4 Data from Table 3 presented as relative percentage of Al, Fe and Ca in intact soil.

Horizon	Acidified OH filtrate		Acidified OH precipitate		
	Al	Fe	Al	Fe	Ca
EN 0-10 cm	0.7	5.4	4.6	14.2	0.5
10-20 cm	1.2	7.5	9.9	31.4	0.3
20-30 cm	0.5	2.3	3.5	10.3	0.2
30-40 cm	0.3	0.5	0.9	3.3	0.0
OW 0-10 cm	0.8	8.4	4.8	16.9	0.1
10-20 cm	0.4	1.0	1.4	5.2	0.1
20-30 cm	0.1	0.0	0.1	0.4	0.1
30-40 cm	0.1	0.0	0.1	0.2	0.0

Blanks and spikes

Solvent blanks and standards were included during regular extract processing. The blanks and standards were shaken (16 hrs), centrifuged, vacuum filtered through 0.45 μ m membrane, stored and analysed for SRP. Triplicate blanks were conducted of each solvent extraction each month. These were averaged and subtracted from the solvent P extracts, assuming the error was due to contamination from some point in the process (Table 5). A very high range was found between months and this was ascribed to common public use of vacuum filter equipment. Recovery of standards are presented in Table 6. Standards were 5 μ g P for resin and 25 μ g P for each solvent.

The high variation and the blanks and generally over recovery rate with the standards suggest consistent contamination within the process. Withholding standards from the filtration step always resulted in <100% recovery (91% n=6 st dev 8%) suggesting the rinsing procedure (triple rinse in RO water) of the vacuum filter manifolds is not sufficient to remove sorbed P from previous filtration. This theory is strengthened by consistently higher P in HCl solvent blanks. HCl would effectively desorb any P on the plastic manifolds.

Table 5 Blanks processed with each extraction process (three monthly) measured in $\mu\text{g P}$.

Solvent	May	June	July	Aug	Sept	Oct	mean $\mu\text{g P}$	St dev
Resin	NA	4.1	1.8	0.2	0.2	0.2	1.32	1.7
HCO ₃	1.9	1.3	2.7	7.3	0.2	6.5	3.31	2.7
OH	5.0	0.3	0.0	0.6	0.4	7.6	2.31	2.9
HCL	0	1.6	1.5	3.3	8.5	7.8	3.76	3.2

Table 6 Percent recovery from standards processed with solvents. Standards were of 5 μg for resin and 25 μg for the others. Standards were also sent for ICP analysis. Coefficient of variation (CV) is the relative standard deviation.

	Resin	HCO ₃	OH	HCl	ICP
	118	113	82	83	88
	235	117	99	80	81
	117	132	102	104	93
	102	117	75	129	92
	98	125	58	121	99
	95	88	73	122	103
	123	-	129	139	98
	113	-	120	117	95
	117	-	122	129	100
	-	-	-	-	108
mean	124	115	96	114	94
st dev	43	15	25	20	7
CV	34	13	26	18	7

Compaction

The sediment corer was calibrated on the side, so that water depth, soft sediment and hard sediment depths could be recorded. These data (Table 7) were compared with actual extruded cores to estimate compaction during sampling and the compaction factor (depth of corer/final core length) compared with core average bulk density but no correlations were found, indicating field sampling had little impact on final bulk density of sediment horizons.

Table 7 Sampling compaction data from open water and enclosure sites. Values are measurements on the side of the sediment corer (in cm), total depth of sediment before removing core, actual core length, and compaction factor. Bulk density for the entire core is in g/cm³.

	Water depth	Soft sediment	Clay	Total depth	Core length	Difference	Compaction	Mean Bulk Density
EN May	75	110	135	60	40	20	1.5	0.654
June	65	100	120	55	25	30	2.2	0.422
July	30	70	80	50	35	15	1.4	0.769
Aug	40	75	100	60	35	25	1.7	0.722
Sept	0	35	95	95	42	53	2.3	0.515
OW May	70	105	130	60	40	20	1.5	0.785
June	75	110	125	50	40	10	1.3	0.749
July	25	65	85	60	40	20	1.5	0.667
Aug	35	80	100	65	35	30	1.9	0.792

Iron, Aluminium, Magnesium and Calcium

Sediment Fe, Al, Ca, and Mg were determined for the May core via complete digestion of sediment and analysis with ICP-ES. Triplicate sub-samples were taken from each horizon and the values were converted from mg/kg of dry weigh to g/m^2 to conform to the measurements of the P extracts. Except for the top horizon where mineral concentrations were about equal within the EN and in the OW section, OW had substantially higher mineral levels for all horizons (Fig. 11 and 12). Mineral abundance also increases with depth, further confirming what was observed in the field.

