SEDIMENT OXYGEN DEMAND AND SEDIMENT NUTRIENT CONTENT OF RECLAIMED WETLANDS IN THE OIL SANDS REGION OF NORTHEASTERN ALBERTA

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A Thesis
Submitted to the Faculty of Graduate Studies
through the Department of Biological Sciences
in Partial Fulfillment of the Requirements for the
Degree of Master of Science at the
University of Windsor

Windsor, Ontario, Canada

2010

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DECLARATION OF CO-AUTHORSHIP

I hereby declare that the majority of this thesis is the product of my own work but also incorporates material that is the result of joint research, as follows:

This thesis incorporates the outcomes of a joint research effort undertaken in collaboration with Jesse Gardner Costa under the supervision of Dr. Jan Ciborowski. The collaboration is covered in Chapter II of the thesis. In all cases, the key ideas, primary contributions, experimental designs and sampling were prepared and conducted by both authors. Data analysis and interpretation along with writing tasks were divided equally among the two authors. All other parts of this thesis are the original work of the primary author.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from the co-author to include the above material in my thesis.

I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

ABSTRACT

Sediment oxygen demand (SOD), nutrient concentrations, and submergent macrophyte biomass were measured in reference wetlands and oil sands process material-affected (OSPM) wetlands in the oil sands region of Alberta. Any or all of these factors could influence the success of wetland reclamation in this area. Gas flux and SOD chambers were deployed to determine the biological and chemical components. Nutrient concentrations were estimated from water and sediment extractions as well as PRS™ probes.

Sediment oxygen demand was slightly higher in OSPM-affected wetlands than in reference wetlands. Over 85% of SOD was due to chemical processes, likely due to ammonium oxidation. High SOD could limit benthic respiration and ultimately affect carbon stores. Reference wetlands had greater submergent macrophyte biomass than OSPM-affected wetlands but phosphorus concentration could not explain this difference. This implies that sediment oxygen demand, phosphorus concentration, and submergent macrophyte biomass are independent of one another among wetland classes.

ACKNOWLEDGEMENTS

There are so many people that need to be thanked for their part in the completion of this thesis. Without each and every one of them, this Masters degree could not have been completed.

I would first like to thank my wonderful supervisor, Dr. Jan Ciborowski. His tough questions, along with his constant encouragement, allowed me to fully understand the scope of my work and appreciate how it fits into the real world. This allowed me to answer the questions I put forth in the most meaningful way. Although he is by far the busiest man I know, he always had time to answer any questions that I had, regardless of what time my emails went out. I knew I could always count on a speedy reply. Without his great dedication to his work and students, I could have never succeeded in completing this degree.

Dr. Dennis Higgs and Dr. Christopher Weisener provided excellent suggestions and feedback for how to improve this thesis. Part of this research was conducted in Dr. Weisener's lab and I thank him for that. Dr. Dennis Higgs helped guide me to a Master's position after finding myself lost once my undergraduate career came to an end. I thank both of these gentlemen for their valuable inputs over the duration of my studies at the University of Windsor.

Field work can be tiresome and unglamorous at times, however, surrounding yourself with a great team always makes it that much more enjoyable. I have only the utmost admiration for the groups of people that helped out in the wetlands of Fort McMurray, Alberta. Jennifer Thoms, Melanie Martin (nee Thoms), Andrew Nelson, Marie-Claude Roy, Hao Chen, Peter and Allison Legg, Kaitlyn Kennedy, Michael "Charlie" Chen, Karolina Jurkowski, Sheeva Nakhaie and particularly Jessica "Ducky" Dowson for keeping me focused and helping me sort through vegetation in the lab as well as Michael Dufour for helping with extractions. Thank you all for your hard work.

A special group of guys gets a special shout out in this section. The trio of Jesse Gardner-Costa, Joshua Martin, and Kurt Frederick were all doing their own Masters projects at the same time I was. That never stopped any of them from lending a helping hand or providing helpful suggestions. Joshua was my roommate my first year in the field and I could always rely on him for guidance. He exemplified what it meant to be a leader,

researcher, and student. He set a good example for me to follow and was a big help with research efforts after he accepted a position with Suncor. Kurt and I started our Masters at roughly the same time and I always knew he would understand the pain and stress I was going through at times. He was always generous with his suggestions and advice. School work aside, I always knew I could count on Kurt to go out for a good time and get away from it all. Never will I forget the outrageous dancing nights in the Mac or the trip to Canmore to see the mountains for the first time. Needless to say, I'm excited for the next adventure. And what can I say about Jesse? We have spent the better part of the last two years in either the same hotel room or office. Surprisingly enough, we never became fed up with one another. Jesse was instrumental in our collaboration as well as helping me with my own writing ideas. He was encouraging, and witnessing his work ethic in the lab, helped me strive to finish my thesis with the same amount of vigour as he had. He was always available for advice and answering questions, and was never too busy to proof-read some of my work. Thank you Josh, Thank you Kurt, and Thank you Jesse.

Many people never made it out to the field but were nonetheless instrumental in the culmination of this thesis. Endless amounts of feedback and constructive criticism were received from lab mates: Mary Sebastian, Katya Kovalenko, Shuba and Laxmi Pandit, Li Wang, Misun Kang, Mirna Jajo, Michelle Dobrin, Nicole Barker, Kyle Swiston, Jessica Cuthbert, Lyndon Barr and my cubby buddy Emily "Pankey" Crisovan. Thank you all for your valuable input.

I would also like to thank people within the Biology department and around the University for their support: Ingrid Churchill, Rodica Leu, Nancy Barkley, Carolin Lekic, Jacqueline Christie, Barbara Zimmerman, Steve Budinsky and Windsor TSC, Christina Smeaton (GLIER), as well as all the other graduate students.

None of this work would have been possible without the hard work of the people working in the oil sands. I would like to convey my considerable amount of appreciation for Clara Qualizza, Terry Van Meer, Chris Beierling, Lori Cyprien, Carla Wytrykush, Tara Penner, Mike Mackinnon (Syncrude), Christine Daly, Joshua Martin, John Hornung (Suncor), and Richard Kavanagh (CNRL).

Last, but certainly not least, I must thank my family and friends. My parents (Richard and Gerda), whose unconditional love, support, and inspiration always drove me

to success. My sister, Sabrina, and brother, Quinten, provided me with the motivation to finish my degree. I would not be who I am today without my wonderful family. Thank you to all of my friends, particularly Curtis Hyra, for his continual visits to my office to make sure that I was staying on task (or perhaps to give me a break from all of my work), and Lukas Wlodarczyk for always offering any help I may need. I also have much appreciation for Michal Kasprowicz and his patience in explaining to me the intricacies of Microsoft Word formatting.

THANK YOU ALL

Funding for this research was provided by grants from Syncrude Canada Ltd, Suncor Energy Inc. (including Petro-Can), Canadian Natural Resources Ltd., Shell (formerly Albian Sands Energy), Imperial Oil Ltd., Total E&P Canada, and the Natural Sciences and Engineering Research Council of Canada (NSERC) along with the Canadian Water Network to Dr. Jan Ciborowski and co-investigators of the CFRAW research initiative. Awards of University of Windsor graduate assistantships, travel awards, and an NSERC Industrial Postgraduate Scholarship to Carsten Slama are gratefully acknowledged.

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LIST OF TERMS & ABBREVIATIONS

SOD – sediment oxygen demand

BSOD – biological sediment oxygen demand

CSOD – chemical sediment oxygen demand

FFT – fine fluid tailings

OSPM – oil sands process material (includes both water and sediment)

OSPW – oil sands process water

OSPS – oil sands process sediment

MFT – mature fine tailings

CT – consolidated tailings

PRS™ probes – plant root simulator probes

SAV – submerged aquatic vegetation

CHAPTER I:
GENERAL INTRODUCTION

Goals and Objectives

The purpose of this study was to evaluate sediment oxygen demand and its components in reclaimed wetlands of the oil sands region and relate it to the concentration of phosphorus and ultimately the submergent macrophyte biomass. This would help determine if sediment oxygen demand could be used as a proxy to explain the lack of submergent macrophytes found in oil sands process material-affected wetlands.

Wetland ecosystems

Wetland ecosystems cover approximately 14% of Canada and 6% of the Earth's land but play a much larger role in the planet's sustainability (Natural Resources Canada 2004). Not only do they provide a habitat for many species of plants and animals that can only flourish in these types of environments but they are also vital for the maintenance of other ecosystem types (Hansson *et al.* 2005). Because of their close link with terrestrial habitats, the benefits of wetland processes are seen in an area much larger than the wetland itself (Kladec and Knight 1996). Wetlands provide a location for flood waters to reside and help regulate water quality by filtering out suspended solids (Zedler et al 2005). Wetlands are a significant site for the cycling of important nutrients such as carbon, nitrogen, and phosphorus (Batzer & Sharitz 2006). They can act as a carbon storage sink, sequestering and preventing its release into the atmosphere (Preston & Bedford 1988). Because of their undeniable contributions to ecological processes and their ability to support high species diversity, it is essential to both conserve wetlands and understand their dynamics and function.

The role of oxygen in wetlands

Oxygen plays a vital role in most biochemical processes. It is a key product of photosynthesis, which in turn allows the respiration of organisms to proceed;

$6CO_2 + 6H_2O \leftrightarrow C_6H_{12}O_6 + 6O_2$ (Campbell & Reece 2005)

Photosynthesis converts carbon dioxide and water into oxygen and a simple sugar. In contrast, respiration (the above reaction in reverse) results in the consumption of oxygen during the breakdown of molecules to provide energy. Oxygen is clearly a central contributor to both of these fundamental biochemical processes.

Furthermore, oxygen can be tracked within an ecosystem to determine whether the system is a net producer or consumer of carbon (del Giorgio 2005, Thormann *et al.* 1999, Drake *et al.* 1996). Carbon accumulates in wetlands when production exceeds decomposition. Therefore, if annual photosynthetic production exceeds annual respiration, carbon will accumulate within the system (Moore *et al.* 2002, Clymo *et al.* 1998). This is generally a case of reduced decomposition rather than an increase in net primary production (Thormann *et al.* 1999). Oxygen is required by many microbes that decompose organic matter. It is also a participant in oxidation-reduction reactions. Clearly, oxygen concentration regulates many wetland functions (Dauer *et al.* 1992). Dissolved oxygen is especially important in wetlands because of their shallow nature (Sweerts *et al.* 1991). Declines in dissolved oxygen concentration in wetlands can influence the survival of fauna and greatly alter community composition (Spieles *et al.* 2003).

Wetland sediments and dissolved oxygen cycling

Studying changes in the dissolved oxygen concentration can lend insight into underlying biological and chemical processes that ultimately govern wetland productivity. Diel fluctuations in dissolved oxygen concentration are a natural occurrence in aquatic systems. Photosynthesis during the day by submergent macrophytes and algae contributes dissolved oxygen to the water column whereas respiration and decomposition of organic matter, as well as the oxidation of reduced chemical constituents, cause a decline (Stratford *et al.* 2004). Many of these reactions occur on or just above the substrate. This is due in large part to the shallow nature of wetlands (Sweerts *et al.* 1991), in which benthic production exceeds pelagic production (Ganshorn 2002). As a result, sediments play a fundamental role as the foundation of the food web in wetlands (Allen *et al.* 1989). Primary producers rely on the sediment and its interaction with the overlying water for nutrients (Donk *et al.* 2002) and consequently generate the energy that supports

the food web. Therefore, processes that occur within, or are affected by the sediment, have the potential to ultimately influence wetland development and sustainability.

Sediment oxygen demand

Processes that occur at the water-sediment interface and cause a decline in the dissolved oxygen concentration contribute to the overall sediment oxygen demand (SOD; Murphy & Hicks 1986), which is defined as the rate at which dissolved oxygen is consumed by biochemical processes at the water-sediment interface. The SOD is usually expressed in terms of units of oxygen consumption per unit surface area per unit time (e.g., $mg/m^2/d$) (Murphy & Hicks 1986). Sediment oxygen demand contributes greatly to oxygen depletion in most water bodies (Truax *et al.* 1995). It was responsible for nearly half of the oxygen consumption in some rivers (Hanes & Irvine 1968), while Burns and Ross (1972) reported that SOD accounted for 81% of the oxygen demand in the hypolimnion of the central basin of Lake Erie (from Veenestra & Nolan 1991). Factors affecting SOD include temperature, sediment depth, reducing substances and dissolved oxygen concentrations (Seiki *et al.* 1994, Utley *et al.* 2008).

Sediment oxygen demand is comprised of two components, the biological sediment oxygen demand and the chemical sediment oxygen demand (Wang & Paula 1984). The biological sediment oxygen demand (BSOD) results from respiration of multicellular benthic organisms as well as the microbial-mediated decomposition of organic matter. The chemical sediment oxygen demand (CSOD) arises from the consumption of oxygen during inorganic chemical reactions, such as the oxidation of molecules such as iron or ammonium (Wang & Paula 1984). The relative importance of the two components is still strongly debated and is discussed further in Chapter II.

Sediment composition

The composition of wetland sediments may greatly influence processes that affect oxygen demand (Barcelona 1983, Utley *et al.* 2008). Sediment type affects both the rate at which reactions proceed and the proportion of the biological and chemical components comprising the total sediment oxygen demand. Conditions such as the quantity of reduced substances or the amount of organic matter may affect the sediment oxygen demand

(Barcelona 1983, Gelda *et al.* 1995, Van Duyl *et al.* 1992). Sediments are also the dominant site for nutrient uptake by submergent macrophytes (Thursby & Harlin 1984, Brix 1994), and therefore a link may exist between SOD and nutrient availability. If so, an increase in oxygen demand may be correlated with a decrease in nutrient levels available to support plant life. Nitrates act as buffers in anaerobic sediments limiting reduction of iron and sulphate (Lucassen *et al.* 2003), which would otherwise release phosphorus. These conditions may regulate overall wetland structure, including the establishment of macrophytes and subsequently the arrangement of the food web (Norlin *et al.* 2005, Bayley & Prather 2003). This may alter the wetland community composition and ultimately its primary and secondary productivity. As such, it is important to understand how sediment attributes influence sediment oxygen demand, especially in wetlands where the sediments are chosen, as is the case in reclaimed wetlands.

Alberta's oil sands

The oil sands region in north-eastern Alberta contains many constructed wetlands. The oil sands deposits found here are some of the largest in the world and cover approximately 140,000 km^2 (Government of Alberta 2009). In 2008, 1.31 million barrels of crude oil were produced per day. Production is expected to increase to 3 million barrels per day by 2018 (Government of Alberta, Alberta Energy 2009). In order to expose the oil-laden sands, the overlying habitat is greatly disrupted. First, the area is clear-cut of all existing trees. The top organic soil horizon is removed along with the underlying overburden (a thick layer above the oil sands comprised of clay and other soil material). This uncovers the desired oil sands which can then be excavated and loaded into trucks, which convey them to the processing plant. Some companies extract bitumen using the Clark extraction method, in which the oil sands are placed in a vat of hot water and sodium hydroxide, which eventually separates the bitumen from the sand (Fine Tailings Fundamentals Consortium (FTFC) 1995). The water and sediments resulting from this process are pumped into large settling basins to allow sedimentation and dewatering to occur. The settled materials are referred to as fine fluid tailings (FFT) when fresh, and mature fine tailings (MFT) when older. To increase the rate of settling, gypsum ($CaSO_4$) is added to the FFT, producing a denser material referred to as consolidated

tailings (CT). The gypsum results in higher concentrations of sulphates and therefore increased salinity in tailings water (MacKinnon *et al.* 2001). The oil sands process materials (OSPM) contain naphthenic acids (NA) and polycyclic aromatic hydrocarbons (PAH). In addition, OSPM also have higher concentration of salts (the marine nature of geological formations contributes as well) and contain residual bitumen and sand. Although the NA and residual bitumen may initially be acutely toxic to aquatic organisms, microbial activity metabolizes some constituents, reducing toxicity over time (Herman *et al.* 1994, Lai *et al.* 1996). This allows these mining by-products, including both OSPM water and sediments, to be used during the reclamation process.

Oil sands reclamation

After open pit mining of the oil sands has been completed, the Environment Protection and Enhancement Act (EPEA) requires the land to be returned to equal or greater productivity than it was prior to mining. In all, 20-40% of the landscape is expected to be reclaimed to wetland ecosystems - marshes, fens, bogs and end pit lakes (OSWWG 2000). The oil companies anticipate that much of the OSPM produced will be incorporated into the reclamation landscape. Both oil sands process affected water and solids are used with the expectation they will eventually contribute to self-sustaining wetlands while at the same time ridding the oil companies of some of their waste product. The overburden and topsoil that was removed and stored during the clearing of the land are once again replaced and incorporated into the wetland. A key issue associated with reclamation efforts is the amount of time needed for wetlands to mature. The high concentrations of salts and other chemical constituents in oil sands process water and sediments hinder the establishment of vegetation (Renault *et al.* 1998). Elevated levels of ammonia and sulphate may also have detrimental affects on macrophyte and invertebrate survival (Hickey & Vickers 1994, Van der Welle *et al.* 2007). The original wetlands of the oil sands region formed and accumulated peat over thousands of years (Vitt *et al.* 1996). Although the reclaimed wetlands may eventually acquire functions similar to those of the original wetlands, efforts are being made to accelerate this progress. Organic topsoil and peat are added to hasten the colonization process and stimulate primary productivity (OSWWG 2000, Turetsky *et al.* 2002). Studies are ongoing to see whether

biofilm from natural wetlands may also act as a surrogate in these attempts to accelerate succession (Frederick 2010).

Oil sands wetlands

Pilot scale demonstration efforts to assess the feasibility of wetland reclamation in the Alberta oil sands mining area have produced a variety of wetland types, varying in construction history and sediment composition. Natural wetlands are those that were formed naturally and have never been disturbed. Reference wetlands are those that either recently formed naturally in low-lying areas ('oppurtunistic wetlands') or were constructed on disturbed land but do not contain any oil sands process material. In contrast, OSPM-affected wetlands are constructed on disturbed land using residual mine process materials (OSWWG 2000). As a result, this landscape provides many opportunities to study the impacts that different sediments have on wetland ecosystem processes.

This research is part of a larger, NSERC-supported collaborative project involving seven oil sands mining partners and four universities. The CFRAW (**C**arbon dynamics, **F**ood web structure and **R**eclamation strategies in **A**thabasca oil sands **W**etlands) research initiative is a project to study and understand the effects of oil sands process materials on reclaimed wetland characteristics and their ability to sustain fully functioning ecosystems (Ciborowski *et al.* 2008). Understanding nutrient cycles and carbon flow through the food web are the major focus of the combined efforts. This research initiative categorizes wetlands based on reclamation class (natural, reference, OSPM-affected), organic matter amendment and age (Figure 1.1). Thus, the CFRAW study design matrix provides wetlands with varying conditions to study these aspects.

A marked feature of OSPM-affected wetlands is that plant colonization rate and zoobenthic community development are impeded (Barr 2009, Cooper 2004). OSPM-affected wetlands have higher salinities than reference wetlands, and contain residual chemical constituents. Submergent macrophytes provide important habitat structure that sustains zoobenthic and zooplanktonic life (De Szalay & Resh 2000). Without these basal resources to support higher trophic levels, many other organisms will be limited in

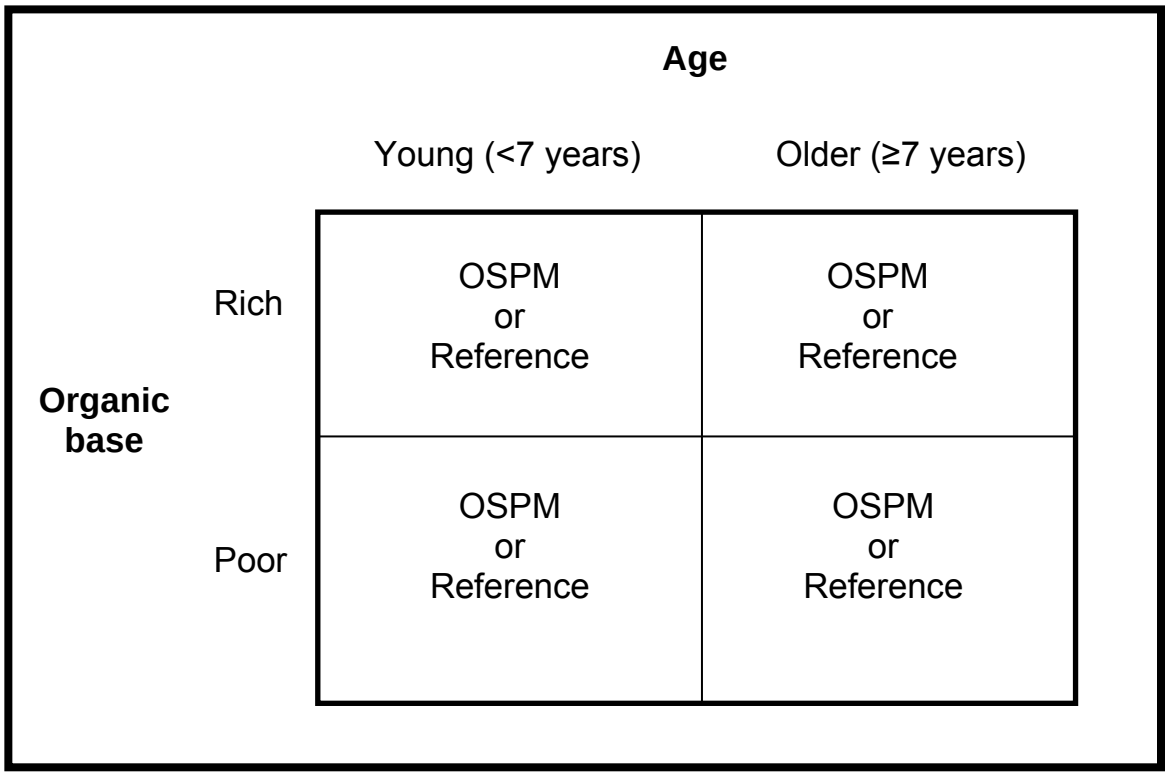

Figure 1.1 The CFRAW matrix. Wetlands are classified according to age (younger or older than 7 years, determined from stabilization of benthic invertebrate community composition), organic matter amendment (whether the substrate of the wetland is rich or poor in organic matter) and class (oil sands process material-affected or not). Wetlands in the oil sands lease area have been classified to provide a suite of focal wetlands in which either one or more of the characteristics can be studied.

numbers or productivity or fewer species will be present in and around these types of wetlands. Consequentially, wetlands that lack submergent macrophytes may be relatively unproductive or less stable than systems that support them (Downing & Leibold 2002, Worm & Duffy 2003, Engelhardt & Ritchie 2001).

Research objectives

I propose that the sediment oxygen demand may play a key role in the development of a substantial submergent macrophyte community through an indirect link involving phosphorus availability. Chemical oxidation, particularly ammonium, can increase the CSOD and the overall SOD as well. This can then affect nutrient concentrations and therefore could limit submergent macrophyte biomass (Figure 1.2). Wetland sediments, particularly those derived from oil sands process materials, may regulate the biochemical reactions that take place and either limit or facilitate nutrient dynamics through a series of redox processes (Reddy & Patrick 1975). High concentrations of sediment-associated chemical constituents may greatly influence sediment oxygen demand (Matthews *et al.* 2002, Barcelona 1983). If this is the case, then the sediments used to reclaim wetlands could regulate overall wetland productivity (Lucassen *et al.* 2004). Ammonia and other reducing compounds in OSPM-affected sediments may exhibit greater rates of oxygen consumption at the water-sediment interface than natural substrates (Matthews *et al.* 2002). Increased levels of chemical constituents have the potential to increase the CSOD and ultimately the overall SOD (Barcelona 1983). There may thus be an indirect link between the proportion of CSOD and the amount of bioavailable phosphorus and ultimately the abundance of submergent macrophytes present. Oxidation of ammonium, through a series of reactions, may prevent phosphorus mobilization from the wetland sediments (Lucassen *et al.* 2004). This will be further explained in Chapter III. As a result, these wetlands may contain less bioavailable phosphorus and therefore are not able to support a submergent macrophyte community. Other than the work of Goudey *et al.* (1990), little SOD research has been done to date on wetlands on the oil sands lease sites and therefore much can be learned about sediment oxygen demand rates in varying wetland types. This research will assess the role SOD

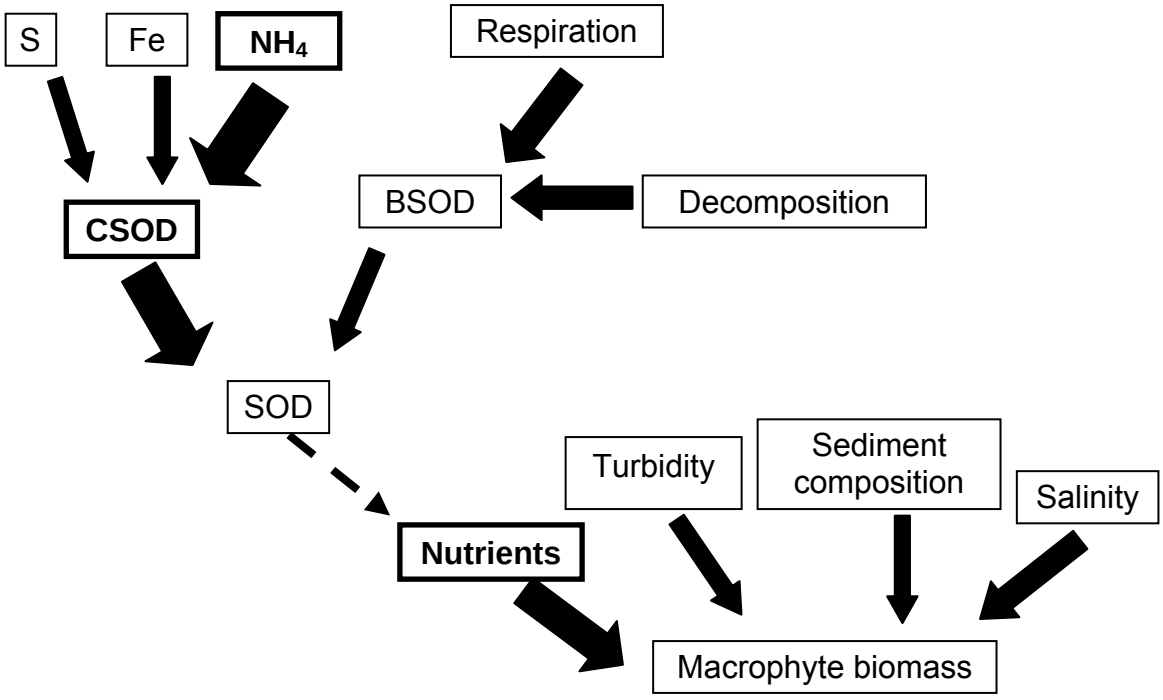

Figure 1.2 Flow chart depicting the series of events by which CSOD may limit submergent macrophyte biomass. Thickness of the arrows shows the postulated proportional impact on the respective factors.

plays in phosphorus availability and ultimately submergent macrophyte establishment and biomass. This knowledge will help environmental biologists better understand the strategies most likely to restore the landscape to its original functions.

Other factors which may inhibit the growth of submergent macrophytes include salinity, turbidity, and sediment suitability. These were all reviewed in depth by Cooper (2004). Of these factors, salinity is likely the most important, while turbidity and sediment type play a lesser role. The combination of theses factors, rather than a single one, probably determines whether submergent macrophytes can become established in an aquatic ecosystem.

The objectives of this research were to determine the impact of reclamation processes and sediments on the sediment oxygen demand. This included contrasting sediment oxygen demand rates of natural and reference wetlands with OSPM-affected wetlands followed by determining the proportion of chemical and biological sediment oxygen demand. Another goal of this project was to determine whether the rate of CSOD can predict and explain the relative dominance of benthic algae compared to submergent macrophytes in OSPM-affected wetlands. My postulate is that residual chemical constituents that increase the CSOD may also cause certain ions to remain bound to the sediments, thus making them unavailable for nutrient uptake by submergent macrophytes. Plant root simulator (PRS^{TM}) probes were used to assess the bioavailability of certain nutrients in the sediments of both reference and OSPM-affected wetlands.

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CHAPTER II:
USING GAS FLUX TO ESTIMATE BIOLOGICAL AND CHEMICAL SEDIMENT OXYGEN DEMAND IN OIL SANDS-AFFECTED WETLANDS

Introduction

The current study investigates the effect of oil sands process materials (OSPM - the residual sediment and water produced during the extraction of bitumen from oil sands) on sediment oxygen consumption rates in wetlands reclaimed using these materials. The objectives were to determine the sediment oxygen demand (SOD - rate of dissolved oxygen consumption at the water-sediment interface (Murphy & Hicks 1986)) in a suite of reference and OSPM-affected wetlands, and to assess the proportion of SOD that could be ascribed to oxygen consumed through biological processes (biological sediment oxygen demand, BSOD) vs. that consumed in redox reactions involving chemical-driven oxidation (chemical sediment oxygen demand, CSOD). I wanted to determine whether chemical or biological oxygen-consuming processes dominate within the study wetlands, and quantify the effect of oil sands process materials (OSPM) on oxygen demands within wetland sediments.

I expected CSOD to be higher in OSPM-affected wetlands than in reference wetlands; likely due to the greater degree of chemical oxidation of ammonia, which is in elevated concentrations in OSPM-affected than reference wetlands. I also expected the SOD to be higher in OSPM-affected than in reference wetlands. Other studies have indicated a dominance of CSOD over BSOD in aquatic systems and so I expected higher CSOD in OSPM-affected wetland and therefore a higher overall SOD in OSPM-affected than in reference wetlands (Neame 1975, Gardner & Lee 1965, Wang 1980).

Secondly, I expected the BSOD to be lower in OSPM-affected than in reference wetlands. Studies had observed lower gas flux in OSPM-affected than in reference wetlands (Gardner Costa 2010) implying less microbial activity, potentially reflecting a low level of oxygen consumption by biological processes. Elevated salinities and conductivities (characteristic of OSPM-affected wetlands) were expected to further limit BSOD.

Sediment oxygen demand is a significant (and perhaps dominant) component of the oxygen budget in shallow water bodies, such as wetlands (Hatcher 1986, Higashino *et al.* 2004). Declines in dissolved oxygen levels have the potential to impede aerobically-mediated biochemical processes, ultimately affecting the community composition (of all aerobically respiring organisms, Dauer *et al.* 1992). Water has a limited capacity to hold dissolved oxygen and so the importance of sediment-associated oxygen-consuming processes increases as the volume of water overlying the sediment is reduced. Processes that occur near the substrate surface are major contributors to dissolved oxygen (DO) depletion in shallow water bodies (Sweerts *et al.* 1991). Respiration by benthic invertebrates, microbially-mediated decomposition of organic matter, and aerobic bacteria are all biological agents of oxygen consumption (Spieles & Mitsch 2003, Hargrave 1972a). Oxygen is also consumed during the oxidation of iron and sulphides as well as other ions (Wang 1980). As a result, the rates at which these processes occur have the potential to limit oxygen availability to biota and therefore alter wetland food webs and ecosystem trajectories.

Oxygen consumed via biological processes is known as the biological sediment oxygen demand (BSOD). This includes the consumption of dissolved oxygen during aerobic respiration - the metabolic catalysis of organic molecules to produce energy while utilizing oxygen. Aerobic decomposition of organic matter by microbes situated on the sediment surface is a respiratory process and contributes to the biological portion of the sediment oxygen demand.

The chemical sediment oxygen demand (CSOD) results from inorganic chemical reactions - reduction-oxidation reactions. These reactions are involved with the transfer of electrons between molecules. During oxidation, an ion loses its electrons and chemically binds to one or more oxygen atoms by sharing their electrons. This binding of formerly free oxygen molecules to create '–ates' (e.g., sulphates, nitrates), produces the demand for molecular oxygen. In the 2-step reaction,

1) $4FeS_2 + 14O_2 + 4H_2O \rightarrow 4Fe^{2+} + 8SO_4^{2-} + 8H^+$

2) $4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O$

the iron molecule donates electrons and becomes oxidized while the oxygen molecule accepts those electrons and is reduced. The reduced oxygen ion can then bind with the positively charged protons to create water molecules. Consequently, the once free oxygen molecule is now unavailable for respiration by aerobically respiring biota and thus increases the oxygen demand.

Simple oxidation reactions also consume oxygen. For example, the oxidation of sulphide yields sulphate, resulting in the loss of two oxygen molecules from the water column.

$$S^{2-} + 2O_2 \rightarrow SO_4^{2-}$$

The sum of the biological and chemical oxygen-consuming components yields overall sediment oxygen demand (BSOD + CSOD = SOD) (Wang & Reed 1984).

There is uncertainty as to whether biological or abiotic chemical factors play a larger role in the overall sediment oxygen demand. Some studies of freshwater sediments have concluded that the biological sediment oxygen demand consumes far more of the dissolved oxygen than its chemical counterpart (Brewer *et al.* 1977, Hargrave 1972b, Liu 1973). Other studies report that the chemical oxygen demand is a larger factor (Neame 1975, Gardner & Lee 1965, Wang 1980). These differences may reflect the amount of organic matter in a system; sediments with higher organic content are able to support more biological activity (Ling *et al.* 2009) and so biological oxygen uptake would be higher in systems with more organic matter. Similarly, young wetlands that have not had time to develop a substantial organic layer may have relatively little BSOD and consequently, proportionally higher CSOD. As such, knowledge of the relative contributions of biological vs. chemical dynamics to the overall sediment oxygen demand is crucial to understanding the role of these processes on overall sustainability in reclaimed wetlands and can serve as a proxy for oxygen dynamics in reclaimed oil sands lakes.

Sediment oxygen demand is also largely affected by the composition of wetland sediments (Barcelona 1983). If sediments contain many reduced substances then the sediments will potentially be dominated by chemical consumption. The amount of organic carbon and temperature may also play a role in the balance between chemical and biological sediment oxygen demand (Seike *et al.* 1994). Both biological and chemical

reaction rates rise with increasing temperatures. The Q_{10} - change in rate for every 10°C increase in temperature for microbial respiration is roughly 1.8 (Winkler *et al.* 1996, Pringault *et al.* 2007). For every 10°C increase in temperature, the respiration rate nearly doubles and oxygen is consumed twice as fast. Ammonium oxidation follows this trend between 10-30°C (Waheed 2010).

The oxygen consumed from the sediments is usually supplied by the overlying water. Dissolved oxygen in the water column is the result of photosynthetic organisms and aeration from the atmosphere. Photosynthesis by submergent macrophytes, phytoplankton, and epibenthic algae replenish oxygen in a diel cycle (Guasch et al. 1998). Dissolved oxygen concentrations rise during the day as submerged macrophytes and epibenthic algae photosynthesize carbon using light energy and produce oxygen. At night, photosynthesis ceases and dissolved oxygen concentrations steadily decline as respiration and chemical oxidation continue. Reaeration, however, is a constant process and the most dominant factor in replenishing dissolved oxygen (Jain *et al.* 2005). This depends on the surface area of the water body and turbulence, especially that generated by wave action as a consequence of wind (Jirka *et al.* 2010). To separate sediment oxygen consumption from water-column sediment consumption, 'control' chambers were used in this experiment to measure oxygen consumption in the water column only. These depletion rates were subtracted from our chambers that measured sediment and water column oxygen demand, giving us a measure of oxygen consumed solely within the sediments.

When the balance between oxygen production and consumption is altered, drastic changes in ecosystem function may occur (Dauer *et al.* 1992). Periods of hypoxia as short as hours may affect organism survival and community structure.(Kladec & Knight 1995). This is especially true in warmer waters, which have reduced capacity to hold oxygen and at the same time, the oxygen demand by all biota increases (Kladec & Knight 1995). Decreased levels of dissolved oxygen limit the survival of aerobically respiring organisms, which may affect interactions within a food web. Although thresholds vary among species (fish typically show impacts at 3-4 mg/L, whereas some krill species may not be affected until levels decline below 0.1 mg/L (Ekau *et al.* 2009)), dissolved oxygen concentrations below 2 mg/L are generally considered poor (Diaz 2001). However, in

species that require high concentrations of dissolved oxygen, e.g. *Hexagenia* mayflies, any decrease in dissolved oxygen will reduce growth and survival rates (Winter *et al.* 1996). In lakes, hypoxic conditions mostly occur near the bottom sediments where oxygenated surface waters cannot diffuse into deeper waters to replenish dissolved oxygen levels. The demand for oxygen is often greater than the supply rate in these areas, which results in hypoxia (Edwards *et al.* 2005). Within our study wetlands, the waters are not deep enough to be stratified and during ice-free periods, wetlands may be reaerated; however, high levels of organic matter or reduced compounds may consume oxygen more quickly than it can be replenished leading to hypoxic conditions (Batzer & Sharitz 2006).

In the oil sands region of north-eastern Alberta (Fig. 2.1), reclaimed wetlands will comprise 20-40% of the reclaimed landscape (Oil sands wetland working group OSWWG 2000). Oil sands process materials (water and solids) are used to create wetlands in the reclaimed landscape (FTFC 1995). This approach not only provides construction materials, but also serves as a means for the disposal of tailings materials. However, oxygen consumption of OSPM-derived sediments in constructed wetlands has not been well studied (but see Goudey *et al.* 2010). The OSPM contains residual hydrocarbons (naphthenic acids, polycyclic aromatic hydrocarbons), which are potentially metabolized by microbes (Del Rio *et al.* 2006, Videla 2007), and elevated concentrations of ammonium and sulphur, which are subject to chemical oxidation. All of these constituents occur naturally in the surrounding area, albeit typically in much lower concentrations (Matthews *et al.* 2002). Elevated salinity is observed in OSPM constructed wetlands due to the use of sodium hydroxide in the oil extraction process, the addition of gypsum ($CaSO_4$) to the tailings to speed flocculation (producing consolidated tailings (Matthews *et al.* 2002)) and the naturally sodic characteristics of the clay overburden that overlies the bitumen deposits for landscape construction reclamation (FTFC 1995, Leung *et al.* 2001).

Oxidation of ammonium and sulphur can increase the chemical sediment oxygen demand and ultimately the overall sediment oxygen demand within a wetland. Adams *et al.* (1982) found that nearly 30% of oxygen consumption at the sediments of Lake Erie

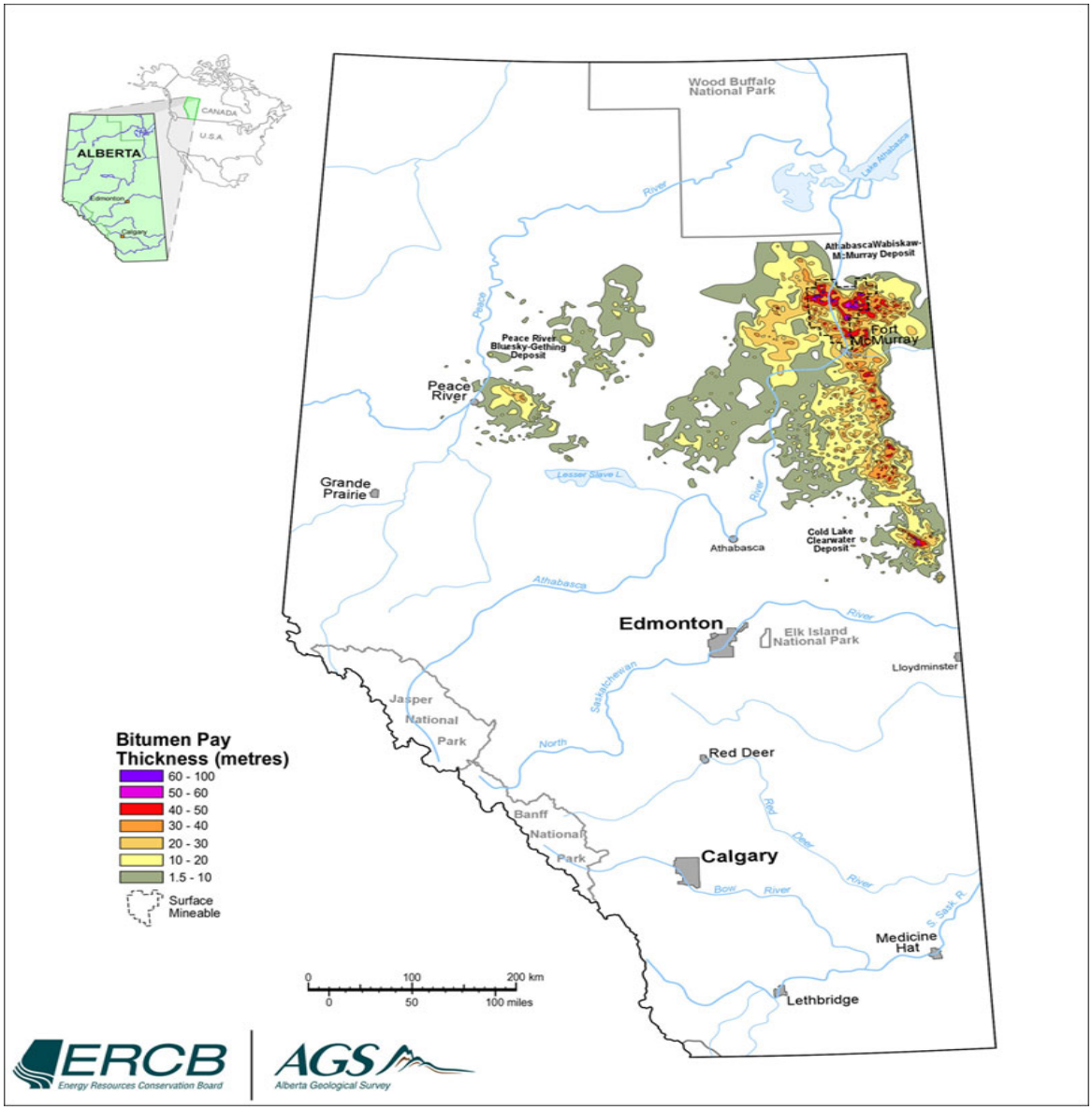

Figure 2.1. Location of Alberta oil sands deposits which cover over 140,000 km^2. Map taken from: http://www.ags.gov.ab.ca/activities/CBM/alberta_oil_sands.html

could be attributed to ammonium oxidation. Consequently, one would expect to observe higher overall SOD rates in OSPM-affected wetlands than reference wetlands. Given the preponderance of these materials in oil sands tailings relative to natural sediments (Matthews *et al.* 2002) we expected to observe a higher CSOD in OSPM-affected wetlands, than in either naturally formed reference wetlands or those constructed with native sediments and fresh water. Based on concurrent studies on relative microbial abundances in the sediments in the study wetlands (Gardner Costa 2010) we expect to find no differences in BSOD between OSPM-affected and reference wetlands. However, the BSOD may be higher in older wetlands, which have had more time to accrue an organic, biologically active surficial sediment layer over the initial inorganic substrate. Few studies have resolved SOD into its biological and chemical components *in situ;* this study will help to determine the importance of chemical and biological activity among wetlands in northeastern Alberta. By using gas flux measurements in conjunction with SOD chambers our goal was to determine the BSOD directly from the amount of carbon dioxide released and then use those values to deduce the CSOD from the SOD in natural wetlands and three classes of constructed wetlands – those built using OSPM (either water or sediment) and reference wetlands constructed or formed without either mine tailings or oil sands process water.

Materials and Methods

Study sites

The study wetlands were located within or near Syncrude Canada Ltd. and Suncor Energy lease areas in north-eastern Alberta, Canada (57.029, -111.585, Figure 2.1). We studied three classes of wetlands; OSPM-affected, reference, and natural.

OSPM-affected wetlands are wetlands constructed in the mined landscape using mine tailings materials as demonstration projects by Suncor Energy, Inc. and Syncrude Canada, Ltd. In addition, OSPM wetlands were either categorized as containing oil sands process sediment (OSPS, n=4) or oil sands process water (OSPW, n=5). If the wetland contained both, it was categorized as OSPS. The waters of these wetlands exhibit elevated salinities and naphthenic acid concentrations and may contain trace quantities of

bitumen. Some wetlands have been amended with varying depths of organic matter, often from peat stockpiles gathered from pre-mining peatlands (descriptions in Table 2.1).

Reference wetlands either formed in depressions in the post-mining landscape or were constructed using natural sediments and local surface water to contrast with the OSPM-affected wetlands. Although they do not contain mine-derived sediments or water, wetlands that have formed in areas with sodic substrate initially had salinities that approach those found in OSPM-affected wetlands. Wetland age ranges from 8-25 y (we are unsure of the age of the natural wetlands. Beaver South wetland is 'young' (less than 5 years old), and Southwest Sands Storage Beaver is at least 30 years 'old'). Boreal wetlands accumulate organic matter slowly over the course of hundreds or thousands of years to eventually form a thick, biologically-derived layer, creating a substantial carbon pool (Bridgham *et al.* 2006). Compared to natural wetlands, which usually take decades or more to form (Bridgham *et al.* 2006), all of our 'non-natural' study wetlands should be considered relatively young (usually no older than 30 years). We used an empirical approach to designate wetland age classes for our study design; Leonhardt (2003) reported that the number of invertebrate families reached an asymptote in wetlands about 7 y after formation. The number of invertebrate families was not statistically different between an 8 or 25 year old wetland and so we set 'older' as 8+ and young as $\leq$ 7 y.

Natural wetlands are areas unaffected by OSPM and are located in areas that had not been mined or deforested. They are typically formed by beaver damming of local creeks. Natural wetlands were studied to test whether reference wetlands, whose age and successional status are similar to those of OSPM-affected wetlands, differed in flux and production from their undisturbed, unconstructed, older counterparts.

A total of 17 wetlands were studied in the summers of 2009 and 2010 (9 OSPM (5 OSPW and 4 OSPS), 5 reference, and 3 natural, Table 2.1). Three wetlands were sampled in both years. The chosen wetlands fit within the CFRAW matrix (Chapter I), allowing one to test for differences among wetlands differing in age, initial sediment organic content and wetland type (OSPM or reference or natural). Each wetland has been mapped (area, depths) and is the subject of complementary ongoing studies of food web processes and carbon dynamics at various trophic levels (Ciborowski *et al.* 2008). Those data will be used to relate carbon flow to this work.

Table 2.1. Wetland descriptions as of 2009. NR: no record available.

Wetland Class	Wetland Name	Original organic base	Age in 2009	Water Source	Sediment type	Lease Area	Additional comments
Natural	South Beaver	Poor	4	Fresh	Native undisturbed	Syncrude	Thin (< 5 cm) organic top layer, clay underneath
Natural	South West Sands	Rich	30	Fresh	Native undisturbed	Syncrude	
Natural	Muskeg	Rich	30	Fresh	Native undisturbed	North of Syncrude	Large amounts of gas seen bubbling up
Reference	High Sulphate	Rich	25	Fresh	Post mining clay/sand mix	Suncor	Lined with sodic overburden, 15 cm of muskeg rests on top
Reference	Golden Pond	Rich	8	Fresh	Post mining clay/sand mix	Syncrude	Lined with Clay/loam (80 cm) with natural, wetland derived organic material on top
Reference	Shallow Wetland	Poor	17	Fresh	Post mining clay/sand mix	Syncrude	1 m clay cap with sodic overburden; no organic material initially added
Reference	Pond 5	Poor	<7	Fresh	Sandy	Suncor	Many iron oxides deposits, bubbling gas
Reference	Waste Area 11	Poor	12	Fresh	Post mining clay/sand mix	Suncor	
OSPW	Test Pond 9	Poor	16	Process water	Clay lining	Syncrude	Clay lined – pockets of bitumen present

							within the clay
OSPW	Mike's Pond	Poor	17	Process water	Clay lining	Syncrude	Clay lined, deeper area (~3 m) has a halocline
OSPW	SWSS Berm	Rich	30	Process water	Sand covered with 30 cm of peat-mineral mix	Syncrude	Water very murky, extreme thermocline
OSPW	Seepage Control Pond	Poor	30	Process and fresh	Post mining sand	Syncrude	Initially fed with process water and later fresh water
OSPW	Natural Wetland	Rich	23	Process water	Post mining sand covered with 15 cm of peat-mineral mix	Suncor	Sandy bottom with a > 15 cm of Muskeg on top
OSPS	4 m CT	Rich & Poor	12	Process water	4 m thicknes of consolidated tailings	Suncor	This wetland contains 2 shallow areas (<20 cm water) with peat. The rest of the wetland has little to no organic layer on top of tailings substrate.
OSPS	Jan's Pond	Poor	10	Process water	Overburden under thin layer of consolidated tailings	Suncor	
OSPS	Demo Pond	Poor	16	Process water	Mature fine tailings	Syncrude	Larger pond stocked with yellow perch for ongoing studies
OSPS	Test Pond 5	Poor	16	Process water	Mature fine tailings	Syncrude	Large pockets of bitumen in sediment

Rationale

Studies of gas flux and SOD were initially two independent projects, measuring biologically released gases from wetland sediments (Gardner Costa 2010) and oxygen consumption by processes (biological or chemical) at the water-sediment interface of wetlands, respectively. We then realized the potential for overlap with our projects.

Oxygen is consumed in biological processes to produce carbon dioxide:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + 36ATP$$

(respiration equation - Madigan *et al.* 2002)

If we could estimate both the amount of oxygen (mg/L) consumed (SOD) and carbon dioxide released (µg/L), we could estimate the amount of oxygen consumed biologically (BSOD), and infer that the remaining oxygen was consumed via chemical processes (CSOD). Essentially, we would have a direct and an indirect measure of sediment oxygen use within our study wetlands. To our knowledge, most SOD studies either only measure SOD *in situ* or when they resolve SOD into BSOD and CSOD, they measure CSOD in the lab by inhibiting biological consumption (Barcelona 1983, Wang 1980, Brewer *et al.*1977, Liu 1973). We wanted to directly measure the biological component of SOD, as it would not only provide information for this project, but also help with eventual carbon mass balance estimates for these systems (Ciborowski *et al.* 2008; Kovalenko *et al.* 2010).

Within each wetland, we measured SOD and carbon dioxide flux in separate, enclosed chambers placed adjacent to each other to ensure that paired measures of SOD and CO_2 were taken under similar conditions. Working in unvegetated sediments, we deployed SOD/CO_2 chamber pairs in a stratified random fashion throughout a wetland. We determined sediment oxygen consumption rate (mg/L) and CO_2 flux (µg/L or µg/m^2/d for daily flux values) for each chamber pair, and then converted CO_2 values to oxygen equivalents (1:1 ratio) that permitted us to estimate biological and chemical SOD. We related these values to wetland water chemistry to determine the effect of OSPM, if any, on SOD and its components. Values for SOD and BSOD chambers were averaged separately for each wetland and then combined to determine CSOD.

Chambers were placed at depths of 20-60 cm in each wetland (Appendix table 2.1). Microcosms were 24 cm tall and needed to be submerged, limiting sampling in

shallow areas. We could also not sample gas or SOD in areas deeper than 1 m because of safety concerns and a lack of equipment needed to place CO_2 flux chambers ("rockets") at deeper depths (a boat and/or SCUBA gear). Although methods have been developed to measure SOD in deeper wetland waters (Goudey 1990), such systems are bulky and were not designed to provide CO_2 flux estimates to resolve biologically consumed oxygen.

Except for the occasional beaver run, our study wetlands were typically 1 m deep or less, permitting one to walk across and sample the entire wetland. Test Pond 9, Mike's Pond and Peat Pond had inaccessible deep areas that could not be sampled. However, gas flux in shallower areas is likely greater than in deeper waters because shallower waters are usually better oxygenated and are more likely to undergo continuous aerobic respiration, potentially producing more carbon dioxide (Batzer & Sharitz 2006). Also, methane is more likely to be metabolized by methanotrophic bacteria in deeper water columns (Blumenberg *et al.* 2007).

We measured SOD in unvegetated sediments because the SOD chambers used were too small to adequately contain submergent vegetation. During early pilot trials, we could not create a tight seal between the chamber and the sediment in vegetated areas. Furthermore, many of the study wetlands (especially OSPM-affected wetlands) were unvegetated or contained only sparse emergent or submergent macrophytes (Shallow Wetland was a notable exception). Consequently we elected to sample similar areas found within a wetland between wetland classes to reduce variability. In addition, assessment of the added effect of gas transfer from the roots of plants to the atmosphere (Kladec & Knight 1995, Chanton 2005, F. Mollard, University of Alberta, in prep.) was beyond the scope of the study design.

Sediment oxygen demand chambers:

The SOD chambers were constructed from Nalgene® plastic desiccators (cat. No.5309). The clear polycarbonate cover (volume 3.5 L) was modified to serve as a light chamber. The blue polypropylene base (volume 3.1 L) was painted black and covered in duct tape to function as a dark chamber. The units were 20 cm high and had an internal diameter of 26.5 cm. Both chambers covered a sediment surface area of 0.0551 m^2. The

top of each dome had a machined hole and was fitted with an attachment to receive a Hydrolab Minisonde 5® temperature, pH, and dissolved oxygen logger.