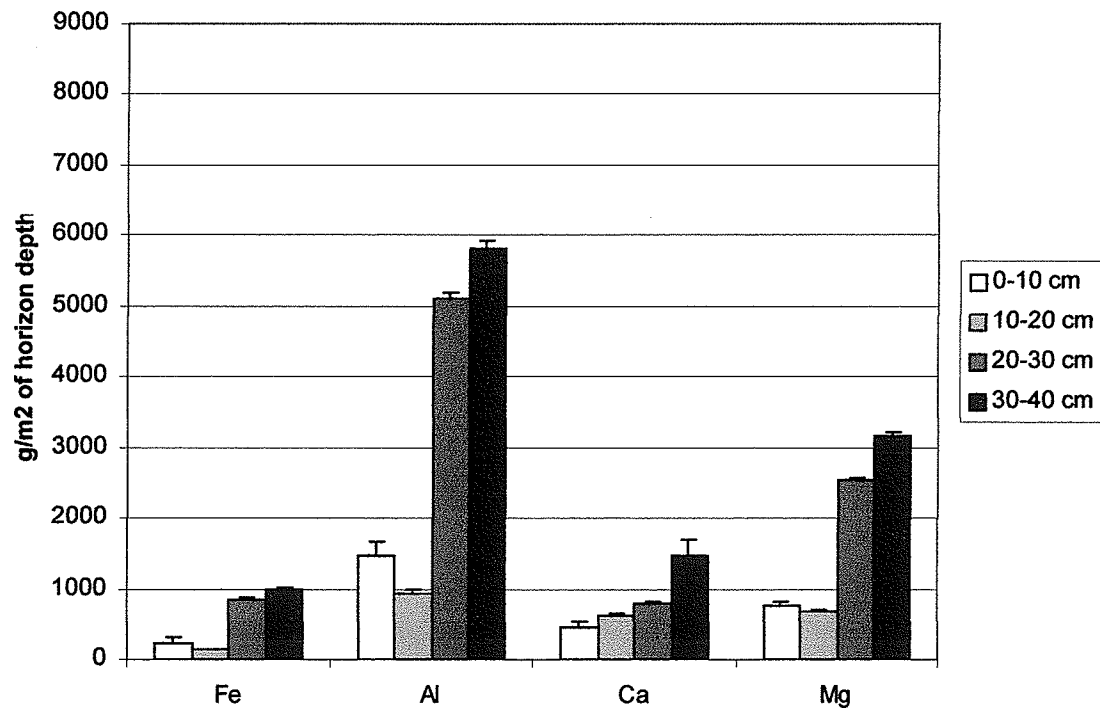


Figure 11 Sediment concentrations of Fe, Al, Mg, and Ca, found in the enclosure in May.