Each Minisonde 5® unit was equipped with a Hach LDO™ sensor. This is a luminescent dissolved oxygen sensor that does not consume any oxygen during the recording period. The probe's sensor cap is coated with a luminescent material, which becomes excited when it is struck by the blue light transmitted from a LED. As the luminescent material relaxes, it emits red light. The amount of oxygen in the water is related to the amount of time it takes for the red light to be emitted. The more oxygen present, the less time that elapses between the two light events. The probe measures this time and then correlates it to the concentration of dissolved oxygen (user's instruction manual). The loggers were also programmed to record the temperature and the pH of the water.

The top half of a 20-L polyethylene bucket (30.25 cm top ID, 26 cm bottom ID) was used as a collar into which the domes could be nested to rest on the substrate and form a tight seal with the sediment (Fig. 2.2). Additionally, the collar supported a tripod that secured the body of the dissolved oxygen logger in place above the chamber.

A collar was inserted 10 cm into the sediment. A sheet of fiberglass window screening was placed across a dome's opening, and the dome was gently inserted into the bucket collar, taking care not to disturb the sediment surface. The window screening permitted circulation between the substrate and water inside the dome but limited organic matter from rising during dome placement and subsequently blocking the probe's sensor. A dissolved oxygen logger was then inserted into the top of each dome and secured to the ring at the center of the tripod resting on the bucket collar (Fig. 2.2).

Domes isolated from the sediment served as controls by which to separate oxygen consumption in the water column from SOD. The standard SOD chamber protocol was used, except that the control domes were placed on a piece of Plexiglas that rested on the sediment surface. Therefore, these chambers were subjected to respiration from only the water column, whereas the experimental chambers lost oxygen due to respiration from both the water column and the sediment. The water column-DO depletion rates were then subtracted from the water column and sediment-DO depletion rates in order to determine the true sediment oxygen demand for each wetland.

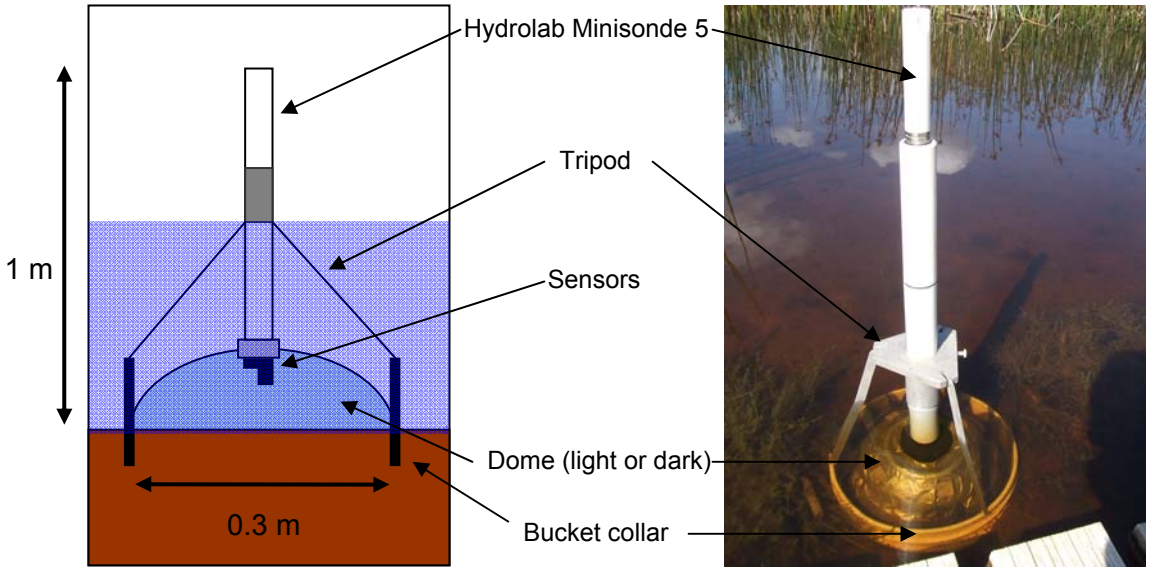

Figure 2.2. Sediment oxygen demand chamber setup; schematic (left) and actual (right).

Chamber deployment

Sediment oxygen demand chambers were deployed on day 3 of gas sampling (see methods below). Chambers were situated within 20 cm of the gas sampling microcosm pairs (placed in a stratified random fashion of unvegetated areas of each wetland). During sampling, we placed 1 light and 1 dark control dome and 2 light and 2 dark SOD domes on the sediment. Dark chambers were used to determine the total gross oxygen consumption due to the sediments while light chambers were used to determine the daytime net rate of depletion - offset of oxygen consumption by photosynthesis. Two 3-h trials were conducted per day (typically 0900 – 1200 and 1300 – 1600 MDT) for 10 days each trial. Three hours was determined to be a sufficient length of time through pilot study work. The time needed to see a change in DO concentration is related to the volume to surface area ratio. Collars and domes were removed and placed in a new location between the two sampling periods.

SOD calculations

The probes recorded DO concentrations (mg/L) every 10 min for the entire 3-h period. The data were retrieved, and line plots of dissolved oxygen concentration (mg/L) vs. time (Fig. 2.3) were examined. For a trial to be deemed valid, initial DO concentration had to be above 3.0 mg/L. Any trials in which the data points quickly dropped below 1.0 mg/L were discarded as these results were deemed to be anomalies caused by the probe sinking into the sediment. The slope of the linear portion of the graphs, expressed as $mgO_2/L/min$, was determined by least squares regression and used for calculations (Utley *et al.* 2008). From these slopes, SOD was calculated using the formula,

$$SOD = 1.44\ (V/SA)(slope_{sediment+water} - slope_{water})$$

where $slope_{water}$ is the slope of the oxygen depletion curve determined from the chambers resting on the Plexiglas (mg/L/min); $slope_{sediment+water}$ is the slope of the curve for a chamber resting on the sediment (mg/L/min); SA is the surface area covered by the domes (m^2); V is the volume of water within the dome (L); and 1.44 is a conversion factor to obtain SOD in $g/m^2/d$ rather than $mg/m^2/min$ (1 d = 24 h).

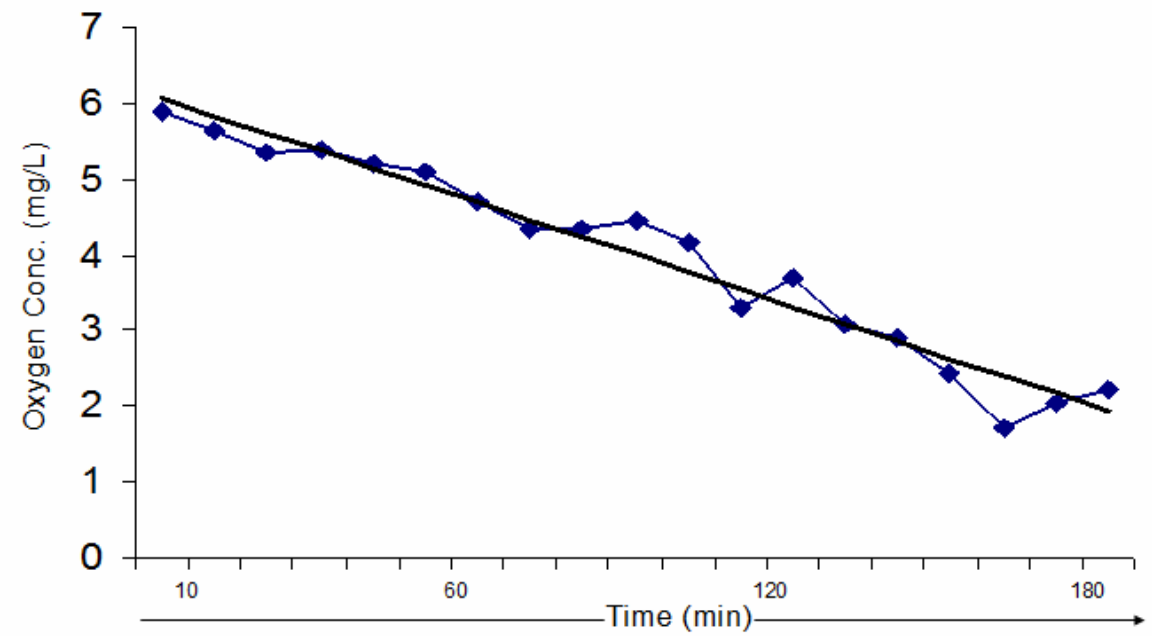

Figure 2.3. Dissolved oxygen depletion curve. Data points were collected every 10 min over a 3-h period. The regression coefficient of the linear regression analysis was used to calculate sediment oxygen demand. The coefficient of determination was always above 0.7.

Sediment oxygen demand was then corrected for temperature and adjusted to 20°C using,

$$SOD_{calc} = SOD_{20}\Theta^{T-20}$$

where SOD_{calc} is the sediment oxygen demand calculated ($g/m^2/d$); SOD_{20} is the sediment oxygen demand at 20°C ($g/m^2/d$); T is the temperature at time of sampling (°C), and Θ is a constant obtained from literature. An appropriate value for this study was deemed to be 1.065 (Truax *et al.* 1995). All oxygen consumption was reported in $g/m^2/d$.

Estimation of biological oxygen demand: Gas flux measurements:

Microcosms were built using black 5 cm ID x 30 cm long PVC piping (Fig. 2.4) to which was attached a 10 cm ID coupling to form the base. The base of the microcosm was inserted 5-8 cm into the sediment, enclosing a surface area of 400 cm^2. The microcosm caps were built using inverted 5-cm polyethylene funnels glued to a machine cut 2-inch ID PVC pipe cap. The microcosm caps (funnel tips) were fitted with 70 cm (this length was used to distance sampling from the microcosm to prevent disturbance) of 0.75 cm Tygon tubing, which was capped with a red rubber stopper or a 3 way stopcock to prevent gas from escaping the microcosm.

Microcosms (rockets) were deployed during spring (June 1-9) 2009 and (June 21-30) 2010. One wetland (Table 2.1 for wetland descriptions) was sampled each day (see the schedule in Table 2.2). Ten microcosms were placed in each wetland in a stratified random fashion. A wetland was divided into 6 radial sections and rockets were placed in each unvegetated (no emergent vegetation with either little or no submerged vegetation) section of a wetland, with the depth of water (cm) recorded. The GPS coordinates of each sampling site were also recorded. Rockets were sampled 72 h after capping.

Each microcosm was carefully capped so as not to jostle the microcosm or disturb the surrounding sediment. The cap tips were lubricated with petroleum jelly to ease with capping as well as to seal the base and cap. Each rocket had zero headspace; upon capping, the caps of the rockets were filled with water and then submerged into the wetland before placing the cap on the shaft of the rocket.

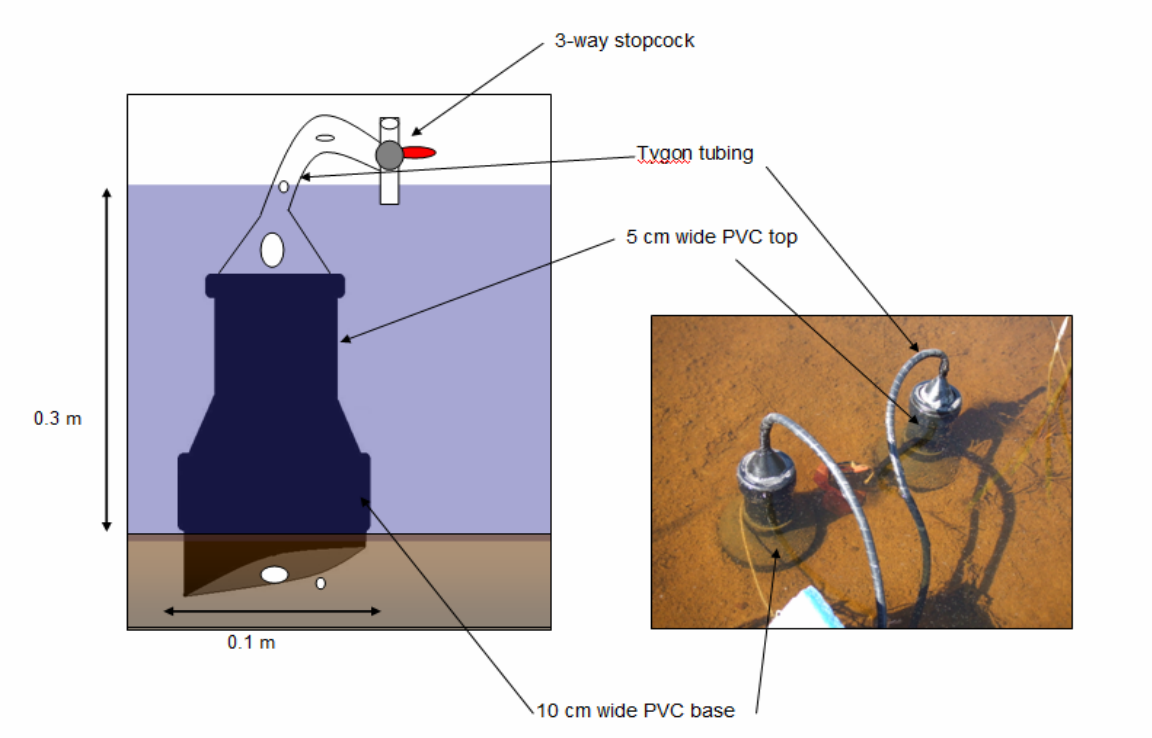

Figure 2.4. Gas flux microcosms; schematic (left) and actual (right).

Table 2.2. Sampling schedule of summer 2009 field sampling for Sediment Oxygen Demand (SOD) and gas flux measurements in our study wetlands.

Sample Component	**Day 1**	**Day2**	**Day 5**
Microcosm placement	- Place rocket base in wetlands, allow sediments to settle - Set out markers for uncapped rockets	- Cap microcosm	- Sample gas @ t= 72h Run sample through Gas Chromatograph
Water chemistry measurements	- Dissolved oxygen, Conductivity, salinity, temperature, pH,		- Dissolved oxygen, Conductivity, salinity, temperature, pH
Covariate measurements	Measure depth of water @ each microcosm site, map out placement of rockets in each wetland	Vegetation sampling → 1 m quadrat, measuring % groundcover of common plant species @ each microcosm site	Remove used rocket to be cleaned for next trial
ATP/ production analysis	18 cm core taken at microcosm site, stored @ 4 ^{o}C		
SOD chamber deployment			Deploy SOD chambers: Placing light and dark chambers into the sediments for 3 hours and then switching each dark for a light and each light for a dark chamber for another 3 hours

Gas collection

Gas sampling was typically done in the morning to mid afternoon (0900- 1500 MDT), although the majority of sampling occurred in the morning hours. Depending on the time the rocket was capped, sampling was not done until 72 h (± ½ h) had passed, assuring consistent sampling times. After 72 h the other microcosm was sampled and removed from the wetland, ensuring that microcosms were sampled independently of each other. Gas samples were taken using a 60-mL airtight syringe, where the needle was inserted into the stopcock or rubber stopper just below the water surface. Using negative pressure, both gas and water were drawn from inside the microcosm. The contents of the syringe were pushed back into the rocket 3 times to purge the line and dislodge any gas bubbles that may have been stuck to the inside of the rocket. The gas collected was kept in the airtight syringe until gas chromatography analysis was performed. Total gas volumes were recorded in the field. Gas samples were analyzed no more than 5 h after collection. Atmospheric gas samples and wetland water samples (for water chemistry) were also taken on site.

Water chemistry

Water quality measurements (dissolved oxygen concentration, conductivity, salinity, and temperature) were taken using a YSI 85 meter at each wetland during gas collection and SOD sampling (see Table 2.2). Water was also collected once from each wetland in July and shipped to an analytical laboratory for full water chemistry analysis (Syncrude 1995). Major ionic and trace metal contents were determined at the Syncrude's Edmonton Research facility using Syncrude Canada's standard analytical methods (Syncrude, 1995). The pH and conductivity of each sample were determined on whole samples. Prior to other analyses, water samples were filtered using 0.45µm Millex® disposable filters. Cations and minor elements were determined by ICP-OES (Inductively coupled plasma optical emission spectrometry using a Varian Vista-PRO RL ICP-OES). Anions (Cl^- and SO_4^{2-}) and ammonium (NH_4^+) concentrations were determined by ion chromatography (Dionex Corporation, Sunnyvale, CA, USA Model DI-300 IC). Alkalinity (HCO_3^- and CO_3^{2-}) was measured by auto-titration using a Metrohm Titriono Model 751 titrator. Total naphthenic acid concentrations were obtained using the FTIR

method, described by Jivraj *et al.*, 1996, in which the carboxylic acids were extracted from H_2SO_4 acidified (pH 2-2.5) water samples with methylene chloride, and absorption at wave numbers 1703 and 1745cm^{-1} were measured with a Thermo Instruments (Canada) Inc. Nicolet Model 8700 FT-IR spectrophotometer.

Redox measurements

Redox potentials were measured using a Thermo Orion 5-Star Portable pH/ORP/DO/Conductivity Meter with a flat surface eH/ORP (RmV/mV) probe. Cores were taken along with a 5-cm water cap to minimize disturbances and to allow the probe to reach the sediment surface. The probe was placed flat on the sediment surface until readings stabilized.

Gas chromatography

Gas Analyses:

Gas samples were analyzed using an Agilent 3000A micro gas chromatograph (GC) equipped with a Plot Q and Molsieve sample columns, a variable sample injector and a thermal conductivity detector (TCD). The GC was calibrated using a Supelco calibration gas composed of 4.008% CH_4, 5.004% CO, 5.006%CO_2, 4.001% H_2, 4.986% O_2, 5.024% N_2 (percent volume) and a balance of helium gas (Sulpeco Ltd; catalog# A-19792). Total gas volumes were recorded in the field and averaged for vegetated and unvegetated zones within each wetland.

We ran all gas samples taken from a wetland on the day of sampling. Thus, we measured one wetland at a time. Samples taken from within a wetland were run in random order. Before measuring any gas samples, 3 atmospheric samples were run through the GC (air taken from the lab in which the GC was set up) as a blank. We also measured atmospheric samples taken from each wetland, but these were treated in the same fashion as the microcosm samples.

Concentrations of carbon dioxide, methane, oxygen and nitrogen were obtained from gas chromatograph (GC) sample elution profiles. The amounts (µg/L) of each gas were calculated from the gas standard used to calibrate the GC. To calculate the molar amount of each gas within the standard, a ratio was determined by dividing the molar

amount (mol/μL) of each gas within the standard by the corresponding areas (from the GC elution profile: area under the curve for each identified gas from an elution profile) with the known area under the curve of a sample (from the GC readout) to determine the sample molar amount. The molar amount of each sample was then divided by the GC sample injection volume (2 μL) to give a concentration of each gas within each sample. Concentrations were then multiplied by the total volume of gas collected in the sample, expressed for the amount of gas released (moles released/ m^2d). These values were then used to determine the total gas flux based on surface area of a wetland taken from this summer's and previous summer's wetland mapping. An example calculation is given below:

e.g. Calculating moles of CH_4 in the gas standard (sample injection volume of 2 μl) CH_4 is 4.008% volume in the standard:

4.008% CH_4 x 2 x 10^{-6} L = 8 x 10^{-8} L CH_4

Using the ideal gas law (PV=nRT),

n(moles) =PV/RT → n= 1 atm x (8 x 10^{-8} L) / (8.233† K*atm*L/mole x 273.15 K) = 3.56x10^{-11} moles of CH_4 in 2 μL of standard.

† = this value was corrected for elevation in Fort McMurray, 0.9901% of standard elevation (1 atm).

To determine moles of a gas in a sample, use the ratio,

Mole CH_4 Std. / Area CH_4 Std. : Mole CH_4 sample / Area CH_4 sample

Rearranged-Mole CH_4 Std. * Area CH_4 sample / Area CH_4 Std. : Mole CH_4 sample

<u>Determining Biological Sediment Oxygen Demand (BSOD)</u>

In order to determine the amount of oxygen consumed by biological processes from unvegetated sediments, the amount of CO_2 collected was used to back calculate oxygen consumption. Using the respiration equation,

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

we used a 1:1 stoicheometric ratio of CO_2: O_2 to convert CO_2 released into oxygen consumed ($g/m^2/day$). Carbon dioxide was measured by collecting gas within microcosms and determining the concentration (mol/L) of CO_2 via gas chromatography and converted to oxygen consumed ($g/m^2/day$).

BSOD ($g/m^2/day$) = moles $CO_{2\ (gas)}$ + moles $CO_{2\ (alkalinity)}$ (convert to O_2 equivalents).

Estimating Chemical Sediment Oxygen Demand (CSOD)

Chemical oxygen demands were estimated by subtracting the BSOD (O_2 $g/m^2/d$) from the overall SOD (O_2 $g/m^2/d$).

SOD-BSOD = CSOD

Statistical analyses

Statistical analyses were performed using STATISTICA version 7.0 (Statsoft, Inc., Tulsa, OK). Statistical significance of differences among the classes of wetlands (OSPS-affected, OSPW-affected, reference constructed, and natural wetlands) with respect to SOD, BSOD and CSOD rates were tested using planned comparison ANOVA of log +1-transformed data. Data were log-transformed to achieve normality. Constructed (reference and OSPM-affected) wetlands were grouped together and compared to natural wetlands. Reference wetlands were then compared to OSPM-affected wetlands. Significance levels were set at $p<0.05$ with wetland as the unit of replication ($n = 5$ OSPS-affected, $n = 4$ OSPW-affected, $n = 5$ reference, $n = 3$ natural). These p values were then adjusted using the Holm modification of the Bonferroni correction to maintain the among-ANOVA test experiment-wise type I error at $p=0.05$.

Results

Wetland water chemistry

Wetland water chemistry values are summarized in Table 2.3. Both conductivity and salinity were higher in OSPM-affected wetlands than in reference and

Table 2.3. Temperature, pH, conductivity dissolved oxygen and salinity were all taken *in situ*, ion and compound concentrations were analyzed from water collected and sent to Syncrude technicians for analysis in 2009. The Syncrude derived values are only a subset of total water chemistry analyses. BDL - below detectable limit, NR - no record available, Cond. - conductivity, DO - dissolved oxygen

Wetland Class	Wetland	YSI Temp. C^{o}	pH	Cond. (µS)	YSI DO (mg/L)	YSI Salinity (ppt)	NO_3 (ppm)	SO_4 (ppm)	Iron (ppm)	NH^{4+} (ppm)	Naphthenic acids (ppm)	water ORP (mV)	Sediment Redox Potential (mV)
Natural	South Beaver	15.9	7.18	313	3.01	0.1	0.47	2.84	0.894	BDL	<1	79	-128.6
Natural	South-west sands Beaver	13.8	7.92	1252	9.82	0.6	BDL	437	BDL	BDL	<1	119	-204.3
Natural	Muskeg	21.2	7.6	424	7.3	0.2	NR	NR	NR	NR	NR	NR	NR
Reference	High Sulfate	15.2	7.35	2980	9.42	1.5	2.2	1650	BDL	0.166	10	124	-350.8
Reference	Golden	19.9	8.52	1737	12.5	0.9	2.3	825	BDL	BDL	2	99	-249.8
Reference	Shallow Wetland	18	7.53	391	7.2	0.2	1.4	6.58	0.036	BDL	<1	113	-151.0
Reference	Pond 5	19.8	7.90	1260	8.95	0.6	NR	NR	NR	NR	NR	66	-151.7
Reference	WA11	19.4	8.13	904	6.33	0.4	NR	NR	NR	NR	NR	153	NR
OSPW	Test Pond 9	23.9	8.87	2050	12.08	1	BDL	97.8	BDL	BDL	19	79	-220.5
OSPW	Mike's Pond	NR	8.45	4550	NR	NR	BDL	565	BDL	0.137	26	106	-188.6
OSPW	SWSS Berm	17.2	9.0	2880	3.55	1.5	NR	NR	NR	NR	NR	NR	NR
OSPW	Seepage	18.6	8.6	2543	11.1	1.3	NR	NR	NR	NR	NR	NR	NR
OSPW	Natural	17.7	8.85	1090	8.27	0.5	27	134	0.499	1.08	33	87	-206.0
OSPS	Test Pond 5	20.9	9.2	2673	9.2	1.4	NR	NR	NR	NR	NR	NR	NR
OSPS	4m CT	15	7.97	2300	6.68	1	1.4	335	0.04	BDL	30	124	-214.1
OSPS	Jan's	20.4	8.2	2815	5.03	1.5	NR	NR	NR	NR	NR	NR	NR
OSPS	Demo	21.4	9.28	1636	9.06	0.8	NR	NR	NR	NR	NR	130	NR

natural wetlands (planned comparison ANOVA, $F=8.106$, $p<0.05$ and $F=6.424$, $p<0.05$ respectively). Reference and natural wetlands showed a wide range of salinities and conductivities (0.1-1.5 ppt, 313-2980 μs/cm, respectively), so mean values calculated across reference and natural wetlands weren't significantly different from the means of OSPM-affected wetlands. The pH in OSPM-affected wetlands was typically higher than reference (7.97-9.28 vs. 7.18-8.52, respectively) although not statistically tested. Naphthenic acid concentrations were 10 times higher on average in OSPM-affected than in other classes. All wetlands were well oxygenated (5.03-12.5 mg/L), with the exception of South Beaver and SWSS Berm (3.01 mg/L and 3.55 mg/L, respectively).

Sediment redox potential values

Sediment redox potentials ranged from -150 to -350 mV. There was no difference among wetland classes. There was however, far more variability among wetlands within the reference class than within the OSPM class (Table 2.3).

Sediment oxygen demands

Values of SOD (O_2 g/m^2/d) can be found in Table 2.4. Only dark chambers were used - they reflect gross SOD estimates. We had planned to calculate net SOD. However, loggers placed in many of the light chambers did not properly record data (logger failure, see Appendix), which left us unable to make our calculations.

Mean (±SE) sediment oxygen demand averages were 0.904 ± 0.003 O_2 g/m^2/d in natural wetlands, 0.847 ± 0.256 g/m^2/d in reference wetlands, 1.337 ± 0.467 g/m^2/d in OSPW-affected wetlands, and 1.18 ± 0.319 g/m^2/d in OSPS-affected wetlands. The regression coefficients of determination for the oxygen depletion curves were always 0.7 mg/L/min or higher. Mean sediment oxygen consumption for dark chamber domes was not significantly different among the classes of wetlands (Table 2.5, Figure 2.5, ANOVA, $F= 0.7196$, $n=15$, $p>0.05$).

Table 2.4. Mean SOD, BSOD and CSOD values ± S.E for our study wetlands for 2009 and 2010. SOD was calculated directly from the sediment oxygen demand chambers. BSOD was estimated from gas flux readings. CSOD was determined by subtraction of BSOD from SOD. BDL - blow detectable limits (<5ml of gas collected), NR - no record available.

Wetland	Wetland Class	SOD ($O_2g/m^2/day$)	BSOD ($O_2g/m^2/day$)	CSOD ($O_2g/m^2/day$)
South Beaver	Natural	0.907	0.049	0.858
SWSS Beaver	Natural	0.901	0.198	0.703
Muskeg	Natural	NR	0.173	NR
	MEAN	**0.904 ± 0.003**	**0.140 ± 0.046**	**0.781 ± 0.077**
Golden Pond	Reference	0.592	BDL	0.592
Shallow Wetland	Reference	1.275	0.134	1.141
High Sulphate	Reference	0.364	BDL	0.364
Pond 5	Reference	0.379	0.1052	0.2738
WA11	Reference	1.626	0.0102	1.6158
	MEAN	**0.847 ± 0.256**	**0.050 ± 0.0288**	**0.797 ± 0.254**
Test Pond 9	OSPW	1.440	0.003	1.437
Mike's Pond	OSPW	0.508	BDL	0.508
Natural Wetland	OSPW	3.022	0.026	2.996
SWSS Berm	OSPW	1.284	0.0024	1.2816
Seepage	OSPW	0.432	0.0508	0.3812
	MEAN	**1.337 ± 0.467**	**0.0164 ± 0.0098**	**1.32 ± 0.467**
4mCT	OSPS	1.687	0.072	1.615
Jan's	OSPS	0.591	0.0194	0.5716
Demo	OSPS	1.263	0.075	1.188
Test Pond 5	OSPS	NR	0.0296	NR
	MEAN	**1.18 ± 0.319**	**0.049 ± 0.014**	**1.12 ± 0.303**

Biological sediment oxygen demands

Carbon dioxide values ranged from 3.0 to 176.5 μg/m^2/d (Test Pond 9- OSPM-affected and Southwest Sands Beaver - natural, respectively). Reference wetlands released more carbon dioxide gas than OSPM-affected wetlands. Differences between classes were statistically significant ($F=4.072$, $n=17$, $p<0.05$) even with the high variability among replicate wetlands. Values of CO_2 converted to O_2 equivalents ranged from 0.0020 to 0.13 g/m^2/d (Test Pond 9 to OSPM-affected and Southwest Sands Beaver - natural, respectively). BSOD in natural wetlands was significantly higher than in constructed wetlands (Table 2.6, Figure 2.6. planned comparison ANOVA, $F=10.48$, $n=17$, $p<0.05$ after Holmes correction). No difference was seen between reference and OSPM-affected wetlands (Table 2.6, Figure 2.6, planned comparison ANOVA, $F=0.56159$, $n=17$, $p>0.05$) or OSPW and OSPS wetlands (Table 2.6, Figure 2.6, planned comparison ANOVA, $F=1.68702$, $n=17$, $p>0.05$). The BSOD values in reference wetlands were more variable than values taken from OSPM wetlands.

Chemical sediment oxygen demands

By subtracting the BSOD from the SOD we were able to estimate oxygen consumption by chemical processes. CSOD ranged from 0.27 g/m^2/d to 2.996 g/m^2/d. We had postulated that CSOD would be higher in OSPM-affected wetlands than reference wetlands. Although this trend was consistently observed, the CSOD was not significantly lower in natural wetlands than in constructed wetlands (Table 2.7, Figure 2.7. planned comparison ANOVA, $F=0.20408$, $n=15$ $p>0.05$) or in reference wetlands compared to OSPM-affected wetlands (Table 2.7, Figure 2.7, planned comparison ANOVA, $F=1.286$, $n=15$, $p>0.05$) or OSPW-affected wetlands compared to OSPS-affected wetlands (Table 2.7, Figure 2.7, planned comparison ANOVA, $F=1.21459$, $n=15$, $p>0.05$). More variability in CSOD was seen in OSPM wetlands than in reference ones.

Table 2.5 Analysis of Variance table for SOD.

Source of Variation	SS	d.f	MS	F	p
Among Treatments	0.63571	3	0.21190	0.40513	0.75232
Natural vs. Constructed	0.08141	1	0.08141	0.14266	0.71283
Reference vs. OSPM	0.50816	1	0.50816	0.89049	0.36561
OSPW vs. OSPS	0.04614	1	0.04614	0.08085	0.78143
Within	6.27715	11	0.57065		
Total	6.91286	14			

Table 2.6 Analysis of Variance table for BSOD.

Source of Variation	SS	d.f	MS	F	p
Among Treatments	0.03710	3	0.01237	3.76905	0.03804
Natural vs. Constructed	0.03381	1	0.02546	9.79130	0.00799
Reference vs. OSPM	0.00094	1	0.00094	0.36229	0.55758
OSPW vs. OSPS	0.00235	1	0.00235	0.90579	0.35859
Within	0.03381	13	0.00260		
Total	0.07091	16			

Table 2.7 Analysis of Variance table for CSOD.

Source of Variation	SS	d.f	MS	F	p
Among Treatments	0.77035	3	0.25678	0.49791	0.69122
Natural vs. Constructed	0.15527	1	0.15527	0.27464	0.61063
Reference vs. OSPM	0.54313	1	0.54313	0.96065	0.34808
OSPW vs. OSPS	0.07195	1	0.07195	0.12726	0.72804
Within	6.21918	11	0.56538		
Total	6.98953	14			

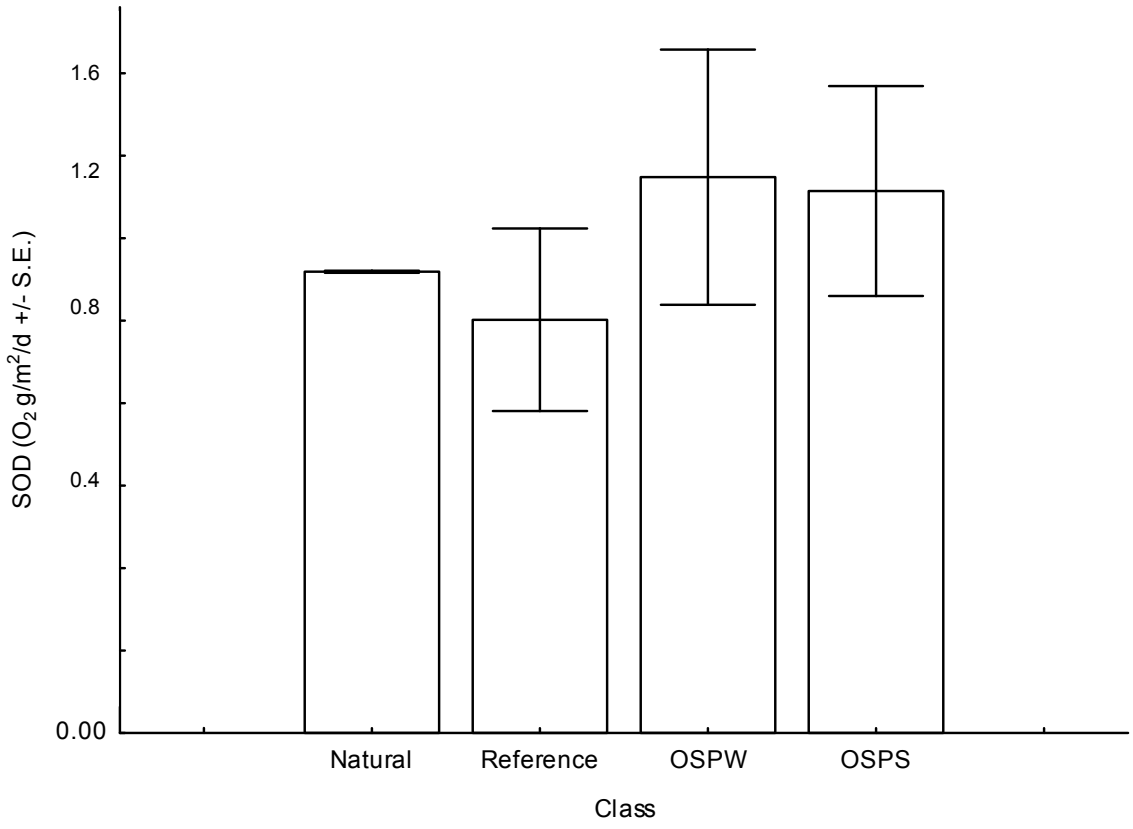

Figure 2.5 Mean (± S.E.) sediment oxygen demand (O_2 g/m^2/d) in natural (n=2), reference (n=5), and OSPW (n=5) and OSPS (n=3) wetlands for dark chambers in unvegetated zones of wetlands. Please note logarithmic scale on Y-axis.

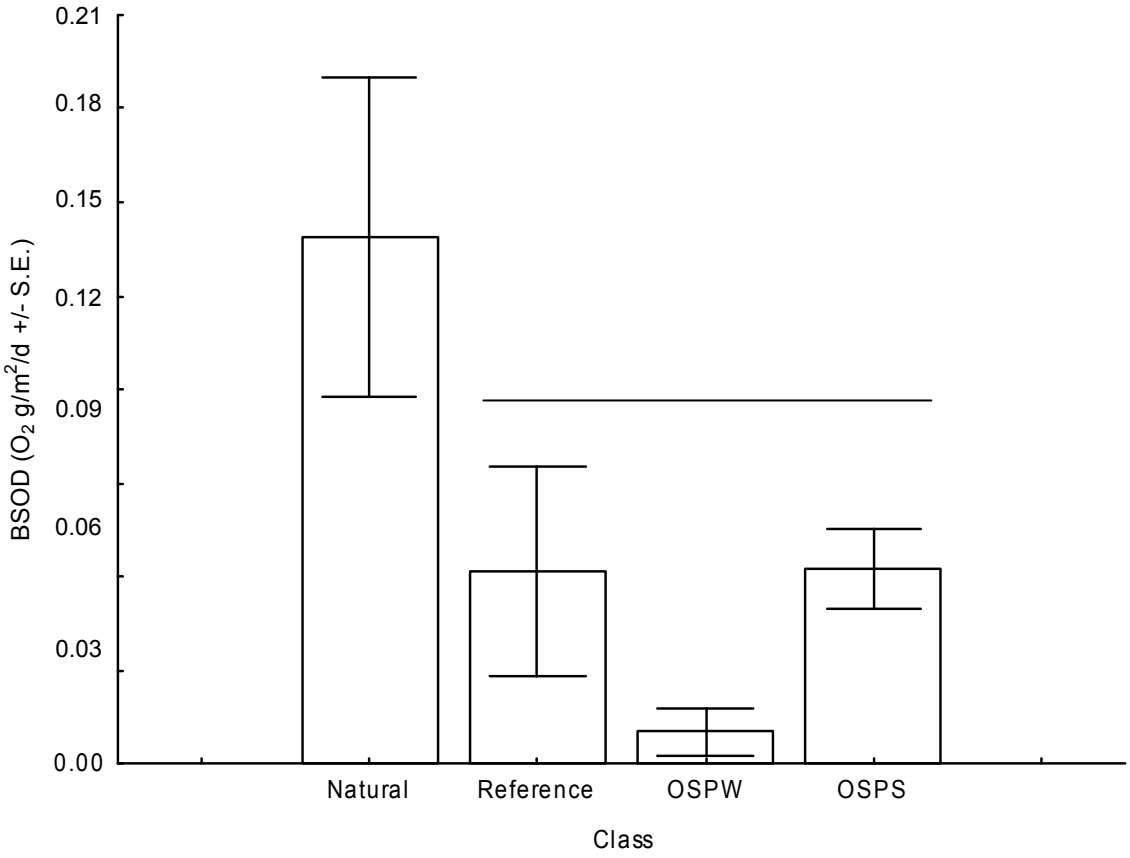

Figure 2.6 Mean (± S.E) biological sediment oxygen demand (O_2 g/m^2/d) in natural (n=3), reference (n=5), and OSPW (n=5) and OSPS (n=4) wetlands for dark chambers in unvegetated zones of wetlands. BSOD in natural wetlands was significantly higher than the other three classes. Horizontal line connects groups that are not significantly different from one another. Please note logarithmic scale on the Y-axis.

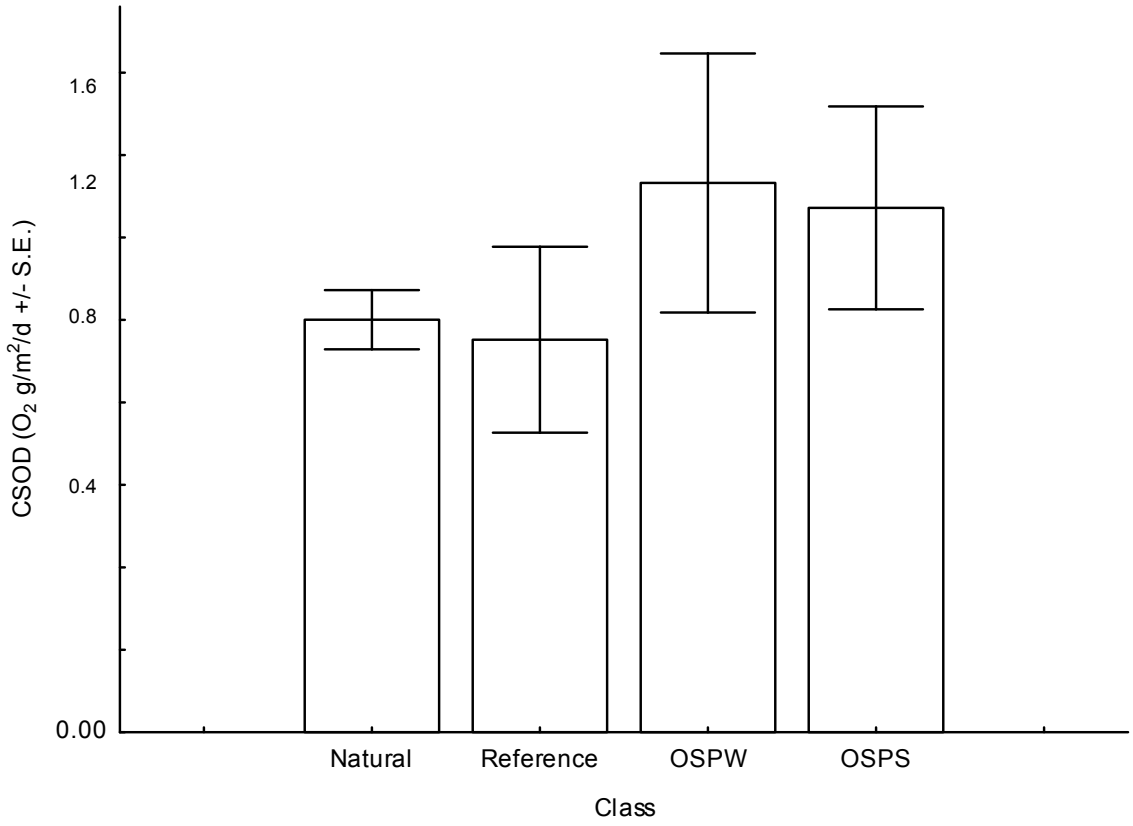

Figure 2.7. Mean ± S.E chemical sediment oxygen demand (O_2 g/m^2/d) in natural (n=2), reference (n=5), and OSPW (n=5) and OSPS (n=3) wetlands for dark chambers in unvegetated zones of wetlands. Please note logarithmic scale on the Y-axis.

Discussion

Sediment oxygen demand

Sediment oxygen demand was not significantly different among classes of wetlands. However, OSPW- and OSPS-affected wetlands showed higher SOD rates than reference wetlands (1.337±0.467 O_2 g/m^2/d compared with 0.847±0.256 O_2 g/m^2/d). It was determined that in order to achieve a power of 80% and detect a difference among class means of 1.0 g/m^2/d, a sample size of 13 per class would be required. To detect a difference of 1.5 g/m^2/d a total of 8 wetlands per class would need to be sampled. Our sediment oxygen demand values fall within range of values reported in the literature. Utley *et al*. (2008) found that SOD varied between 0.1-2.3 O_2 g/m^2/d in Georgian streams, while Matlock *et al.* (2003) showed a range of 0.15-1.36 O_2 g/m^2/d for the Colorado River. Veenstra & Nolan (1991) concluded a range of 0.34 O_2 g/m^2/d to as high as 9.02 O_2 g/m^2/day in southwestern U.S. lakes. Little work has been done in northern wetlands. However, it appears that SOD varies greatly depending on the study system.

The higher trend in SOD rates observed in OSPM-affected wetlands suggest that oil sands process material could possibly have an effect on the rates of oxygen consumption at the sediment surface. The presence or absence of OSPM rather than the type of OSPM (water or sediments) appeared to influence SOD rate. The majority of the demand was apparently due to chemical processes rather than by biological demand. This suggests that chemical constituents play a larger role than sediment composition in CSOD and ultimately SOD. Sediment composition was also not an important factor for differences in the relative biomass of heterotrophic bacteria within these study wetlands (Gardner Costa 2010). Since CSOD values are ~ 10 times higher than BSOD in both reference and the two OSPM-affected wetlands, we conclude that the oxidation of chemical constituents within reference and OSPM-affected wetlands is the primary mode of oxygen consumption within our study wetlands.

Dissolved oxygen concentrations in reference and OSPM-affected wetlands declined by averages of 30% and 44% from their initial concentrations, respectively. Differing percentages of oxygen depletion indicate that the increased SOD is the result of more rapid DO consumption in OSPM-affected wetlands. An increase in SOD can limit

aerobic processes within a wetland food web by depleting DO concentrations at an accelerated rate (Connolly *et al.* 2004).

Biological sediment oxygen demand

Any differences observed in SOD are attributable to differential use of oxygen (biological vs. chemical) in reference and OSPM-affected wetlands. We estimated biological sediment oxygen demand using carbon dioxide released from sediments as a proxy for oxygen consumed. We measured CO_2 released from wetlands as flux and CO_2 dissolved within wetland water. These values were converted to moles of oxygen consumed using the cellular respiration equation (a 1:1 ratio). Oxygen consumed by biological processes was significantly higher in natural wetlands than in constructed wetlands.

The difference in BSOD between OSPW-affected wetlands and reference wetlands is almost 7-fold, with the difference being much higher for natural wetlands. However, it is nearly the same in OSPS-affected wetlands compared to reference wetlands. This strongly indicates that respiring organisms are more affected by the condition of the water rather than the sediment. It seems that the chemical constituents in the sediment have little to no effect on epibenthic biological processes while those in the water have drastic ones. We had expected that reference wetlands cycled carbon more quickly or had more microbial biomass than OSPM-affected wetlands, which would result in more respiration and ultimately more biologically consumed oxygen. Daly (2007) found no significant differences in bacterioplankton production rates within our study wetlands in 2005. If wetlands do not differ in terms of turnover rates, then it is likely that more microbial biomass is seen in natural wetlands. This would suggest that the differences in gas release were the result of higher abundances of organisms respiring, rather than higher turnover rates. Relative microbial biomass was determined for these wetlands in 2009 (Gardner Costa 2010) but was not significantly different among wetland classes. Although microbes may not be the source of increased BSOD, there is evidence of higher abundance of organisms in reference wetlands, observed in several studies conducted within oil sands constructed wetlands. Larger biomass of sediment algal biofilm (measured by chlorophyll *a* analysis (Frederick 2010)), have been found. All of

these organisms respire CO_2 aerobically. Thus, more organisms would result in higher CO_2 release. In addition, OSPM-affected wetlands typically look less productive (pers. obs.) than reference wetlands. Water in OSPM-affected wetlands is typically clear, and there are noticeably fewer plants and invertebrates than in reference wetlands.

Utility of CO_2 as proxy for biological oxygen consumption

In our study, we assumed that any gas trapped within our microcosms was biologically produced and released via aerobic respiration. We did not address precipitation of carbonates in these wetlands, which could potentially lead to underestimates of biologically consumed oxygen. Alternatively, not all of the CO_2 collected was necessarily aerobically respired. Any anaerobically produced CO_2 would also be included with our measurements, overestimating the biologically consumed oxygen in all of the wetland classes. Gardner Costa (2010) examined the differences in the amount of heterotrophic bacteria within sediments of reference and OSPM-affected wetlands using concentrations of adenosine triphosphate (ATP) as a proxy for relative cell abundance. The samples were sealed and kept in the dark for 4 months, meaning he would predominantly find non-photosynthetic, anaerobic bacteria within the samples. He found no significant differences in microbial abundance between wetland classes and thus we would expect to see no differences in the amount of carbon dioxide released from either wetland class. In 2009 we did observe higher (2x) CO_2 flux in reference wetlands and almost 10x more in natural wetlands compared with OSPM-affected wetlands. Combined with evidence of higher abundances of algae, invertebrates and plants found in reference wetlands, we argue that anaerobically produced CO_2 was not a sufficient factor to explain differences in BSOD among wetland classes. This is not to say that anaerobically produced CO_2 is not important. Microcosm studies using aerobic and anaerobic chambers could give estimates for the proportion of aerobically/anaerobically produced carbon dioxide. If the sources of carbon and oxygen were different for aerobic and anaerobic bacteria, stable isotope carbon and oxygen signatures could resolve this issue (Daly 2007 used stable isotopes to identify bacterial carbon sources). Determining the isotope signatures of respiratory gas would be more expensive and complicated than estimating signatures from biomass (Andrea Farwell, University of Waterloo, pers.

comm.). Further studies should resolve wetland respiration into the various sources of gases and relate it to microbial communities found within the wetlands.

There was some concern over whether our results (or lack of differences) may have been an artifact of changes in barometric pressure throughout the course of the study. Changes in pressure would alter the partial pressure of CO_2 in the water and therefore alter gas released or dissolved into a wetland. To minimize this, we stratified the sampling order of wetlands, alternating reference and OSPM-affected. Also we checked our field notes, and the weather did not vary throughout the 9 day study – it was warm and sunny for the duration.

Chemical sediment oxygen demand

Our estimates of BSOD, suggest that oxygen consumption in OSPM-affected wetlands was dominated by the chemical oxidation. Roughly 86% and 90% of the oxygen was consumed chemically in natural and reference wetlands respectively, while close to 98% was chemically consumed in OSPW-affected wetlands and 93% was consumed chemically in OSPS-affected wetlands. Although these values were calculated indirectly from the biological demand, they are consistent with ratios obtained by other researchers. Kladec and Knight (1995) suggest that a 100:5 mg/L ratio of CSOD:BSOD is common in wetlands. Neame (1975) showed that CSOD was responsible for 95% of the oxygen uptake in a Californian lake. However, the dominance of CSOD vs. BSOD is still highly debated in the scientific community, and different systems seem to have large variations in the ratio. In all classes of study wetlands, particularly in OSPM-affected wetlands, chemical sediment oxygen demand was the largest component of the overall sediment oxygen demand. As a result, consumption of oxygen is driven by the oxidation of chemical constituents. These chemicals (Fe(II), NH_4^+, S^{2-}) are oxidized directly at the sediment-water interface where reduced chemicals from the sediments come into contact with oxygen.

The increased trend of CSOD (although not statistically significant) could potentially be attributed to the higher concentrations of these chemicals in OSPM-affected wetlands. Ammonium and sulphate are concentrated in OSPM as result of extraction and upgrading of oil sands (FTFC 1995). Heavy hydrocarbons are ‘cracked’

whereby unsaturated bonds are hydrogenated, releasing sulphur and nitrogen (FTFC 1995). Sulphate concentrations are also increased when gypsum ($CaSO_4$) is used as a settling agent, producing consolidated tailings. Plant root simulator (PRS™) probes (although only showing bioavailable concentrations of compounds in the sediment) indicated the presence of twice as much ammonium in OSPM-affected wetlands (Chapter III).

Sulphide concentrations were variable among the wetlands. Higher concentrations of sulphides were expected (but not seen) in OSPM-affected wetland sediments. Differences in concentrations of iron (Table 3.3) were minimal among wetland classes; this observation was expected as mining extraction does not appear to concentrate metals in tailings (M. Mackinnon pers. comm.). Redox potentials just below the sediment surface were measured in the range of -150 to -350 mV (Table 3.3). This range suggests that any ammonium, sulphides, manganese or iron would be reduced, only to be oxidized at the sediment surface when oxygen is present. Our observations suggest that only ammonium was significantly higher in OSPM-affected than in reference wetlands (Chapter III). Despite variability seen in concentrations of sulphides in OSPM and reference wetlands, the sediments we measured support oxidation of ammonium as a likely source for increased CSOD in OSPM-affected wetlands.

Although we classify ammonium oxidation under the chemical component of the sediment oxygen demand, it is widely understood that ammonia can be oxidized microbially (biologically) via nitrification (Fisher *et al.* 1952). Ammonium, which becomes ammonia at higher pH, is oxidized first into nitrites by *Nitrosomonas* and ultimately into nitrates by *Nitrobacte*r (Belser 1979); oxygen is consumed at the ammonia → nitrite step (Batzer & Sharitz 2006). Since we assumed biologically-consumed oxygen only resulted in CO_2 release, we are underestimating the biological sediment oxygen demand and therefore overestimating the chemical sediment oxygen demand. Most studies involving the fractionation of SOD into BSOD and CSOD are done in the lab where a toxicant (formaldehyde, phenol) is used to inhibit all biological activity while measuring dissolved oxygen, thus determining the chemical component of the sediment oxygen demand (Wang 1980). This design is not feasible *in situ*; as a result, we had to

define biological oxygen demand as solely aerobic respiration and anything else under chemical oxygen demand.

In order to measure nitrifying bacteria's contribution to SOD we would need to determine rates of nitrate production from the sediments. One would also need to separate microbially produced nitrate from nitrates produced by other organisms or external sources. Stable isotope analyses may provide this resolution, as the source of ammonia would likely be from the oil sands cracking process with a more depleted N^{15} signature than nitrogen sources in contact with the atmosphere. With an estimate of microbially produced nitrate one could back calculate to determine how much oxygen should have been consumed using the same methods and calculations found in this chapter to measure SOD and estimate BSOD and CSOD.

Conclusions

Rates of sediment oxygen demand and its components differ among natural, reference and the two classes of OSPM-affected wetlands. Although not statistically significant (possibly due to our small sample size), OSPM-affected wetlands had an increased trend in rates of CSOD and SOD and the lowest rate of BSOD. Our level of replication was insufficient to detect a difference of 1.5 $g/m^2/d$ or less as significant. The only significant difference was seen when BSOD from natural wetlands was compared to constructed wetlands. CSOD was the largest factor in all cases but consumed oxygen at a higher rate in OSPW-affected wetlands. We conclude that this is due to increased concentrations of reduced molecules, particularly ammonium. The oxidation of ammonium may be chemically or biologically driven but it appears to have an important role related to SOD. High rates of CSOD and overall SOD have the potential to alter ecosystem function. With dissolved oxygen being depleted at a higher rate, organisms may become stressed due to hypoxic conditions. This may be seen in the reduced biological activity in OSPM-affected wetlands. Reducing the amount of residual chemical constituents that contribute to CSOD will be beneficial to the reclamation process. This is especially important for lake reclamation if the same tailings materials constitute lake sediments. Chapter III also looks at how ecosystem trajectories may be altered as CSOD potentially affects submergent macrophyte growth and ultimately community

composition. As a result, we suggest that reducing ammonium and therefore the CSOD will increase the probability and/or rate of successful reclamation. All in all, not much difference was seen in SOD rates and its components. As a result, OSPM-affected wetlands seem to resemble natural wetlands (with regards to oxygen demand) to a far larger extent than had been anticipated.