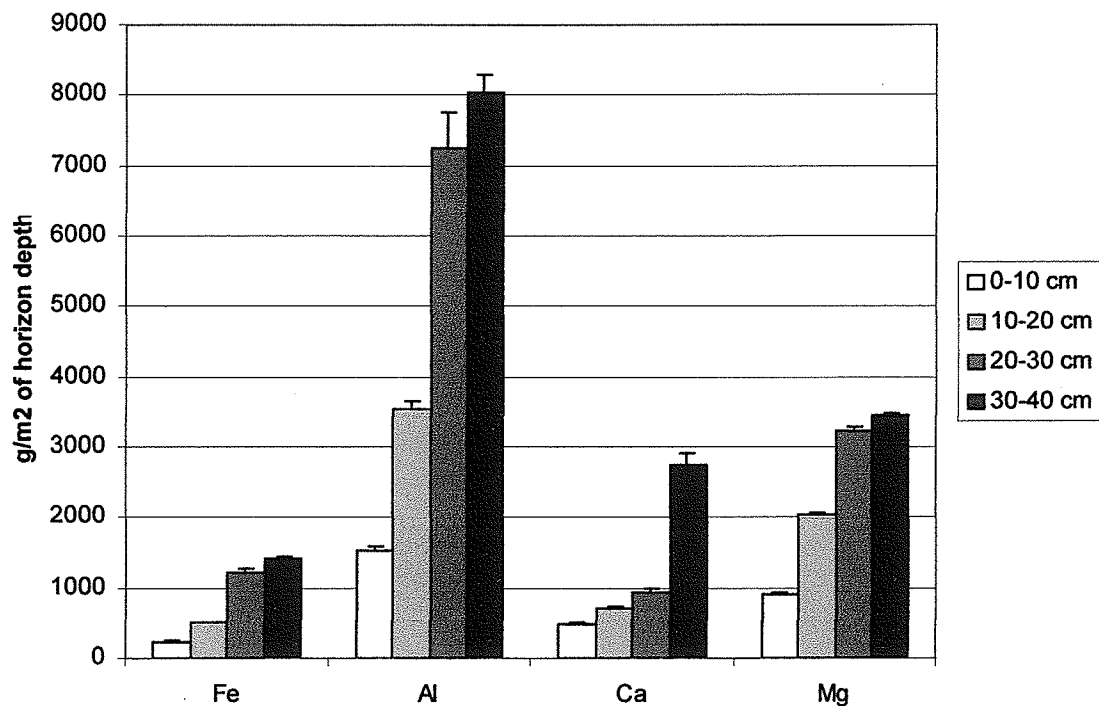


Figure 12 Sediment concentrations of Fe, Al, Mg, and Ca, found in the open water site in May.

Soil texture

Conventional sand silt and clay soil textural analysis was performed (McKeague 1978) with each horizon of the October core using ashed soil (500° C 4 hrs). The results are presented in conventional manner as percent of dry ashed soil in Table 8 and as g/m^2 as has been the norm in this thesis (Fig. 13 and 14). The latter presentations take into consideration water content and organic matter and give an accurate view of the sediment make-up.

Table 8 Soil texture as a percentage of ashed dry sediment (October core).

	<i>% sand</i>	<i>% silt</i>	<i>% clay</i>
EN 0-10 cm	17.3	63.6	19.1
10-20 cm	43.2	42.6	14.2
20-30 cm	47.1	35.5	17.4
30-40 cm	43.4	36.8	19.7
OW 0-10 cm	8.3	73.6	18.1
10-20 cm	63.8	28.7	7.5
20-30 cm	45.8	40.7	13.5
30-40 cm	35.1	43.0	21.9

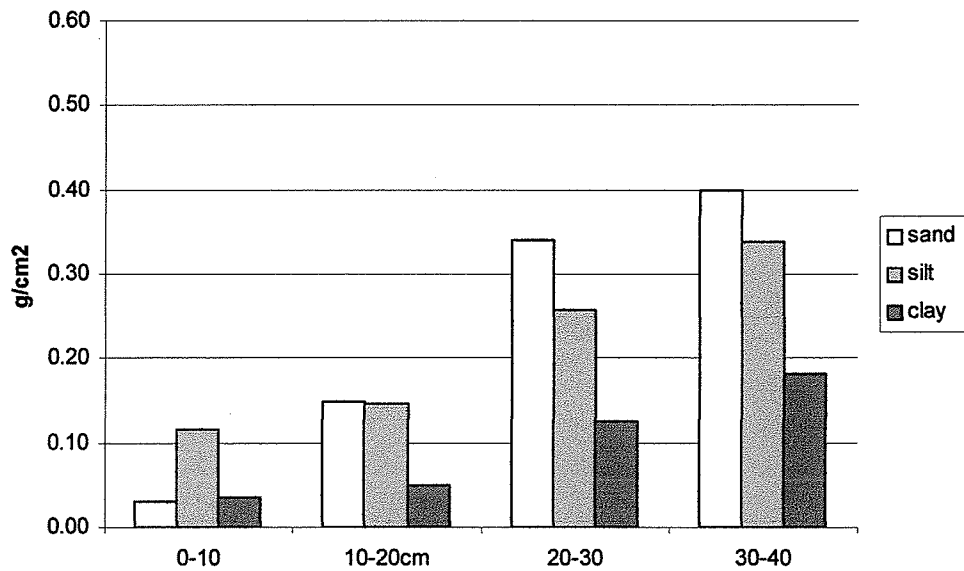


Figure 13 Soil texture components measured in grams/m² of horizon depth in the enclosure (October core).