APPENDIX

Light chambers were deployed in the same manner as mentioned in the methods section. We used these chambers to determine the impact of photosynthetically produced oxygen on offsetting the sediment oxygen demand. This would allow us to estimate the role that macrophytes play in replenishing dissolved oxygen throughout the day;. However, few of the light chambers yielded any results. Light SOD values listed (Appendix Table 2.2) are taken from one reading only (rather than an average) within the wetland and therefore may not represent the true light SOD for that wetland. We did not feel comfortable in drawing any conclusions from these data. In addition, many of the oxygen-consumed-values were higher in the light chambers than in the dark chambers within the same wetland. One would expect the rate of oxygen consumption to be less in the light chambers due to oxygen being produced via photosynthesis. This may have been caused by the settling of sediment on top of the domes and inhibiting light penetration from occurring. This would in essence make them dark chambers. As a result, the data collected from the light chambers was not used for analyses.

Appendix Table 2.1. Mean wetland water depth (cm ± S.D.) of each study wetland for 2008 and 2009 sampling. Wetland zone refers to the area depth and gas samples were taken – unvegetated = areas with no emergent vegetation, possibly some submerged vegetation; vegetated = areas with emergent vegetation.

Year	Wetland class	Wetland	Wetland zone	Mean water depth (cm ± S.D.)
2008	OSPM	4 m CT	unvegetated	21.0 ± 8.6
			vegetated	19.7 ± 7.0
		Natural	unvegetated	37.3 ± 9.2
			vegetated	24.5 ± 5.1
		Test Pond 9	unvegetated	55.4 ± 7.2
			vegetated	34.8 ± 6.6
	Reference	Beaver South	unvegetated	48.9 ± 6.8
			vegetated	42.5 ± 6.7
		High Sulphate	unvegetated	55.6 ± 7.9
			vegetated	36.7 ± 6.5
		Peat Pond	unvegetated	48.5 ± 11.7
			vegetated	29.3 ± 11.2
		Shallow	unvegetated	59.1 ± 10.6
			vegetated	53.2 ± 7.0
		Southwest sands Beaver	unvegetated	45.8 ± 9.9
			vegetated	29.3 ± 6.3
2009	OSPM	4 m CT	unvegetated	24.4 ± 6.5
		Mikes Pond	unvegetated	38.7 ± 8.4
		Natural	unvegetated	40.7 ± 9.0
		Test Pond 9	unvegetated	48.5 ± 9.5
	Reference	Golden	unvegetated	45.4 ±10.7
		High Sulfate	unvegetated	58.6 ± 5.0
		Shallow	unvegetated	56.9 ± 10.5
		South Beaver	unvegetated	52.9 ± 8.0
		Southwest sands Beaver	unvegetated	36.7 ±11.0

Appendix Table 2.2 Mean SOD (both light and dark) values ± S.E. and percent biological and chemical demand.

Wetland	Wetland Class	Dark SOD ($O_2g/m^2/day$)	% BSOD	% CSOD	Light SOD ($O_2g/m^2/day$)
South Beaver	Natural	0.907	5.4	94.6	NR
SWSS Beaver	Natural	0.901	22.0	78.0	1.682
Muskeg	Natural	NR	NR	NR	NR
	MEAN	**0.904 ± 0.003**	**13.7 ± 8.3**	**86.3 ± 8.3**	**1.682**
Golden Pond	Reference	0.592	0	100	0.041
Shallow Wetland	Reference	1.275	10.5	89.5	3.021
High Sulphate	Reference	0.364	0	100	0.779
Pond 5	Reference	0.379	27.7	72.3	NR
WA 11	Reference	1.626	0.63	99.37	NR
	MEAN	**0.847 ± 0.256**	**7.76 ± 5.37**	**92.2 ± 5.37**	**1.28 ± 0.896**
Test Pond 9	OSPW	1.440	0.21	99.79	1.902
Mike's Pond	OSPW	0.508	0	100	NR
Natural Wetland	OSPW	3.022	0.86	99.14	NR
SWSS Berm	OSPW	1.284	0.19	99.81	NR
	MEAN	**1.564 ± 0.527**	**0.32 ± 0.187**	**99.68 ± 0.187**	**1.902**
4mCT	OSPS	1.687	4.27	95.74	0.134
Jan's	OSPS	0.591	3.28	96.72	NR
Demo	OSPS	1.263	5.94	94.06	NR
Test Pond 5	OSPS	NR	NR	NR	NR
Seepage	OSPS	0.432	11.76	88.24	NR
	MEAN	**0.993 ± 0.293**	**6.31 ± 1.89**	**93.69 ± 1.89**	**0.134**

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CHAPTER III:
DETERMINING THE BIOAVAILABILIY OF NUTRIENTS AND THEIR IMPACT ON SUBMERGENT VEGETATION BIOMASS IN WETLAND SEDIMENTS USING PLANT ROOT SIMULATOR™ PROBES.

Introduction

This study investigates the role of phosphorus in the growth of submergent macrophytes within wetlands of the oil sands region. The objectives were to determine how the relative concentrations of phosphorus in the sediment and water vary among four wetland classes in parallel with surveys to document submergent vegetation biomass. The goal of the study was to assess the bioavailablity of phosphorus to submergent vegetation and determine whether or not phosphorus concentration could account for variation in the extent and biomass of submergent vegetation among classes of reclaimed wetlands.

Based on the results of previous work (Cooper 2004: personal observations), we expected to see substantially more submergent macrophyte biomass in wetlands unaffected by oil sands process material compared to those that contain process material because of differences in phosphorus concentrations.

Secondly, we expected phosphorus concentrations to be higher in wetlands unaffected by oil sands process material (OSPM) than those that contained OSPM due to chemical constituents (such as iron) binding and making it unavailable for plant use.

Lastly, we expected that redox-sensitive phosphorus would constitute the largest proportion of phosphorus, particularly in oil sands process material-affected wetlands. Phosphorus fractions that are bound to iron are unattainable to plants (Schachtman *et al.* 1998).

Through photosynthesis, primary producers (algae and plants) are able to use the sun's energy to convert carbon dioxide into organic compounds that can be stored.

$$6CO_2 + 6H_2O \leftrightarrow C_6H_{12}O_6 + 6O_2$$ (Campbell & Reece 2005)

This is one of the most important biological processes on earth, and is the primary driver of the carbon cycle (Black 1973). The oxygen produced replenishes atmospheric oxygen consumed during aerobic cellular respiration (the reverse of the above reaction). Primary producers are the initial source of carbon and therefore form the base of any food web

(Campbell & Reece 2005). In addition to their undeniable impacts on carbon fixation, primary producers (particularly plants) also provide shelter for many life forms seeking refuge. As a result, photosynthetic plants are a cornerstone for the formation, viability, and sustainability of any ecosystem.

Plant colonization and establishment requires sufficient quantities of light, nutrients, and appropriate soil composition. Although carbon dioxide and oxygen can be obtained from the atmosphere, most nutrients are obtained from the soil (Vance *et al.* 2003). Nitrogen and phosphorus are two essential plant macronutrients (Gusewell & Koerselman 2002). Nitrogen is an important constituent of amino acids, which form proteins. These proteins can be found in structural components such as plant walls or as enzymes governing many biochemical processes (Lipson & Nasholm 2001). Nitrogen is also vital for growth and reproduction as it is a large component of nucleic acids making up DNA. Much of the nitrogen in the environment resides in unavailable organic forms (Barbarick 2006). For plants to be able to take up the nutrients, nitrogen must either be in the form of ammonium (NH_4^+) or nitrates (NO_3^-) (von Wiren *et al.* 1997). These forms are created through nitrogen fixation by bacteria although some nitrogen is oxidized into nitrates by lightning strikes. Ammonium may also be converted to nitrates through a two step process known as nitrification wherein nitrites are created as an intermediate product (Howard & Rees 1996).

$$2NH_4^+ + 3O_2 + 4HCO_3^- \rightarrow 4CO_2 + 6H_2O + 2NO_2^-$$
$$2NO_2^- + O_2 \rightarrow 2NO_3^- \qquad \text{(Siegrist \& Gujer 1987)}$$

Phosphorus is another key element in both the genetic makeup of plants and in catalyzing biochemical reactions, such as photosynthesis where ATP (adenosine triphosphate) is the major energy unit resulting from harnessing the sun's energy (Schachtman *et al.* 1998). In most freshwater systems, phosphorus is typically the limiting nutrient for plant growth (Elser *et al.* 2007). However, different species have different requirements (Barko & Smart 1980). Although phosphorus can be found in many different forms or bound to a number of compounds, only some forms are available for plant use (Schachtman *et al.* 1998). Most phosphorus is taken up in the form of

orthophosphate ions ($H_2PO_4^-$), which are needed in relatively high concentrations for the initial growth of plants (de Groot *et al.* 2003, Brondani *et al.* 2008).

Vegetation is abundant in most aquatic environments. Plants that are found in aquatic ecosystems and are visible to the naked eye are referred to as macrophytes. This study focuses solely on submergent macrophytes; those that are anchored to the sediment and do not protrude past the water's surface. In aquatic environments such as wetlands, macrophytes play a major role in ecosystem function. Photosynthesis by submerged aquatic vegetation (SAV) produces dissolved oxygen, which is vital for respiration by benthic invertebrates and other aquatic life forms (Dauer *et al.* 1992). Vegetation provides both a source of food and shelter for creatures in the wetland (De Szalay & Resh 2000). The roots of some anchored plants release oxygen while also allowing oxygenated water to penetrate into the sediment layer where conditions are otherwise mostly anoxic (Bodelier *et al.* 1996, Jespersen *et al.* 1998). As a result, biogeochemical processes of microbes are affected and benthic invertebrates are able to survive in these areas. Although some nutrients can be absorbed through the leaf tissue directly from the water column, most nutrients are taken up by macrophytes' root systems (Thursby & Harlin 1984, Shilla *et al.* 2006). As a result, chemical reactions in the soil are important determinants of plant health in an aquatic ecosystem.

Reclaimed wetlands in the oil sands region can be classified as either natural, reference, or oil sands process material-affected (see Chapter II). Algae and plants have a large influence on shaping newly constructed wetland communities as they can affect invertebrate distribution by providing shelter, food, and a place for egg attachment (De Szalay & Resh 2000). However, the amount of submergent macrophytes varies greatly among the wetland classes (personal observation). M.C. Roy (Univ. of Alberta, Ph.D. in progress) showed that OSPM-affected wetlands had a different vegetation community composition as well as reduced biomass than naturally occurring wetlands. The reason for this limitation in plant growth is unclear. Oil sands mine tailings contain elevated levels of ammonium, sulphur, salts, and naphthenic acids compared to natural wetland sediments, which may adversely alter the environment in which plants tend to grow (Matthews *et al*. 2002*)*. Oil sand reclamation efforts aim to construct wetlands with function equivalent to those that were present before the mining activities commenced.

The absence of submergent macrophytes in reclaimed wetlands may hinder primary production (Hoagland *et al.* 2001), limiting the accumulation of organic carbon (Bridgham *et al.* 2006) and consequent secondary productivity. Ultimately, this may reduce the complexity of the food web, an undesirable outcome (Batzer & Sharitz 2006).

In Chapter II, sediment oxygen demand was shown to be affected by residual chemical constituents in the system. Elevated levels of oxidizable material increased the overall sediment oxygen demand. These sediments, particularly those derived from oil sands process materials, alter the biochemical reactions that take place. Because these wetlands contain higher concentrations of chemical constituents, their sediment oxygen demand seemed to be affected. There was a direct link between the oxidation of chemicals and wetland SOD. Consequently, the materials used to reclaim wetlands could influence productive capacity. Wetlands containing oil sands process materials had nearly twice the rate of oxygen consumption at the water-sediment interface as wetlands with other substrates (Chapter II). A goal of this project was to determine whether the amount of CSOD can predict and explain the relative lack of submergent macrophytes in OSPM-affected wetlands.

Ammonia's oxidation not only contributes to the CSOD, but it can also potentially interfere with other complexes. Nitrates can act as a buffer and prevent the reduction of iron in these systems by being a more favourable electron acceptor. This causes the iron to remain bound to the phosphorus (Lucassen *et al.* 2004), rendering it unavailable to plants. Thus, there may be an indirect relationship between a wetland's CSOD and the amount of macrophytes that exist there. If so, the magnitude of chemical sediment oxygen demand may explain the relative lack of submergent macrophytes seen in OSPM-affected wetlands. We postulate that the sediments of wetlands with a higher CSOD portion will support a diminished biomass of submergent macrophytes due to limited nutrient availability.

The objectives of this study were to determine if there were differences in the amount of nutrients available to macrophytes in the varying classes of wetlands and whether this affected the composition and biomass of the submergent macrophyte community. Phosphorus was extracted from sediment samples to determine its chemical form present and in what quantities. We sampled SAV community composition and

biomass in 17 wetlands. We concurrently estimated absolute and bioavailable concentrations of nutrients in the sediments and water.

Materials and methods

Submergent vegetation surveys

Ten wetlands were sampled in each of August 2009 and 2010. Five randomly-selected locations were sampled in each wetland. The locations were selected from shore to ensure that no bias was present in selecting areas, as submergent vegetation could not be seen from shore. We sampled only open water areas where no emergent vegetation was present at a depth of approximately 40-60 cm. At each location, a 1-m^2 quadrat was placed on the surface of the water and a thatching rake was used to scrape the substrate within the delineated area until all of the submergent vegetation had been gathered. Each sample was gently squeezed to remove excess water, placed in a labelled polyethylene bag and frozen until analysis in September of each year.

Submergent vegetation survey analysis

In the lab, macrophyte samples were thawed at room temperature over 24 h. They were then immersed in a tray of water to facilitate separation. Each plant was identified to species and pooled with other conspecifics from the sample. Each species was placed in an aluminum foil tray and dried in a drying oven at 110°C for 24 h. Samples were then weighed to the nearest 0.1 g. The trays were placed back in the oven for a few h and then reweighed. If the mass changed by <0.05 g, the sample was deemed to be moisture-free and the dry mass was recorded. Otherwise, the sample was returned to the oven and reweighed. This process was repeated until constant mass had been achieved for each sample. This resulted in estimates of biomass for each species collected, expressed as g/m^2.

Plant root simulator probes™

The bioavailability of sediment-associated cations and anions was assessed using plant root simulator probes (PRS^{TM}) produced by Western Ag Innovations Inc. These plastic probes were 10 cm long and contained an adsorptive membrane (Fig. 3.1), which

Figure 3.1 PRS™ probes. One cation, one anion.

is chemically pre-treated to mimic the "surface characteristics and nutrient sorption phenomena" of plant roots (Western Ag Innovations Inc., Operations Manual). When probes are buried, charged ions in the available form adsorb to the membrane, which can later be eluted for analysis to determine the potential nutrient supply (Western Ag Innovations Inc., Operations Manual). Two different types of probes were used, one of which was used to detect anions while the other, cations.

Ten wetlands were sampled over a 3-d period in August of 2009. A second set of 10 wetlands was sampled in the same manner over 3 d in June 2010. Plant root simulator (PRS™) probes were received from Western Ag Innovations a day prior and were stored refrigerated until used. Thirty-two probes (16 anion, 16 cation) were placed in each wetland. Each anion probe was paired with (buried adjacent to) a cation probe. Members of the pair were fastened together with nylon fishing line through a hole at the end of each probe. A plastic fishing bobber was tied to the other end of the fishing line to mark the probes' location after they had been buried in the sediment. Sixteen pairs were placed in 10 separate Ziploc bags (one per wetland).

On the days of sampling, bags were stored on ice in a cooler until deployment. Wetlands were radially divided into 8 sections and 2 pairs of probes were buried in the sediment of each section where the water was 30-50 cm deep. Within each section one pair of probes was placed in the emergent macrophyte zone ("veg" zone) and the other was buried in the submergent macrophyte zone ("open" zone). Therefore, each wetland contained 8 pairs in each of the two zones throughout the wetland. However, 4 probes of each type were always analyzed together in to provide a composite value. As such, each wetland contributed to four values of nutrient concentrations, two from the emergent zone and two from the submergent zone. To deploy the probes into the sediment, a finger was used to create a small 30 cm long groove into the sediment. The probes were placed horizontally into the groove with the faces of the adsorptive membranes perpendicular to the sediment surface. The PRS™ probes were placed roughly 2-3 cm below the sediment surface in an attempt to keep the distance between the entire length of the adsorptive membrane and the sediment surface constant. Once the probes were placed in the grooves, the sediment was gently patted back together to ensure that the entire membrane

surface was in direct contact with the wetland sediments. Probes were deployed between 0900-1200 and were recovered after 96 ± 2 h.

The PRS™ probe pair was located by following the fishing line down to the sediment, and was carefully removed. The fishing line was cut, and each probe was scrubbed with a soft nylon-bristled brush to remove as much sediment from the membranes as possible and then placed in a Ziploc bag. Each bag received a total of either 4 anion or 4 cation probes. Probes were placed in alternating bags during collection to ensure that all analyses would represent the entire wetland. This resulted in 4 bags from each wetland for a total of 2 anion analyses and 2 cation analyses. Again these bags were placed in coolers with ice and brought back to the lab for thorough cleaning. Distilled water and a toothbrush were used in the lab to remove the minute amounts of sediment that was trapped in the cracks surrounding the membrane. Cleaned probes were placed in new bags, and stored refrigerated until all units had been collected at the end of the third day. Subsequently all of the samples returned to Western Ag Innovations for analysis. Results were returned in approximately two weeks.

Sediment core collection

Cores of sediment were collected from the same wetlands in which the SOD studies were performed (see Chapter II) and the PRS probes deployed. Four cores were collected per wetland. We used Lucite coring tubes 5 cm in diameter and 21 cm long. A coring tube was pushed roughly 2/3 of its length into the sediment, forcing a 14-cm long plug of substrate into the tube. The top of the tube was capped and the coring tube along with the sediment within was pulled up. The sediment was retained in the core tube due to negative pressure. The bottom of the tube was then capped. The top and bottom of each tube was capped beneath the water surface, trapping the wetland water overlying the sediment surface. Capped tubes were sealed using Parafilm and electrical tape and were transported on ice and then stored refrigerated at 4°C until extractions could be performed in the lab.

Water sample collection

Water samples were collected from the same wetlands in which the PRS™ had been deployed, in August 2010. Water was collected in 1 L opaque plastic Nalgene® containers. The containers were rinsed and then submersed upside down in the wetland. The container was then slowly inverted to allow all air to escape and then capped, resulting in no head space. The water was stored in a refrigerator until all the samples were taken. They were then placed into a cooler with ice packs and sent to the University of Alberta for nutrient analysis within 48 h of collection.

Sediment phosphorus extractions

Sequential phosphorus extractions were performed to determine the chemical forms of phosphorus bound in the sediments. Of the four cores collected, three were randomly selected for extraction analysis following procedures developed by Psenner *et al.* (1984) and were later modified by Jensen and Thandrup (1993). The method followed for these extractions came from Lukkari *et al.* (2007a), as it was the most recent edition of the procedure as well as providing the most explanation of the steps.

Preparation: Cores from the wetland were placed in a N_2-filled glove box (anaerobic chamber) to prevent exposure to the oxygen in the atmosphere. This was necessary as Lukkari *et al.* (2007b) reported changes in phosphorus fractionation concentrations between aerobic and anaerobic atmospheres. This was due to the fact that oxygen has the potential to interact with reduced species. As a result, every precaution was taken to minimize the core's exposure to the atmosphere. Once in the glove box, the cores were opened and a sediment sample was taken roughly 2 cm beneath the surface. This depth was chosen as the PRS™ probes were deployed at a similar depth, allowing for comparison between the two results. One gram of sediment from each core was weighed out and placed in a 50 mL polypropylene centrifuge tube while another gram was used for total phosphorus determination.

Step One: 25 mL of 0.46 M NaCl was added to the centrifuge tube containing 1 g of sediment. The sample was then mixed in an orbital shaker at 400rpm for 1 hour, after which it was centrifuged for 15 minutes at 400rpm. The supernatant was poured off and the pellet was rinsed with another 25 mL of NaCl to collect any remaining phosphorus.

The two supernatants were combined and their pH was adjusted to 2.0 using H_2SO_4 to ensure certain metals would remain dissolved as well as to optimize colour formation during analysis. Half of the sample was then filtered using a 0.45 μm syringe filter with the other half remaining unfiltered. Phosphorus concentration was then determined using the ascorbic acid method (see below).

Step Two: The remaining pellet from step one was resuspended using 12.5 mL of 0.11 M sodium dithionite ($Na_2S_2O_4$) and 12.5 mL of 0.11 M sodium bicarbonate ($NaHCO_3$). The sample was once again shaken for 1 hour in an orbit shaker at 400 rpm with a centrifugation cycle of 15 minutes at 4000 rpm. The supernatant was poured off and the pellet was rinsed for 15 minutes with another 12.5 mL of each compound above. The supernatant and the rinsings were mixed together and pH adjusted to 2.0. Half of the sample was filtered using a 0.45 μm syringe filter, resulting in 25 mL of filtered sample and 25 mL of unfiltered sample. Phosphorus concentration was determined using the ascorbic acid method (see below).

Step Three: The remaining pellet from step two was resuspended using 25 mL of 1.0 M NaOH and placed in the orbital shaker for a duration of 18 h (overnight). The sample was centrifuged at 4000rpm for 15 minutes and the supernatant poured off. Another rinsing step was performed for 15 minutes with an additional 25 mL of 1.0 M NaOH. The supernatants were combined and the pH was adjusted to 2.0 and half of the supernatant was filtered through a 0.45 μm syringe filter. Phosphorus concentration was determined using the ascorbic acid method (see below).

Ascorbic acid method: In order to create the molybdenum blue reagent, 125 mL of 5*N* sulphuric acid (H_2SO_4) was mixed with 37.5 mL of ammonium molybdate solution. Seventy-five mL of ascorbic acid solution was then added followed by the addition 12.5 mL of potassium antimonyl tartrate solution. This reagent was created everyday as it was only stable for a period of about 24 hours.

To determine phosphorus concentration for each of the sequential extractions, 4 mL of the reagent was added to roughly 25 mL of both the filtered and unfiltered sample. After ten minutes of allowing the reagent and sample to mix, 3 mL of the mixture was added to a cuvette and placed in a spectrophotometer. Absorbance readings were

conducted at 880 nm and recorded. These could then be converted to phosphorus concentrations using a standard curve (Murphy & Riley, 1962).

Standard Solution: 0.1757 g of potassium dihydrogen phosphate (KH_2PO_4) was dissolved in 1 litre of MilliQ resulting in a concentration of 40mg of P per L. Dilutions were performed and the known concentrations were graphed against absorbance in order to create a standard curve.

Total Phosphorus: Total phosphorus was determined using the persulfate digestion method. Forty mL of MilliQ water was added to the one gram of sediment taken from the cores initially and mixed thoroughly. One drop of phenolphthalein indicator solution was added and if a red colour appeared, it was neutralized with the drop-wise addition of H_2SO_4. Once the colour was discharged, 1 mL of H_2SO_4 solution was added along with 0.5 g of solid potassium persulfate ($K_2S_2O_8$). Samples were autoclaved for 30 minutes at 125°C. Once cooled, another drop of phenolphthalein was added and any colour formation was neutralized with the addition of NaOH (American Public Health Association *et al.* 1995). Phosphorus concentrations were determined using the ascorbic acid method described above.

Statistical analyses

Statistical analyses were performed using STATISTCA version 7.0 Statsoft, Inc., Tulsa, OK. Composite samples in each wetland were averaged and then log-transformed to ensure normality among data points. Statistical significance of differences between natural, reference, OSPW-affected, and OSPS-affected wetlands with respect to nutrient concentrations and submergent vegetation biomass were tested using ANOVA. Specific hypothesis were tested by planned comparisons. Significance levels were set at $p<0.05$ with wetland as the level of replication (n=3 natural, n=5 reference, n=4 oil sands process water-affected (OSPW) and n=4 oil sand process sediment-affected (OSPS)). A principal component analysis (PCA) was performed on the PRS™ probe data (log transformed values) to determine how the groups of ions interacted with one another.

Results

Submergent vegetation survey

Natural and reference wetlands accumulated more submergent macrophyte biomass than OSPW- and OSPS-affected wetlands (Figure 3.2, planned comparison ANOVA, $F=15.74$, $p<0.005$). These wetlands had more than four times the biomass of submergent macrophytes than the other two classes of wetlands. Because there was no difference seen between natural and reference wetlands or between OSPW-affected and OSPS-affected wetlands, they were grouped together when looking at the effects of age on submergent macrophyte biomass. Wetlands unaffected by process material supported a larger biomass of submergent macrophytes than those containing tailings and/or process water. These wetlands established a submergent macrophyte community far sooner than process-affected wetlands (Figure 3.3). Process-unaffected wetlands also had more species richness than process-affected wetlands. Wetlands that contained process material had at most two species present whereas most of the natural and reference wetlands had at least four species present. In addition, the community composition also differed as OSPM-affected wetlands contained mostly species of *Potamogeton.*

Plant root simulator probes™

A total of 16 ions were recorded by the PRS™ probes (Appendix Table 3.1). Differences were seen in some nutrient absorption rates, however the five most relevant ions that were considered for comparison were NO_3^-, NH_4^+, PO_4^-, S^{2-}, and Fe^{2+} (Table 3.1). These were selected as they were thought to have potential to affect either sediment oxygen demand or submergent macrophyte growth. Nitrate rates ranged from 0.1 to 4.0 µg/10 cm^2/96 h, ammonium from 3.7 to 72.0 µg/10 cm^2/96 h, phosphate from 0.2 to 11.6 µg/10 cm^2/96 h, sulphide from 2.1 to 1496.5 µg/10 cm^2/96 h and iron from 7.9 to 1385.5 µg/10 cm^2/96 h. Pilot study work showed that 96 h was a sufficient time frame over which to determine adsorption rates without fear of saturating the probes**.** Phosphate was the only ion whose concentrations differed significantly among the wetland classes (Figure 3.4, planned comparison ANOVA, $F=21.39$, $p<0.0001$). Natural wetlands had significantly more bioavailable phosphorus than the other wetland classes (more than

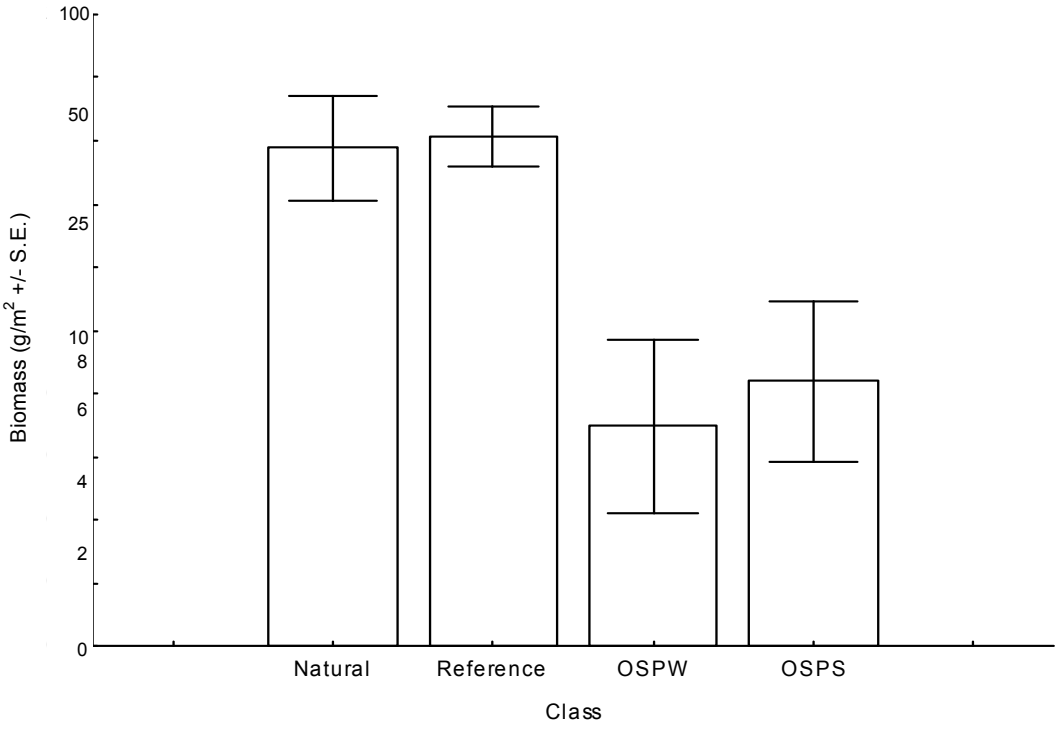

Figure 3.2 Mean plant biomass (g/m^2 dry weight ± S.E.) in natural (n=3), reference (n=5), OSPW (n=5), and OSPS (n=4) wetland classes. Biomass was significantly higher in natural and reference wetlands than in OSPW and OSPS-affected wetlands ($F=15.74$, $p<0.005$). Please note logarithmic scale on Y-axis.

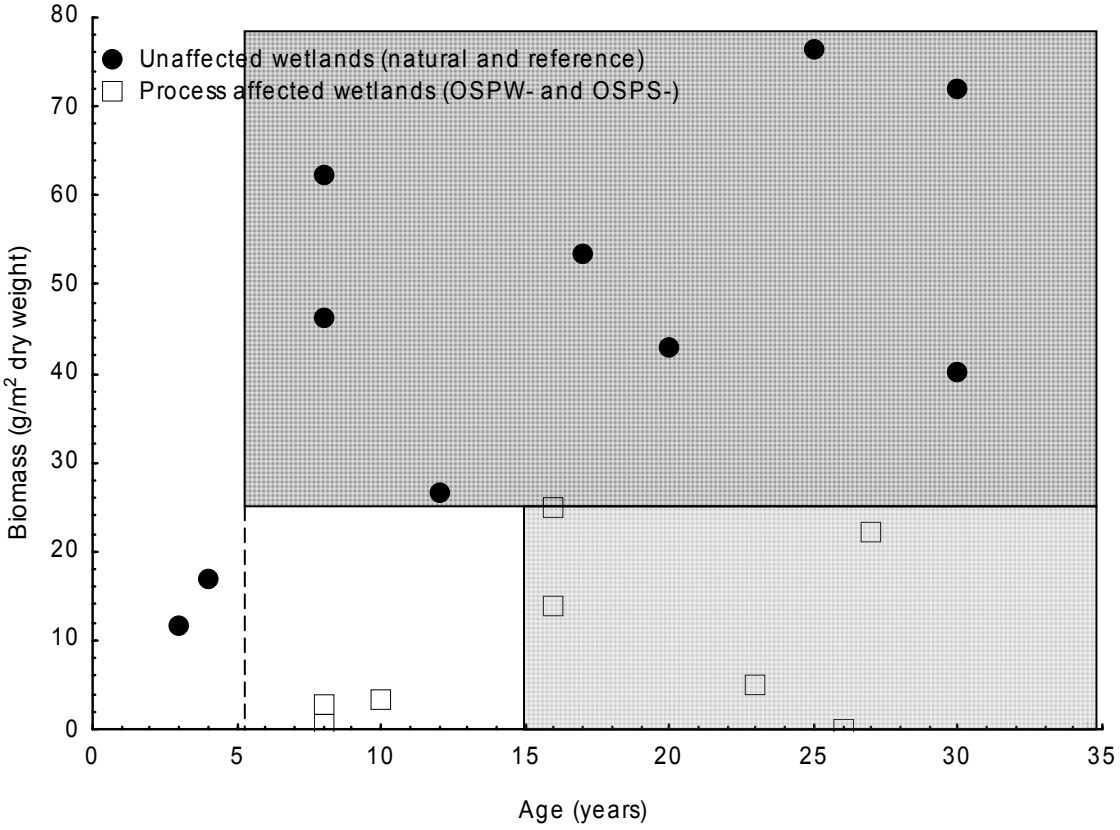

Figure 3.3 The relationship between submergent macrophyte biomass (g/m^2) and the age (years) of a wetland. Process unaffected wetlands (natural and reference) accumulate biomass at a faster rate as well as have more biomass than process affected wetlands of similar age (OSPW- and OSPS-) with the same age. The shading illustrates thresholds for submergent macrophyte development; 5 years and >25 g/m^2 for unaffected wetlands (dark box), 15 years and <25 g/m^2 for affected wetlands (light box). No relationship is apparent within the boxes.

Table 3.1 PRS™ probe data for open zones. Values in μg/10 cm^2/96 h.

Wetland	Class	NO_3^-	NH_4^+	PO_4^-	S^{2-}	Fe^{2+}
South Beaver	Natural	2.0	15.9	6.6	2.6	1362.2
SWSS Beaver	Natural	2.7	13.1	11.6	313.5	255.4
Muskeg	Natural	NR	NR	NR	NR	NR
	MEAN	**2.35**	**14.5**	**9.1**	**158.1**	**808.8**
	±SE	**±0.35**	**±1.4**	**±2.5**	**±155.5**	**±553.4**
Golden Pond	Reference	1.8	11.6	2.8	740.8	82.8
Shallow Wetland	Reference	2.7	14.2	4.1	2.8	1063.5
High Sulfate	Reference	1.4	8.1	1.3	1318.1	7.9
Pond 5	Reference	2.2	8.5	0.4	1270.2	471.1
WA11	Reference	0.1	12.6	0.7	1286.2	81.5
Peat Pond	Reference	1.3	13.8	5.5	340.4	291.1
S. Bison Ditch	Reference	0.8	10.6	1.4	688.0	366.2
	MEAN	**1.47**	**11.34**	**2.31**	**806.6**	**337.7**
	±SE	**±0.33**	**±0.91**	**±0.72**	**±194.5**	**±136.8**
Test Pond 9	OSPW	2.0	5.1	0.3	62.6	322.6
Mike's Pond	OSPW	2.1	3.7	0.2	988.0	95.3
Natural Wetland	OSPW	0.8	72	6.6	55.6	768.2
SWSS Berm	OSPW	1.0	9.9	5.2	148.6	405.4
Seepage	OSPW	2.5	19.3	2.1	72.2	637.9
	MEAN	**1.68**	**22.0**	**2.88**	**265.4**	**445.8**
	±SE	**±0.33**	**±12.8**	**±1.3**	**±181.4**	**±118.4**
4mCT	OSPS	1.6	8.3	0.3	615.5	113.6
Jan's Pond	OSPS	1.1	17.6	0.2	1401.6	23.0
Demo	OSPS	2.7	5.6	0.5	213.0	567.9
Test Pond 5	OSPS	NR	NR	NR	NR	NR
	MEAN	**1.8**	**10.5**	**0.33**	**743.4**	**234.8**
	±SE	**±0.47**	**±3.6**	**±0.08**	**±349.0**	**±168.6**

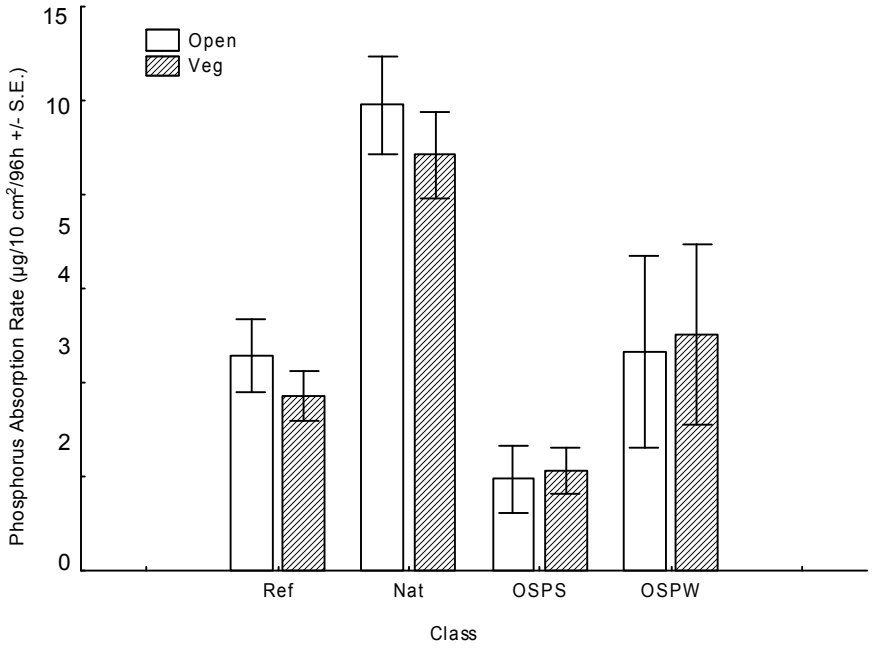

Figure 3.4 Mean ±S.E. phosphorus adsorption rate (µg/10 cm^2/96 h) in reference (n=5), natural (n=3), OSPS (n=4) and OSPW (n=5) wetland classes. Phosphorus adsorption rates were higher in natural wetlands compared to the others ($F=21.39$, $p<0.0001$). There was no difference in adsorption rates between zones. Please note logarithmic scale on Y-axis.

twice as much). Planned comparison analysis showed that none of the other ions differed significantly in concentration among wetland classes, although iron concentration was much higher in natural wetlands while NH_4^+ showed an increase in OSPW-affected wetlands. No difference was seen between PRS™ probes placed in open areas and those placed in vegetated ones.

Principal component analysis was performed on the ion absorption rates from the PRS™ data. PCA showed that five factors explained 83.4% of the original variation in the original variables (Table 3.2). Ions that were positively correlated with PC1 were manganese, phosphorus, nitrates, and calcium. No ions were negatively correlated with this factor. Positively correlated ions associated with PC2 were total nitrogen, ammonium, and potassium. Again no ions were negatively correlated. For PC3, boron and aluminum were both negatively related while no ions were positively associated with this component. Lead and iron were positively associated with PC4 while magnesium and sulphur were negatively related. Zinc was the negatively correlated to PC5 with no ions positively associated with it.

Sediment phosphorus extractions

Results from the sequential extractions show that soluble phosphorus concentration was fairly similar across wetland classes with the exception of OSPS-affected wetlands. The OSPS-affected wetlands had lower concentrations of this fraction, although the difference was not statistically significant (Figure 3.5). The second step separates out redox-sensitive phosphorus (iron-bound) and shows that OSPS-affected wetlands along with natural wetlands had the largest concentration (Figure 3.6). The formation of a precipitate interfered with some of the analyses and so only the filtered portion of the sample was analysed. If the filtered portions gave readings above the total phosphorus concentrations, they were discarded. No error bar is seen for natural wetlands as only one wetland was usable for this reason. Organic phosphorus concentrations again were highest in OSPS-affected and natural wetlands but the differences were not statistically significant (Figure 3.7). Total phosphorus concentration was highest in natural wetlands (Figure 3.8).

Table 3.2 Principal component analysis for ion concentrations from PRS data.

Ion	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Mn	**0.878**	-0.078	0.185	0.282	0.166
P	**0.841**	0.328	0.198	0.069	0.008
NO3-N	**0.620**	-0.051	-0.389	0.091	-0.490
Ca	**0.467**	-0.343	0.557	0.089	0.102
Total N	0.160	**0.954**	0.051	0.108	-0.031
NH4-N	0.113	**0.961**	0.080	0.101	0.006
K	-0.299	**0.814**	-0.390	-0.109	0.060
B	-0.266	0.155	**-0.862**	-0.161	-0.031
Al	0.103	-0.339	**-0.717**	-0.216	0.226
Pb	0.056	-0.058	0.079	**0.890**	0.043
Fe	0.242	0.168	0.124	**0.857**	0.141
Mg	-0.091	-0.041	-0.247	**-0.792**	0.177
S	-0.369	-0.357	0.163	**-0.557**	-0.481
Zn	-0.072	0.043	0.062	-0.059	**-0.880**
Expl. Var.	2.500	3.031	2.067	2.675	1.394
Prp. Total	0.179	0.217	0.148	0.191	0.100
Cumulative Prp. Total	0.179	0.396	0.543	0.734	0.834

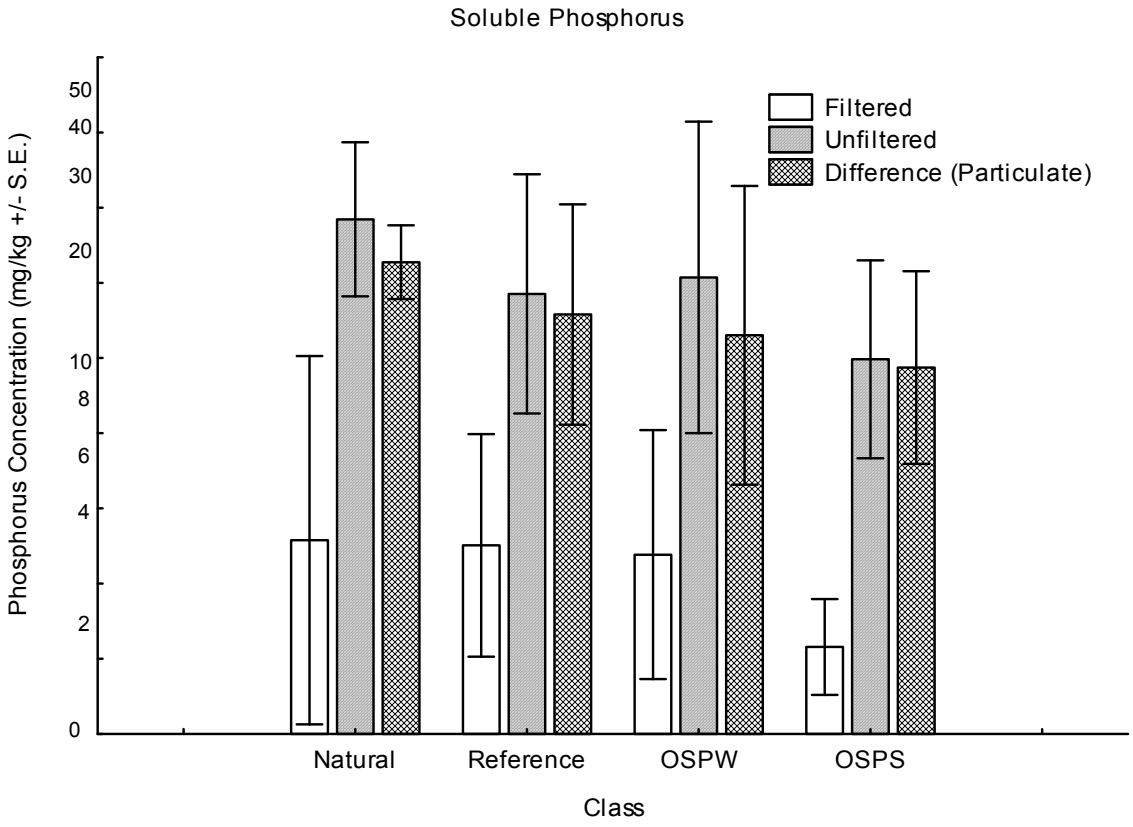

Figure 3.5 Step one of the sequential extraction shows mean soluble phosphorus concentration (mg/kg ± S.E.) in natural (n=3), reference (n=5), OSPW (n=5), and OSPS (n=4) wetlands. Please note logarithmic scale on Y-axis.

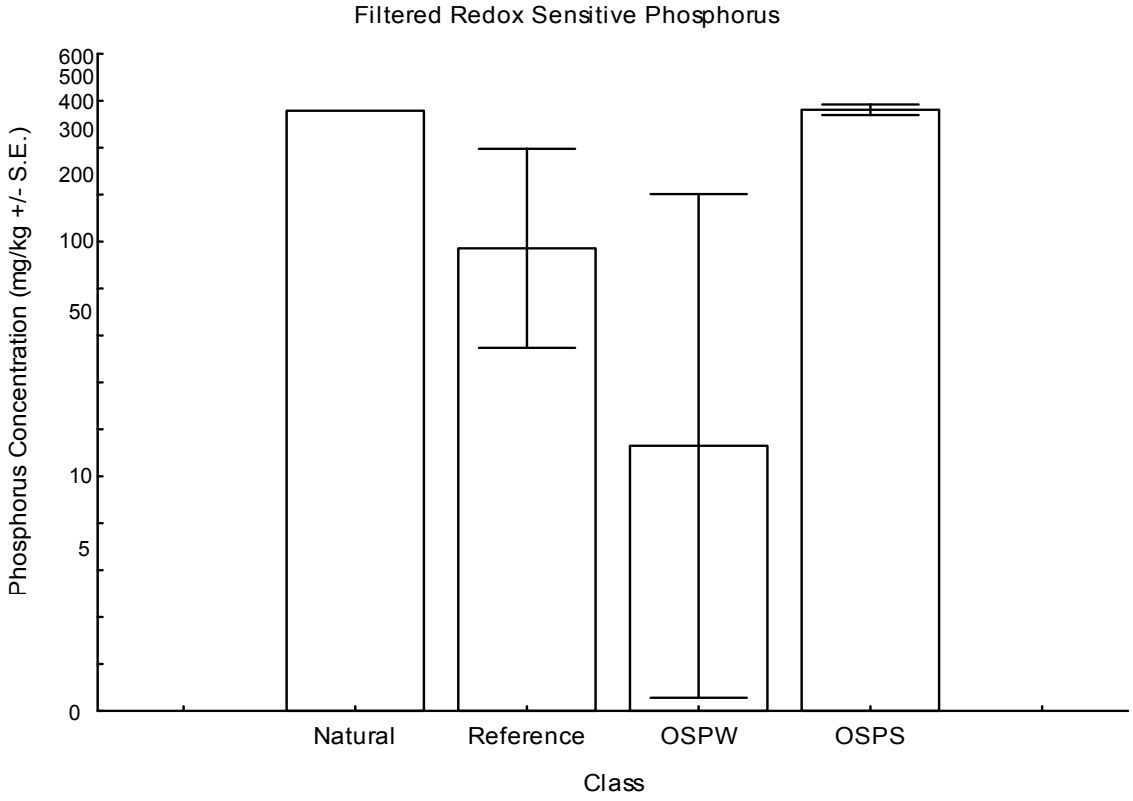

Figure 3.6 Mean redox-sensitive phosphorus concentration (mg/kg ± S.E.) in natural (n=1), reference (n=3), OSPW (n=3), and OSPS (n=3) wetland classes. No error bar is seen for natural wetlands as only one wetland's data was usable. Please note logarithmic scale on Y-axis.

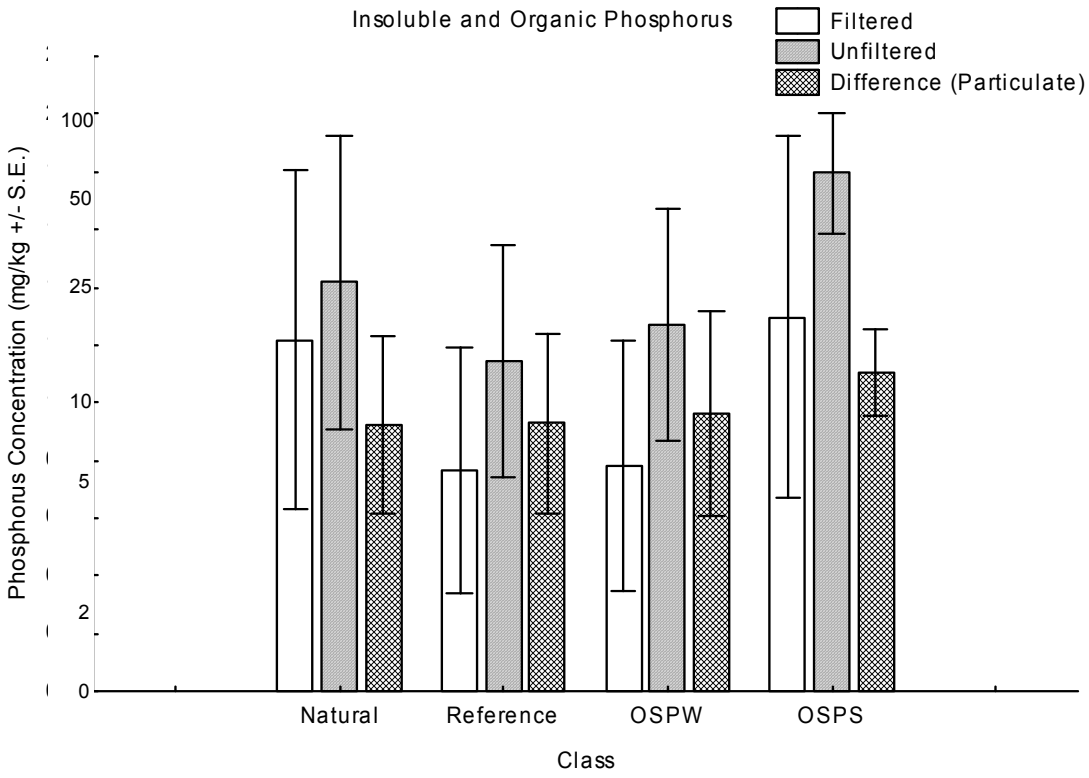

Figure 3.7 Mean organic phosphorus concentration (mg/kg ± S.E.) in natural (n=3), reference (n=5), OSPW (n=5), and OSPS (n=4) wetland classes. Please note logarithmic scale on Y-axis.

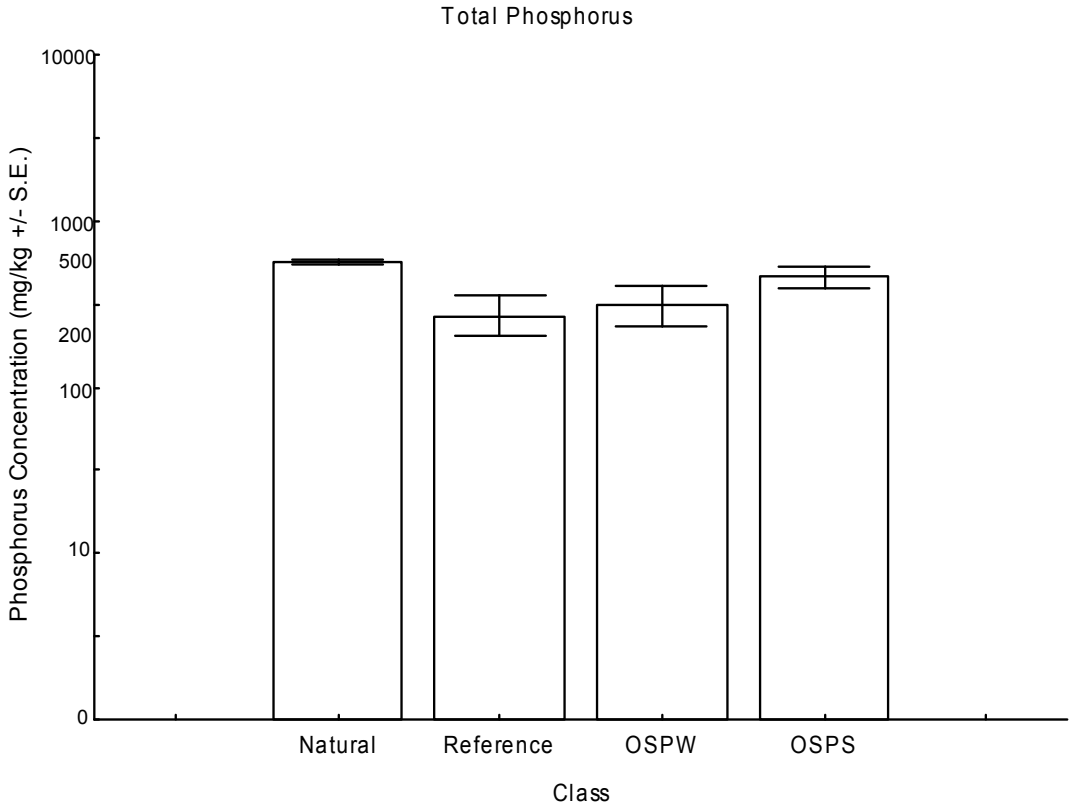

Figure 3.8 Mean total phosphorus concentration (mg/kg ± S.E.) in natural (n=3), reference (n=5), OSPW (n=5), and OSPS (n=4) wetland classes. Please note logarithmic scale on Y-axis.

Water nutrient analysis

Data from the water nutrient analysis show that soluble reactive phosphorus, total dissolved phosphorus, and total phosphorus concentrations were all highest in OSPW-affected wetlands (Table 3.2, Figure 3.9). However, two wetlands in this class, SWSS Berm and Natural Wetland had extremely high values for these categories. Both of these wetlands were constructed with peat-amended substrate whereas the other two OSPW wetlands had a solid clay substrate. If the two peat-amended wetlands are removed from the analysis, natural wetlands show the highest concentrations in each of the three categories (Figure 3.10). In both cases, reference wetlands had significantly reduced concentrations of soluble reactive phosphorus (Figure 3.9, $F=9.08$, $p<0.01$ and Figure 3.10, $F=6.19$, $p<0.01$).