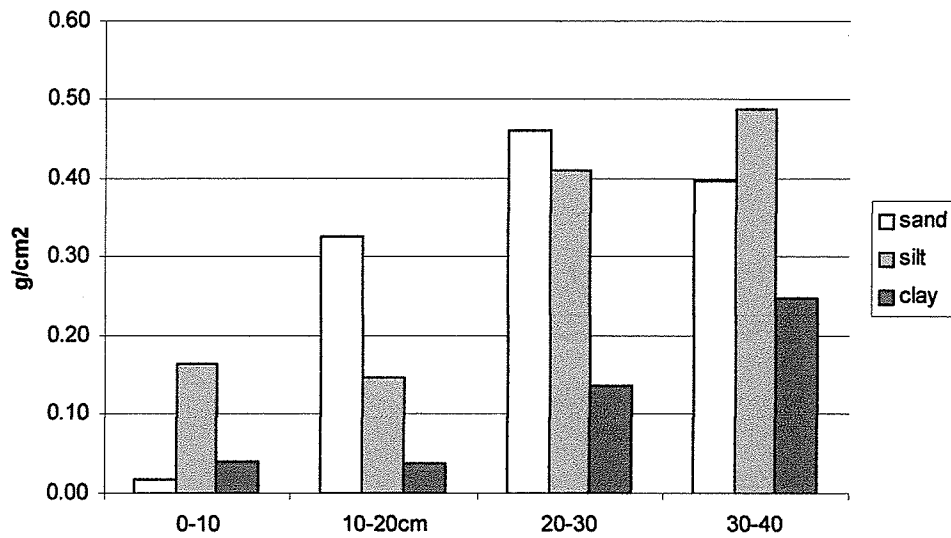


Figure 14 Soil texture components measured in grams/m² of horizon depth in open water (October core).

Presentation of P extract data

Most sediment P studies present P extract data as ppm (wt/wt) but this project found $\mu\text{g}/\text{vol}$ to be a better measure. The need to express P as mass/area of marsh, necessitated conversion to wt P/vol sediment but when this was done, we found the picture of P within the horizons changed markedly (Table 9) as did the correlations between extracts and other factors such as sediment water content (Table 10).

Table 9 Total P in sediment profiles in the enclosure (EN) and open water (OW) expressed $\mu\text{g/g}$ (ppm) and g/m^2 (calculated from $\mu\text{g/cm}^3$) with the Coefficient of variation ($100 \times (\text{St dev}/\text{mean})$) abbreviated to CV.

Horizon	$\mu\text{g P / g}$			g P /m^2 of horizon		
	mean	St Dev	CV	mean	St Dev	CV
EN 0-10 cm	600	124	21	15.2	3.4	23
10-20 cm	508	126	25	22.9	6.3	28
20-30 cm	415	78	19	34.2	7.6	22
30-40 cm	368	47	13	38.1	5.9	15
OW 0-10 cm	617	94	15	18.5	2.2	12
10-20 cm	479	52	11	30.6	9.0	29
20-30 cm	369	77	21	40.2	9.2	23
30-40 cm	340	63	19	40.9	7.8	19

Table 10 Correlation between percent water and P extracts for all horizons from sampling in the enclosure (n=6). Data is presented as both $\mu\text{g/g}$ (ppm) and g/m^2 (calculated from $\mu\text{g/cm}^3$).

% water and P extract:	$\mu\text{g P / g}$		g P /m^2 of horizon	
	Spearman's Rho	p	Spearman's Rho	p
TP	0.91	<.0001	-0.91	<.0001
HCO3	0.80	.002	0.22	0.25
HCO3 organic	0.96	<.0001	0.88	.0002
OH	0.81	.001	-0.41	.17
OH organic	0.99	<.0001	0.50	.10
HCl ICP	0.74	.006	-0.92	<.0001
Residual	0.20	.23	-0.99	<.0001