Discussion

Submergent vegetation

Mean submergent macrophyte biomass was significantly (4X) higher in wetlands that did not contain oil sands process material than those that did (Figure 3.3). Our estimates of biomass in reference constructed wetlands are similar to those of others. Hoagland *et al.* (2001) reported values of 276-1380 g/m^2 in constructed wetlands of Illinois while the mean weight of our samples from references wetlands prior to drying was just below 600 g/m^2. Studies by Carr & Chambers (1998), illustrate a macrophyte biomass of 200-800 g/m^2 in the Saskatchewan River. These comparative data suggest that OSPM-affected wetlands have reduced biomass compared to other wetland systems. Wetlands that contained any form of oil sands process material had markedly lower amounts of vegetation beneath the water's surface. Although macrophyte biomass per unit area increased with wetland age, the rate of increase was much slower in oil sands-affected wetlands than in those that lacked oil sands materials (Fig 3.4). In wetlands lacking any OSPM (natural and reference), a substantial submergent macrophyte community seems to become established after 5 y. After this period, no linear trend exists between age and submergent macrophyte biomass. However, dry biomass was consistently >25 g/m^2. In contrast, submergent macrophytes were entirely absent from

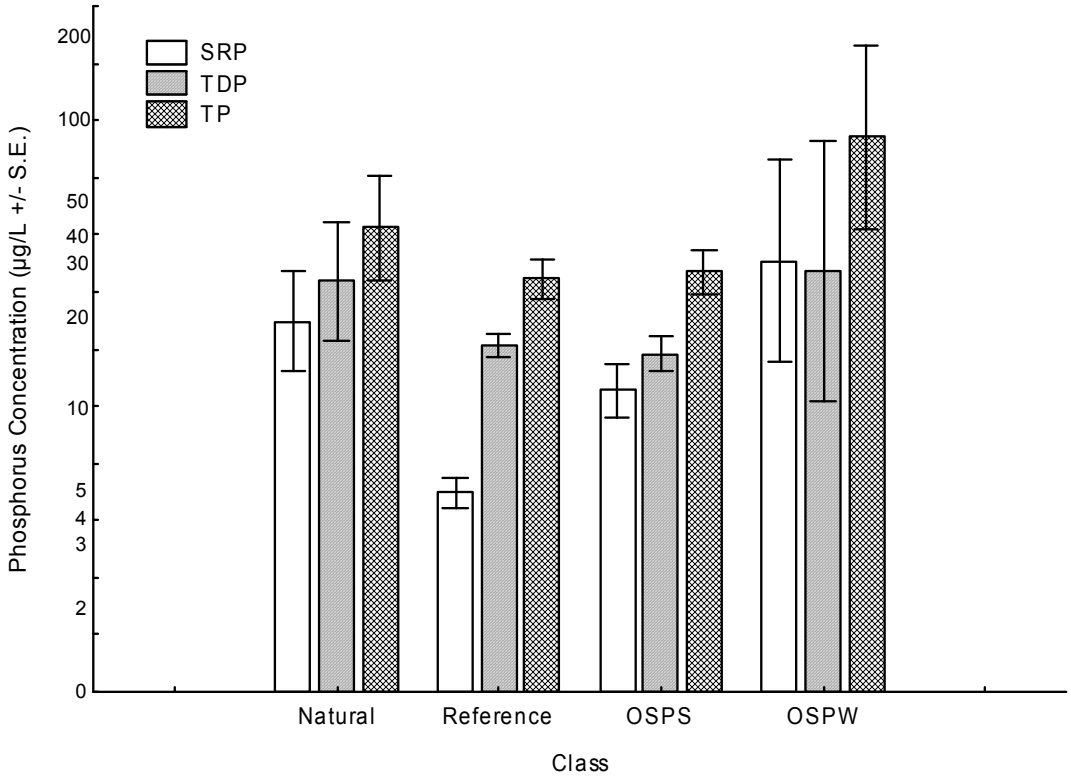

Figure 3.9 Mean phosphorus concentration (µg/L ± S.E.). Three different forms of phosphorus are examined (SRP – soluble reactive phosphorus, TDP – total dissolved phosphorus, TP – total phosphorus). SRP was significantly lower in reference wetlands ($F=9.08$, $p<0.01$). Natural (n=3), reference (n=5), OSPW (n=5), and OSPS (n=4). Please note logarithmic scale on Y-axis.

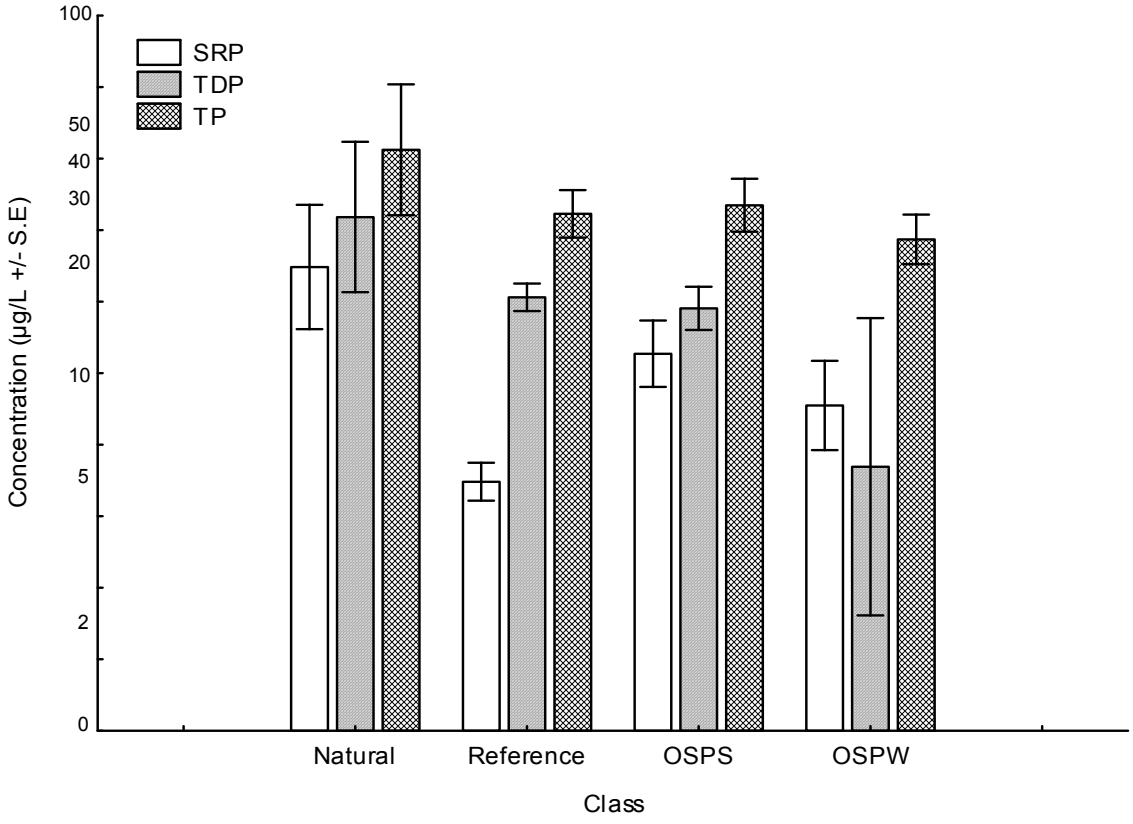

Figure 3.10 Mean phosphorus concentration (µg/L ± S.E.) Three different forms of phosphorus are examined (SRP – soluble reactive phosphorus, TDP – total dissolved phosphorus, TP – total phosphorus). Two OSPW-affected wetlands were removed from this analysis due to extremely high results in order to show the drastic change in means. SRP was still significantly lower in reference wetlands ($F=6.19$, $p<0.01$). Natural (n=3), reference (n=5), OSPW (n=5), and OSPS (n=4). Please note logarithmic scale on Y-axis.

OSPM-affected wetlands, <15 years of age; and older OSPM-affected wetlands never had a submergent macrophyte biomass >25 g/m^2. This indicates that these types of wetlands take longer to acquire a substantial macrophyte community. However, the fact that no trend or asymptote is seen in the graphs speaks to the large variability among wetlands even within classes. For example, although both over the age of 15 and both containing OSPW, SWSS Berm wetland has nearly no submergent macrophytes while Test Pond 9 had a biomass of roughly 24 g/m^2. Therefore, factors other than simply the presence or absence of oil sands process material must influence submergent macrophyte growth.

In addition to containing less SAV biomass, OSPM-affected wetlands supported fewer macrophyte species. The mean number of species seen did not differ significantly among wetlands, although a maximum of 2 species was seen in any OSPM-affected wetland whereas most reference wetlands supported 4 species, and one wetland had 5 species. This pattern is consistent with data of Roy and Foote (2010). Engelhardt & Ritchie (2001) found that wetlands which have greater species richness, help to sustain ecosystem processes and increase productivity. Species such as *Myriophyllum sibiricum* and *Ceratophyllum demersum* were never found in OSPM-affected wetlands. These wetlands tended to support species in the Potamogetonaceae (pondweed) family.

Plant root simulator probes™

The goal of this study was to determine the putative causes for the reduced submergent biomass seen in OSPM-affected wetlands. The initial postulate was that perhaps the amount of available phosphorus differed among wetland classes, which could limit plant growth in the most deficient wetlands. Data from the PRS™ probes showed that natural wetlands did indeed have higher levels of bioavailable phosphorus (Fig. 3.2). However, there was no consistent difference among the other three classes. We had expected to see similar phosphorus concentrations in reference wetlands as they had nearly the same coverage and biomass of submergent macrophytes but this was not the case. Bioavailable phosphorus in reference wetlands was similar to that of some OSPM-affected wetlands. Because reference wetlands consistently supported more submergent macrophytes with the same amount of phosphorus as OSPM-affected wetlands, perhaps phosphorus is not limiting the growth in these wetlands. Alternatively, some other factor

may be limiting macrophyte establishment – salinity, sediment composition, or possibly even biofilm cover (which may be able to develop on the clay and CT substrates that may be too impermeable to sustain macrophyte roots).

Another major nutrient, nitrogen, did not differ significantly among wetland classes. Consequently, nitrogen variation among wetland classes cannot account for the differences in submergent macrophyte biomass. Ammonium levels (a byproduct of the oil sands refining process) however, were variable but were in higher amounts in OSPW-affected wetlands than in reference wetlands. As mentioned in the previous chapter, the oxidation of this ion may increase the chemical sediment oxygen demand and ultimately the overall sediment oxygen demand. This was in fact seen as OSPW-affected wetlands did show a trend toward greater rates of sediment oxygen demand (Chapter II). Natural Wetland had both the highest ammonium concentration and the highest SOD rate. It was initially thought that ammonium oxidation could also affect phosphorus availability to plants by inhibiting its release from its Fe-bound state (Lucassen *et al.* 2004). Oxidized ammonium, resulting in nitrates, were thought to prevent the reduction of sulphates to sulphides, which could then no longer interact with iron-phosphate complexes preventing the phosphorus from becoming available for plant use. We did not see a relationship between the amount of phosphorus in the wetlands and the amount of ammonium present. Consequently, it is unlikely that this pathway is the cause for reduced plant growth.

All of the other ions showed marked variability within wetland classes, and no statistically significant differences among classes were apparent. Iron concentration was highest in natural wetlands while sulphides were the lowest. Sulphides have been indentified as one of the major components when conductivity is high (Renault *et al.* 1998), and those may reveal why natural wetlands have more submergent macrophyte biomass. Again, this does not explain the pattern in reference wetlands, as they had the highest sulphide concentrations, although the difference was not statistically significant.

This was the first attempt at using PRS™ probes in flooded wetland sediments and as such it was difficult to compare our results to the findings of others. However, converting our absorption rates into N:P ratios gave us the best basis for comparison (Table 3.3). Studies by Stanek & Jeglum (1977) reported N:P ratios ranging from 11.8-25.3 in Canadian wetlands. Focusing solely on marshes, which our wetlands most

Table 3.3 Nitrogen to phosphorus ratios in the four wetlands classes.

Wetland Class	Nitrogen Adsorption Rate (µg/10 cm^2/96 h)	Phosphorus Adsorption Rate (µg/10 cm^2/96 h)	N:P Ratio
Natural	16.85	9.1	1.85
Reference	12.81	2.31	5.55
OSPW	23.68	2.88	8.22
OSPS	12.3	0.33	37.27

resemble, Simpson *et al.* (1983) reported a ratio of 5.1 and Bowden (1984) conveyed a ratio of 10.6. A compilation of data suggests a mean ratio of 8.7 for marshes in temperate North America (Bedford *et al.* 1999). Most of our ratios fall below this average (natural - 1.85, reference - 5.55, OSPW – 8.22) with the exception of OSPS wetlands (37.27). N:P ratios below 14 suggest that the system is N limited (Koerselman & Meuleman 1996, Verhoeven *et al.* 1996). This may once again indicate that phosphorus may not be the reason for reduced submergent macrophyte growth. Phosphorus is the limiting nutrient in most freshwater systems. Our ratios must be carefully scrutinized as they came from results of bioavailable phosphorus and nitrogen. It is entirely possible that total nutrient concentrations would have yielded different N:P ratios.

Water nutrients

Water column concentrations of nitrogen and phosphorus were both higher in OSPW-affected wetlands than any other class. Two wetlands in this class (those that had been amended with peat substrate) had exceedingly high values for both variables, which undoubtedly raised the class means (Table 3.4). If these values are outliers, phosphorus concentrations become highest in natural wetlands whereas nitrogen concentrations become similar in every wetland (Figure 3.9, 3.10). This matches the patterns seen in the PRS™ data. However, doing so reduces the sample size to two, and ultimately the power of the test. If these results are read as they are, they may indicate that most of the phosphorus in natural wetlands is sediment-associated whereas OSPW-affected wetlands have the majority of their phosphorus in the water column. This may possibly explain the lower number of plants seen in OSPW-affected wetlands. Although some submergent macrophytes can take up nutrients through their leaves, the majority of nutrient transfer is accomplished through the root system (Thursby & Harlin 1984, Shilla *et al.* 2006). If nutrients are in the water column, they may be of little use to the plants growing in the wetland. Natural wetlands, which have most of their phosphorus in the sediment rather than in the water column, support a larger biomass of submergent species than their constructed counterparts. This is one possible explanation for why few submergent macrophytes are seen in the OSPM-affected wetlands. This difference in nutrients in the

Table 3.4 Water nutrient concentrations. TDN-total dissolved nitrogen, TN-total nitrogen, SRP-soluble reactive phosphorus, TDP-total dissolved phosphorus, TP-total phosphorus.

Wetland	Class	TDN (µg/L)	TN (µg/L)	SRP (µg/L)	TDP (µg/L)	TP (µg/L)
South Beaver	Natural	1480	1550	45	63	79
SWSS Beaver	Natural	880	981	12	28	51
Muskeg	Natural	540	606	12	10	17
	MEAN	**966.6**	**1045.7**	**23.0**	**33.7±**	**49.0±**
	±SE	**±274.8**	**±274.4**	**±11.0**	**15.6**	**17.9**
Golden Pond	Reference	1400	1620	4	17	49
Shallow Wetland	Reference	1180	1230	5	17	26
High Sulfate	Reference	1290	1420	5	13	21
Pond 5	Reference	NR	NR	NR	NR	NR
WA11	Reference	1180	1320	3	11	16
Peat Pond	Reference	1630	2390	6	22	38
S. Bison Ditch	Reference	1010	1180	2	14	23
	MEAN	**1281.7**	**1526.7**	**4.2**	**15.7**	**28.8**
	±SE	**±87.5**	**±184.1**	**±0.6**	**±1.6**	**±5.0**
Test Pond 9	OSPW	1540	1590	10	14	27
Mike's Pond	OSPW	858	947	5	1	19
Natural Wetland	OSPW	6100	7380	66	73	201
SWSS Berm	OSPW	3630	4460	245	353	514
Seepage	OSPW	995	1100	23	21	40
	MEAN	**2624.6**	**3095.4**	**69.8**	**92.4**	**160.2**
	±SE	**±1001.0**	**±1246.7**	**±45.1**	**±66.3**	**±94.6**
4mCT	OSPS	1800	1840	12	16	27
Jan's Pond	OSPS	1600	1780	9	15	36
Demo	OSPS	1280	1420	9	14	34
Test Pond 5	OSPS	1510	1560	5	8	14
	MEAN	**1547.5**	**1650.0**	**8.75**	**13.25**	**27.75**
	±SE	**±107.8**	**±97.5**	**±1.45**	**±1.8**	**±4.97**

water column could ultimately affect phytoplankton growth and turbidity. This change in the nutrient-turbidity relationship could shift the steady state of these types of wetlands. At a certain point, phytoplankton in the water column could theoretically increase the turbidity to a point at which macrophytes can no longer persist (Scheffer 1990). If two steady states exist in these systems, then the location of nutrients could potentially play a large role in whether submergent macrophytes become established.

Sediment phosphorus extractions

Sequential phosphorus extractions were used to determine what form of phosphorus was present and in what proportions within the four wetland classes. During the first step, loosely bound phosphorus and pore water phosphorus were extracted (Fig. 3.5). Dissolved phosphorus was always present in lower concentrations than the particulate form. The soluble phosphorus fraction is bioavailable to plants and can be easily used (Schachtman *et al.* 1998). OSPS-affected wetlands had the least amount of soluble phosphorus, though the mean was not statistically different from other classes. Although this may possibly be an indication of why fewer plants are seen, the argument falls apart as OSPW-affected wetlands had just as much of this form of phosphorus. The difference seen in OSPS-affected wetlands however, could indicate that certain chemical constituents are affecting the availability of phosphorus (as these wetlands contain OSPM in their sediment), regardless of whether or not the phosphorus affects plant growth.

Iron-bound phosphorus was analyzed in the second step and was found to be the largest proportion of phosphorus (Fig. 3.6). Unfortunately, due to the formation of a precipitate, which occurred during the extraction process, only the filtered extract was looked at. This precipitate was the result of dithionite breaking down into thiosulfate and hydrogen sulfite (Lukkari *et al.* 2007a). These interfere with the molybdenum blue reagent and spectrophotometric analysis rather than affecting phosphorus concentrations directly. By filtering out the precipitate and then adding the reagent, phosphorus concentrations remain the same and can still be analysed. Natural and OSPS-affected wetlands had similar levels of redox-sensitive phosphorus while OSPW-affected wetlands had the lowest concentrations. OSPW-affected wetlands had only 80.1mg/kg (23%) of their total phosphorus in this form while the other three wetland classes were all higher

Table 3.5 Concentration and relative abundance of phosphorus types in each extract in the four wetland classes.

Wetland Class	Total Phosphorus (mg/kg)	Loosely bound P (mg/kg)	Iron bound P (mg/kg)	Organic and insoluble P (mg/kg)	Other forms of P (mg/kg)
Natural	565.87	30.0±16.1 (5.3%) n=3	350.3 (61.9%) n=1	75.3±44.6 (13.3%) n=3	110.27 (19.5%)
Reference	307.9	32.0±14.5 (10.4%) n=5	180.4±97.8 (58.6%) n=3	20.0±12.5 (6.5%) n=5	75.4 (24.5%)
OSPW	343.74	40.2±23.6 (11.7%) n=4	80.1±80.1 (23.3%) n=2	45.0±18.3 (13.1%) n=4	178.4 (51.9%)
OSPS	477.23	11.9±4.9 (2.5%) n=4	350.3±19.5 (73.4%) n=3	70.2±28.2 (14.7%) n=4	44.9 (9.4%)

than 180 mg/kg or 58% (Table 3.5). Initially, we had expected to see higher concentrations of iron-bound phosphorus in OSPM-affected wetlands. However, this was not the case as natural wetland sediments contained just as much of this fraction as OSPW-affected wetlands. In addition, reference wetland sediments contained higher concentrations than OSPS-affected wetlands. This once again, disproves the theory that iron-bound phosphorus not being available to plants is the cause of fewer submergent macrophytes seen in OSPM-affected wetlands.

In the last extraction step, organic phosphorus was extracted (Fig. 3.7). This insoluble, non-reactive phosphorus form is not available for use by plants, but could potentially be converted to useable phosphorus. This makes this extraction component an important pool for phosphorus as it could possibly increase the amount of phosphorus already available to plants. Natural wetlands again had the highest concentration of this form of phosphorus, which may seem detrimental to the wetland. However, almost all of the classes at roughly 13% of their phosphorus concentration tied up in this manner and so the increase is only due to their increase in total phosphorus concentrations. Reference wetlands only had 6% organic phosphorus.

General thoughts

Despite the variety of data that was collected regarding phosphorus, few conclusions could be reached due to the lack of significant differences among wetland classes. Although a few minor trends seemed to have a possibility of explaining the lack of plants seen, firm conclusions cannot be drawn regarding phosphorus concentrations and submergent macrophyte establishment due to the large amount of variation seen within wetland classes. That being said, it is clear that OSPS-affected wetlands are most affected by the presence of oil sands process material. Not only do they support the fewest macrophytes, but they also contain the lowest amount of usable phosphorus for plants (not necessarily limiting). Although total phosphorus in OSPS-affected wetlands was similar to that of natural wetlands, only 2.5% of that is easily used by vegetation (both PRS and extraction data support this). This may indicate that chemical constituents in the sediment are far more important than those in the water when it comes to plant growth. Although the data for OSPS-affected wetlands seem to support our hypothesis

(they had few macrophytes, the least bioavailable phosphorus and the highest proportion of iron-bound phosphorus) there are just as many findings in this study that contradict it. Two possibilities exist. Firstly, phosphorus concentrations may be driving submergent macrophyte establishment but this relationship is being masked by the large amount of variability seen with each wetland class; or secondly, phosphorus concentrations are not limiting the plant growth in these wetlands. Undoubtedly, many factors influence plant colonization and establishment and therefore the latter may be a more reasonable explanation at this point. High conductivity and salinity measures impede macrophyte growth (Batzer & Sharitz 2006, Levigneron *et al.* 1995) and the high concentration of salinity in OSPM-affected wetlands is the largest factor impeding successful reclamation, at least with regard to plant life (Renault *et al.* 1998). Sodium and sulphate ions are the major components of salinity increase in the oil sands wetlands (Renault *et al.* 1998). Both of these ions have detrimental effects on plants at higher concentrations (Datta *et al.* 1993, Bernstein 1975). Although phosphorus is the limiting nutrient in these systems, it does not necessarily mean that it is the limiting factor. Variables such as light, sediment composition, and water chemistry may also affect growth (Madsen & Sand-Jensen 2006, Barko & Smart 1986, Jin *et al.* 2006), while other factors affect phosphorus uptake directly (Jin *et al.* 2006). If one of these variables exceeds a certain threshold, then the concentration of phosphorus becomes insignificant. In the case of these wetlands, salinity may be the most important limiting factor. Saline waters may be unfavourable for macrophyte establishment even if more than enough nutrients are present (SWSS Berm wetland for example). That is not to say that once this factor is detected or dealt with that phosphorus will once again be the driving force behind successful submergent macrophyte colonization. If so, the relationships found in this chapter may change. OSPS- and OSPW-affected wetlands both had few established submergent macrophytes. However, if the factor presently limiting plant growth is removed, OSPW-affected wetlands may develop a macrophyte community resembling those of natural or reference wetlands while OSPS-affected wetlands may remain the same. OSPW-affected wetlands had higher bioavailable phosphorus than reference wetlands, the highest soluble phosphorus, and the lowest iron-bound phosphorus. This shows that they should be able

to support a substantial macrophyte community once the current limiting factor is removed.

The initial goal of this study was to relate CSOD to nutrient concentrations and eventually submergent macorphyte biomass. Scatterplots (not shown) indicate that there was little or no relationship between CSOD and either of the variables. This indicates that changes in CSOD due to residual chemical constituents may not affect phosphorus concentrations as initially hypothesized.

Conclusions

Submergent macrophyte dry biomass was significantly higher (4x) in oil sands process-unaffected wetlands than in affected wetlands. Phosphorus studies using PRS™ probes indicate that natural wetland sediments had the highest concentration of bioavailable phosphorus while OSPS-affected wetlands had the lowest. Phosphorus in the water column was highest in OSPW-affected wetlands but showed large amounts of variation. The sequential extractions that were performed indicate that iron-bound phosphorus was the most abundant form of phosphorus in each wetland class, while OSPS-affected wetlands had the lowest amount of useable phosphorus for plant growth.

Many of the studies performed in this chapter yielded nonsignificant differences among wetland classes. This undoubtedly reflects the large amount of variation among wetlands. The variation in biomass within a single wetland itself was relatively small. However it was considerable for both the PRS™ studies and the sediment extraction studies of phosphorus. This illustrates the incredible variability one encounters when trying to study such dynamic systems as wetlands. Categorizing wetlands into classes according to the presence or absence of OSPM is only one of many possible ways and did not reveal the results that were expected. A different classification, possibly of salinity gradient would have produced more meaningful results with regards to submergent macrophyte establishment.

In conclusion, phosphorus availability may play a smaller role in the establishment of submergent macrophytes *at this time* than initially thought, while nitrogen availability apparently had no influence. It is possible however that this relationship is simply being masked by the effects of other variables. Phosphorus could

easily become the most important issue once the other limiting factors are identified. In addition, ammonium was shown to have no influence on phosphorus availability and therefore the link between the CSOD and phosphorus availability was non-existent.

Appendix Table 3.1 PRS™ supply rate data stated in µg/10 cm^2/96 h.

Wetland	Zone	Total N	NO3-N	NH4-N	Ca	Mg	K	P	Fe	Mn	Cu	Zn	B	S	Pb	Al	Cd
Detection limits:		**2**	**2**	**2**	**2**	**4**	**4**	**0.2**	**0.4**	**0.2**	**0.2**	**0.2**	**0.2**	**2**	**0.2**	**0.4**	**0.2**
HS	Open S1	11.8	2.2	9.6	1643.0	510.6	32.8	2.0	7.2	4.2	0.2	0.2	2.8	1290.8	0.0	54.2	0.0
HS	Open S2	7.2	0.6	6.6	1405.0	418.4	28.0	0.6	8.6	5.6	0.0	0.2	1.6	1345.4	0.0	39.8	0.2
	AVG	9.5	1.4	8.1	1524.0	464.5	30.4	1.3	7.9	4.9	0.1	0.2	2.2	1318.1	0.0	47.0	0.1
HS	Veg S1	10.4	3.0	7.4	1233.4	398.6	27.2	2.0	12.2	4.8	0.0	2.8	3.0	1436.6	0.0	42.4	0.0
HS	Veg S2	6.2	0.0	6.2	1431.2	457.8	29.2	1.2	10.6	6.2	0.0	0.8	2.2	1556.4	0.0	49.8	0.0
	AVG	8.3	1.5	6.8	1332.3	428.2	28.2	1.6	11.4	5.5	0.0	1.8	2.6	1496.5	0.0	46.1	0.0
Golden	Open S1	9.8	0.0	9.8	1750.2	459.2	13.6	2.6	26.6	65.2	0.0	0.0	1.2	751.8	0.0	46.2	0.0
Golden	Open S2	17.0	3.6	13.4	1575.0	408.6	13.2	3.0	139.0	63.8	0.0	0.8	1.6	729.8	0.0	45.0	0.0
	AVG	13.4	1.8	11.6	1662.6	433.9	13.4	2.8	82.8	64.5	0.0	0.4	1.4	740.8	0.0	45.6	0.0
Golden	Veg S1	11.0	2.0	9.0	1614.2	377.2	10.2	2.2	229.6	29.4	0.0	0.6	2.8	936.2	0.2	53.0	0.0
Golden	Veg S2	9.8	2.4	7.4	1753.8	424.2	11.0	1.8	160.8	44.8	0.0	0.2	1.8	946.0	0.2	50.4	0.0
	AVG	10.4	2.2	8.2	1684.0	400.7	10.6	2.0	195.2	37.1	0.0	0.4	2.3	941.1	0.2	51.7	0.0
SWSS	Open S1	16.6	3.4	13.2	1805.8	385.6	9.8	12.0	234.6	191.2	0.0	0.2	1.4	361.6	0.2	47.8	0.0
SWSS	Open S2	15.0	2.0	13.0	1901.6	465.0	14.0	11.2	276.2	214.4	0.0	0.2	1.4	265.4	0.2	41.4	0.2
	AVG	15.8	2.7	13.1	1853.7	425.3	11.9	11.6	255.4	202.8	0.0	0.2	1.4	313.5	0.2	44.6	0.1
SWSS	Veg S1	21.0	4.2	16.8	1578.4	352.4	9.8	13.0	288.2	227.4	0.0	0.4	1.8	260.6	0.0	40.6	0.0
SWSS	Veg S2	8.4	1.8	6.6	1673.6	420.4	7.8	4.2	218.4	82.8	0.0	0.4	1.2	863.8	0.2	37.8	0.0
	AVG	14.7	3.0	11.7	1626.0	386.4	8.8	8.6	253.3	155.1	0.0	0.4	1.5	562.2	0.1	39.2	0.0
4mCT	Open S1	11.6	0.2	11.4	1356.4	657.2	38.4	0.2	81.4	8.2	0.0	0.2	2.8	600.0	0.0	43.6	0.0
4mCT	Open S2	14.0	3.0	11.0	1360.8	676.8	37.4	0.4	145.8	11.2	0.0	0.4	4.4	631.0	0.0	53.4	0.0
	AVG	12.8	1.6	11.2	1358.6	667.0	37.9	0.3	113.6	9.7	0.0	0.3	3.6	615.5	0.0	48.5	0.0
4mCT	Veg S1	10.6	0.4	10.2	1360.6	644.8	35.4	0.4	68.8	12.4	0.0	0.2	3.2	677.6	0.0	43.2	0.0

4mCT	Veg S2	12.8	2.0	10.8	1437.2	761.8	35.2	0.4	99.2	7.2	0.0	0.6	3.0	826.6	0.0	53.2	0.0
	AVG	11.7	1.2	10.5	1398.9	703.3	35.3	0.4	84.0	9.8	0.0	0.4	3.1	752.1	0.0	48.2	0.0
Pond 5	Open S1	9.0	2.4	6.6	1448.0	281.4	6.0	0.4	489.2	8.2	0.0	0.8	1.4	1256.6	0.4	34.8	0.0
Pond 5	Open S2	12.4	2.0	10.4	1790.4	298.8	8.4	0.4	453.0	8.2	0.0	0.4	1.6	1283.8	0.2	39.2	0.0
	AVG	10.7	2.2	8.5	1619.2	290.1	7.2	0.4	471.1	8.2	0.0	0.6	1.5	1270.2	0.3	37.0	0.0
Pond 5	Veg S1	6.2	1.0	5.2	1697.6	320.0	8.0	0.4	232.8	18.4	0.0	0.8	1.6	1575.0	0.2	38.4	0.0
Pond 5	Veg S2	10.0	0.0	10.0	2114.0	401.6	8.8	0.4	436.0	9.6	0.0	0.6	1.6	919.8	0.2	44.4	0.0
	AVG	8.1	0.5	7.6	1905.8	360.8	8.4	0.4	334.4	14.0	0.0	0.7	1.6	1247.4	0.2	41.4	0.0
Natural	Open S1	63.2	0.0	63.2	1155.8	355.6	75.8	7.8	735.6	29.6	0.0	0.4	2.0	44.8	0.2	33.0	0.2
Natural	Open S2	82.4	1.6	80.8	1267.6	351.2	88.0	5.4	800.8	22.6	0.0	0.6	2.2	66.4	0.2	36.8	0.0
	AVG	72.8	0.8	72.0	1211.7	353.4	81.9	6.6	768.2	26.1	0.0	0.5	2.1	55.6	0.2	34.9	0.1
Natural	Veg S1	46.6	2.2	44.4	1425.6	357.2	77.8	3.0	662.8	29.0	0.0	0.4	3.6	83.2	0.2	51.8	0.0
Natural	Veg S2	48.8	2.8	46.0	1368.8	338.4	70.2	6.2	514.0	47.2	0.0	0.4	3.2	143.2	0.2	44.4	0.0
	AVG	47.7	2.5	45.2	1397.2	347.8	74.0	4.6	588.4	38.1	0.0	0.4	3.4	113.2	0.2	48.1	0.0
S. Beaver	Open S1	15.4	1.4	14.0	1449.0	253.2	11.6	8.0	1400.6	125.6	0.0	0.4	2.0	3.4	0.6	46.8	0.0
S. Beaver	Open S2	20.4	2.6	17.8	1537.6	280.6	11.4	5.2	1323.8	126.2	0.0	0.4	2.4	1.8	0.6	48.8	0.0
	AVG	17.9	2.0	15.9	1493.3	266.9	11.5	6.6	1362.2	125.9	0.0	0.4	2.2	2.6	0.6	47.8	0.0
S. Beaver	Veg S1	14.0	1.0	13.0	1762.0	287.6	11.2	2.2	1342.8	98.8	0.0	0.4	1.2	2.2	0.6	40.2	0.0
S. Beaver	Veg S2	17.6	3.0	14.6	1827.6	305.8	11.4	8.0	1428.2	148.6	0.0	0.4	1.6	3.4	0.6	47.6	0.0
	AVG	15.8	2.0	13.8	1794.8	296.7	11.3	5.1	1385.5	123.7	0.0	0.4	1.4	2.8	0.6	43.9	0.0
Mike's	Open S1	6.0	2.8	3.2	1058.0	384.6	38.4	0.2	112.4	15.8	0.0	0.2	4.6	1088.4	0.2	52.2	0.0
Mike's	Open S2	5.6	1.4	4.2	1120.4	466.0	48.2	0.2	78.2	14.8	0.0	0.2	2.0	887.6	0.0	51.6	0.0
	AVG	5.8	2.1	3.7	1089.2	425.3	43.3	0.2	95.3	15.3	0.0	0.2	3.3	988.0	0.1	51.9	0.0
Mike's	Veg S1	6.6	2.4	4.2	1055.2	393.6	61.0	0.4	78.6	14.8	0.0	0.2	2.8	917.2	0.8	39.2	0.0

Mike's	Veg S2	8.4	2.4	6.0	1265.4	424.4	32.6	0.4	87.6	18.0	0.0	0.0	2.6	770.4	0.0	47.4	0.0
	AVG	7.5	2.4	5.1	1160.3	409.0	46.8	0.4	83.1	16.4	0.0	0.1	2.7	843.8	0.4	43.3	0.0
Shallow	Open S1	19.4	3.8	15.6	1786.8	284.4	23.2	1.2	1095.4	77.0	0.0	0.4	1.4	1.8	0.4	44.2	0.0
Shallow	Open S2	14.4	1.6	12.8	1573.8	282.8	16.8	2.4	1031.6	102.4	0.0	0.2	2.2	3.8	0.4	39.2	0.0
	AVG	16.9	2.7	14.2	1680.3	283.6	20.0	1.8	1063.5	89.7	0.0	0.3	1.8	2.8	0.4	41.7	0.0
Shallow	Veg S1	10.4	0.0	10.4	1808.8	307.0	13.2	2.0	974.2	88.4	0.0	0.2	1.8	2.4	0.4	43.8	0.0
Shallow	Veg S2	14.6	2.6	12.0	1890.4	324.0	18.2	1.0	970.6	99.2	0.0	0.2	2.2	1.8	0.4	45.2	0.0
	AVG	12.5	1.3	11.2	1849.6	315.5	15.7	1.5	972.4	93.8	0.0	0.2	2.0	2.1	0.4	44.5	0.0
TP9	Open S1	5.0	1.0	4.0	1196.6	376.8	39.4	0.2	329.8	46.4	0.0	0.2	2.6	80.0	0.2	51.2	0.0
TP9	Open S2	9.2	3.0	6.2	1169.8	337.2	37.8	0.4	315.4	43.0	0.0	0.2	2.8	45.2	0.0	46.4	0.0
	AVG	7.1	2.0	5.1	1183.2	357.0	38.6	0.3	322.6	44.7	0.0	0.2	2.7	62.6	0.1	48.8	0.0
TP9	Veg S1	4.6	0.0	4.6	1157.0	310.6	25.4	0.6	287.2	22.6	0.0	0.2	3.2	61.0	0.2	51.2	0.0
TP9	Veg S2	6.6	0.0	6.6	1204.4	335.8	27.6	0.4	375.0	37.0	0.0	0.2	2.6	27.0	0.2	43.4	0.0
	AVG	5.6	0.0	5.6	1180.7	323.2	26.5	0.5	331.1	29.8	0.0	0.2	2.9	44.0	0.2	47.3	0.0
Jan's	Veg S1	8.2	1.0	7.2	1161.0	530.2	39.6	0.4	31.8	4.6	0.2	0.6	1.0	1394.8	0.0	15.4	0.0
Jan's	Veg S2	19.0	3.0	16.0	1408.6	653.8	38.2	0.4	14.8	7.4	0.2	1.2	1.0	1369.2	0.0	16.8	0.0
	AVG	13.6	2.0	11.6	1284.8	592.0	38.9	0.4	23.3	6.0	0.2	0.9	1.0	1382.0	0.0	16.1	0.0
Jan's	Open S1	18.8	1.4	17.4	1075.8	579.6	38.6	0.2	25.4	5.8	0.0	0.8	1.0	1535.6	0.0	8.2	0.0
Jan's	Open S2	18.6	0.8	17.8	963.4	492.2	36.8	0.2	20.6	5.0	0.0	0.8	0.8	1267.6	0.0	12.8	0.0
	AVG	18.7	1.1	17.6	1019.6	535.9	37.7	0.2	23.0	5.4	0.0	0.8	0.9	1401.6	0.0	10.5	0.0
WA 11	Veg S1	21.4	1.4	20.0	1821.4	530.8	48.2	1.4	196.0	62.0	0.0	0.6	0.8	936.4	0.0	18.0	0.0
WA 11	Veg S2	15.2	0.0	15.2	1768.2	444.2	38.4	0.4	136.4	59.6	0.0	0.4	0.6	690.4	0.0	17.6	0.0
	AVG	18.3	0.7	17.6	1794.8	487.5	43.3	0.9	166.2	60.8	0.0	0.5	0.7	813.4	0.0	17.8	0.0
WA11	Open S1	10.2	0.0	10.2	1869.8	541.2	43.8	0.6	114.2	50.2	0.0	0.6	0.8	1124.0	0.0	21.0	0.0
WA 11	Open S2	15.2	0.2	15.0	2276.0	716.0	65.2	0.8	48.8	27.4	0.0	0.6	1.0	1448.4	0.0	27.0	0.0
	AVG	12.7	0.1	12.6	2072.9	628.6	54.5	0.7	81.5	38.8	0.0	0.6	0.9	1286.2	0.0	24.0	0.0

SWSS Berm	Veg S1	18.8	3.6	15.2	1245.0	392.2	60.4	9.2	229.2	46.8	0.0	0.6	1.6	67.4	0.0	26.0	0.0
SWSS Berm	Veg S2	7.8	0.2	7.6	1135.0	324.8	49.4	6.2	258.4	22.6	0.0	0.6	1.2	97.4	0.0	19.4	0.0
	AVG	13.3	1.9	11.4	1190.0	358.5	54.9	7.7	243.8	34.7	0.0	0.6	1.4	82.4	0.0	22.7	0.0
SWSS Berm	Open S1	13.4	2.0	11.4	852.2	266.4	43.0	5.0	392.6	45.6	0.0	0.4	2.2	234.6	0.0	19.0	0.0
SWSS Berm	Open S2	8.4	0.0	8.4	909.8	267.4	44.0	5.4	418.2	31.0	0.0	0.4	1.6	62.6	0.0	16.8	0.0
	AVG	10.9	1.0	9.9	881.0	266.9	43.5	5.2	405.4	38.3	0.0	0.4	1.9	148.6	0.0	17.9	0.0
4mCT	Veg S1	5.2	0.6	4.6	1253.4	706.8	33.2	0.4	79.4	5.2	0.0	0.6	1.2	523.2	0.0	21.6	0.0
4mCT	Veg S2	7.0	0.0	7.0	1120.8	743.8	33.8	0.2	101.4	5.8	0.0	0.4	1.0	712.0	0.0	10.4	0.0
	AVG	6.1	0.3	5.8	1187.1	725.3	33.5	0.3	90.4	5.5	0.0	0.5	1.1	617.6	0.0	16.0	0.0
4mCT	Open S1	8.4	0.0	8.4	1282.2	729.4	44.8	0.2	49.6	4.4	0.0	0.6	0.6	934.2	0.0	24.2	0.0
4mCT	Open S2	9.6	1.4	8.2	1333.6	779.0	49.6	0.4	210.0	7.0	0.0	0.2	2.2	367.6	0.0	24.8	0.0
	AVG	9.0	0.7	8.3	1307.9	754.2	47.2	0.3	129.8	5.7	0.0	0.4	1.4	650.9	0.0	24.5	0.0
SW	Veg S1	23.8	3.4	20.4	1949.4	344.4	27.0	3.6	909.6	92.6	0.0	0.4	1.0	17.6	0.2	11.8	0.0
SW	Veg S2	20.6	4.6	16.0	1762.6	322.6	18.8	3.2	904.0	112.0	0.0	0.4	1.2	11.6	0.2	24.6	0.0
	AVG	22.2	4.0	18.2	1856.0	333.5	22.9	3.4	906.8	102.3	0.0	0.4	1.1	14.6	0.2	18.2	0.0
SW	Open S1	17.0	0.4	16.6	1708.0	305.6	39.2	4.4	977.2	130.4	0.0	0.4	0.0	19.8	0.2	18.4	0.0
SW	Open S2	19.4	1.8	17.6	1897.8	334.4	38.8	3.8	1330.6	145.8	0.0	0.4	1.6	7.0	0.2	26.8	0.0
	AVG	18.2	1.1	17.1	1802.9	320.0	39.0	4.1	1153.9	138.1	0.0	0.4	0.8	13.4	0.2	22.6	0.0
Demo	Veg S1	9.6	0.6	9.0	1323.0	532.0	25.4	0.6	445.4	36.8	0.0	0.6	1.2	264.4	0.2	20.8	0.0
Demo	Veg S2	12.2	2.0	10.2	1528.8	524.0	33.6	1.0	416.4	58.4	0.0	0.4	1.4	88.2	0.2	23.0	0.0
	AVG	10.9	1.3	9.6	1425.9	528.0	29.5	0.8	430.9	47.6	0.0	0.5	1.3	176.3	0.2	21.9	0.0
Demo	Open S1	7.4	4.0	3.4	993.2	566.8	33.6	0.6	433.8	63.0	0.0	0.6	1.8	95.2	0.0	21.6	0.0
Demo	Open S2	9.2	1.4	7.8	1311.8	502.8	24.6	0.4	702.0	48.4	0.0	0.6	2.0	330.8	0.0	23.2	0.0
	AVG	8.3	2.7	5.6	1152.5	534.8	29.1	0.5	567.9	55.7	0.0	0.6	1.9	213.0	0.0	22.4	0.0

Seepage	Veg S1	16.6	1.0	15.6	929.0	362.2	28.8	1.0	428.6	23.2	0.0	0.4	0.6	283.8	0.0	6.0	0.0
Seepage	Veg S2	15.0	2.0	13.0	908.6	379.2	33.0	2.0	526.4	20.2	0.0	0.6	0.6	51.0	0.2	12.2	0.0
	AVG	15.8	1.5	14.3	918.8	370.7	30.9	1.5	477.5	21.7	0.0	0.5	0.6	167.4	0.1	9.1	0.0
Seepage	Open S1	24.8	3.2	21.6	1122.8	383.2	44.8	3.2	837.0	29.8	0.0	0.4	1.4	67.2	0.2	23.4	0.0
Seepage	Open S2	18.8	1.8	17.0	910.2	352.8	42.6	1.0	438.8	25.0	0.0	0.4	1.0	77.2	0.0	15.6	0.0
	AVG	21.8	2.5	19.3	1016.5	368.0	43.7	2.1	637.9	27.4	0.0	0.4	1.2	72.2	0.1	19.5	0.0
PP	Veg S1	22.6	1.6	21.0	1739.0	437.2	11.8	2.0	521.2	37.6	0.0	0.6	0.2	424.4	0.0	14.6	0.0
PP	Veg S2	10.4	3.0	7.4	1420.6	381.8	10.6	0.8	339.4	24.0	0.0	0.6	0.0	923.6	0.0	10.2	0.0
	AVG	16.5	2.3	14.2	1579.8	409.5	11.2	1.4	430.3	30.8	0.0	0.6	0.1	674.0	0.0	12.4	0.0
PP	Open S1	13.4	0.8	12.6	1448.6	403.8	15.0	3.8	333.6	66.6	0.0	0.4	0.4	396.0	0.0	12.2	0.0
PP	Open S2	16.8	1.8	15.0	1732.6	445.0	19.4	7.2	248.6	154.6	0.0	0.4	0.4	284.8	0.0	14.6	0.0
	AVG	15.1	1.3	13.8	1590.6	424.4	17.2	5.5	291.1	110.6	0.0	0.4	0.4	340.4	0.0	13.4	0.0
S. Bis. Dit	Veg S1	7.6	0.6	7.0	1271.2	473.0	37.6	0.6	240.4	18.0	0.0	0.4	0.4	1391.4	0.0	14.4	0.0
S. Bis. Dit	Veg S2	7.8	0.0	7.8	1293.2	511.0	43.4	0.8	347.4	24.2	0.0	0.6	0.8	985.2	0.2	17.6	0.0
	AVG	7.7	0.3	7.4	1282.2	492.0	40.5	0.7	293.9	21.1	0.0	0.5	0.6	1188.3	0.1	16.0	0.0
S. Bis. Dit	Open S1	14.8	1.2	13.6	1457.2	531.0	42.6	1.6	302.2	22.6	0.0	0.2	1.4	900.2	0.0	20.8	0.0
S. Bis. Dit	Open S2	8.0	0.4	7.6	1399.2	509.2	48.8	1.2	430.2	34.8	0.0	0.4	0.4	475.8	0.0	15.4	0.0
	AVG	11.4	0.8	10.6	1428.2	520.1	45.7	1.4	366.2	28.7	0.0	0.3	0.9	688.0	0.0	18.1	0.0
ETB	Veg S1	10.0	0.2	9.8	1160.2	317.4	30.0	0.4	564.8	61.2	0.0	1.0	0.2	198.0	0.4	1.2	0.0
ETB	Veg S2	8.2	0.6	7.6	909.4	227.4	19.8	0.4	666.0	64.8	0.0	1.2	0.4	323.0	0.4	2.4	0.0
	AVG	9.1	0.4	8.7	1034.8	272.4	24.9	0.4	615.4	63.0	0.0	1.1	0.3	260.5	0.4	1.8	0.0
ETB	Open S1	10.0	0.6	9.4	1079.4	295.4	40.4	0.2	320.0	165.0	0.2	1.4	0.2	230.4	0.4	4.6	0.0
ETB	Open S2	14.6	1.4	13.2	883.4	275.4	40.4	0.2	599.2	134.8	0.2	1.6	0.4	381.8	0.6	3.6	0.0
	AVG	12.3	1.0	11.3	981.4	285.4	40.4	0.2	459.6	149.9	0.2	1.5	0.3	306.1	0.5	4.1	0.0

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CHAPTER IV:
GENERAL DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

Overview

The goal of this study was to quantify sediment oxygen demand (SOD) in both natural and constructed wetlands in the oil sands region of North-eastern Alberta. Some constructed wetlands contained oil sands process material (OSPM), allowing determination of the effect of this residual material on the sediment oxygen demand. We also determined the proportion of this demand that could be ascribed to biological processes as opposed to chemical reactions. Additionally, we wanted to determine if the observed trends in SOD rates across wetland classes could explain the limited number of submergent macrophytes that become established in OSPM-affected wetlands. Phosphorus concentration was measured as a potential pathway linking SOD to submergent macrophyte growth.

Findings suggest that,

1) Sediment oxygen demand, was nearly twice as high in OSPM-affected wetlands, but this difference was not significantly different among wetland classes.
2) Chemical sediment oxygen demand dominates over biological sediment oxygen demand and was responsible for most (at least 85%) of the oxygen depletion observed.
3) Among the wetland classes, biological sediment oxygen demand was significantly higher in natural wetlands than in the other three classes.
4) Process unaffected wetlands had four times more submergent macrophyte biomass (dry weight) than process-affected wetlands.
5) Concentrations of phosphates and other ions in sediments suggest that the mechanism for phosphorus binding does not exist in these systems, as no relationship was found between the levels of phosphorus and ammonium in these wetlands. As a result, phosphorus is not likely limiting submergent plant growth in oil sands process-affected wetlands.

Limitations and Concerns

The largest issue concerning this project was the large amount of variability among replicates within each wetland class. Sediment oxygen demand was nearly twice has high in OSPM-affected wetlands, yet the difference was not statistically significant. Sampling more wetlands as well as finding covariates that account for some of the among-wetland variation could help to reduce the variability and would increase the power of the comparison. These wetlands are unquestionably unique systems, and classifying them into certain categories is difficult. We categorized the wetlands based on the presence or absence of oil sands process material and whether they were constructed or not. Wetlands grouped in the same class varied tremendously in the water chemistry, age, and even the substrate used during reclamation. For instance, OSPW-affected wetlands contain water that has been affected by the mining process but does not infer anything about the sediment type, which may have consisted of clay or peat; and if this has an impact on the sediment oxygen demand or submergent macrophyte growth, then the variability within the class would be drastically increased.

Another issue that will draw criticism is the fact that we had to classify ammonium oxidation under chemical sediment oxygen demand. Ammonium is oxidized within sediments by nitrifying bacteria and perhaps should therefore be considered partially a biological process rather than completely a chemical process (Fisher *et al.* 1952, Belser 1979, Batzer & Sharitz 2006). However, we did not distinguish between this biologically-driven form of oxygen demand, and the chemical process that occurs when ammonium diffusing from the substrate becomes oxidized in the surface water. This project was novel in attempting to quantify the BSOD and CSOD components in the field. However, without being able to interfere with biological processes in the wetlands, we had to classify the oxygen used in nitrification entirely as CSOD. To the extent that microbial nitrification is a biological process we are overestimating the CSOD and underestimating the true BSOD in these systems by an unknown amount. This could account for the marked dominance of chemical demand over its biological counterpart. Bringing sediment back to the lab setting could allow one to separate the components using certain chemical inhibitors of biological processes (Wang 1980, Brewer *et al.*1977, Liu 1973).

Lastly, light sediment oxygen demand chambers did not give reliable results and so we were unable to assess net oxygen consumption due to the sediments. This could have been the result of the clear polycarbonate dome filtering out part of the light spectrum or of light being blocked out from disturbed sediments settling on the dome or remaining suspended in the water column. In addition, a few of the chambers also tended to sink into sediment, causing the probes to malfunction. Creating SOD chambers as one-piece units with 'stabilizer fins' could help resolve this issue. Rethinking deployment techniques of light chambers to minimize sediment disruption could possibly yield net sediment oxygen demand data.

Conclusions and Recommendations

Although the differences observed were not statistically significant, SOD tended to be greater in OSPM-affected wetlands than in reference or natural wetlands. We found that the chemical constituents characterizing OSPM-affected wetlands have the potential to drive this demand. In our case, the elevated levels of ammonium are the most important factor behind the differences in sediment oxygen demand. Ammonium is one of the major ions in tailings material and it occurred at higher concentrations in OSPM-affected wetlands. Regardless of whether ammonium oxidization is classified as a chemical or biological contributor to sediment oxygen demand, ammonium oxidation increases the oxygen consumption rates in these wetlands. Greater oxygen demand ultimately reduces oxygen availability to the biota and therefore increases the stress they are under (Dauer *et al.* 1992). Efforts to reduce the sediment oxygen demand will ultimately ensure that benthic processes and trophic interactions are not altered in these wetlands (Breitburg *et al.* 1997). Presumably, reducing the amount of oxidizable chemical constituents in tailings results in less oxygen consumption on the sediment surface. In turn, this reduces epibenthic hypoxia, which can limit wetland benthic production. Reducing the amounts of ammonium released into the environment should be beneficial to reclamation efforts in the oil sands region. Aerating water entering tailings ponds could help convert ammonium to nitrates before it is reintroduced to the environment. The oxidized, form of these molecules would no longer contribute to sediment oxygen demand. Although SOD may not be as central a measure as originally

postulated for wetland reclamation, the oxidation processes in and on the sediments clearly have a large impact on the food web and overall community establishment.

Phosphorus concentration within the wetland classes was also quite variable. The only statistically significant difference came from bioavailable phosphorus concentrations being higher in natural wetlands, although other marked trends were observed. The absence of a relationship between sediment oxygen demand and the amount of phosphorus in the wetlands or between ammonium and phosphorus concentrations indicates that it is unlikely that a major pathway leading to the binding of phosphorus exists. Consequently, it seems that SOD cannot be used to explain the lack of submergent macrophytes seen in OSPM-affected wetlands. The lack of a difference in phosphorus concentrations among three of the wetland classes also indicates that phosphorus is not the limiting factor for plant growth in OSPM-affected wetlands. Reference wetlands had four times more plant biomass than OSPS- and OSPW-affected wetlands, despite phosphorus concentrations being comparable. Accordingly, other factors must be more important in controlling the growth of submergent macrophytes.

Of other candidate factors, components of salinity, especially sodium and sulphate ions most likely play a large part in plant establishment. Sodium is a by-product of the extraction process and may be difficult to contend with (Renault *et al.* 1998). Sulphate on the other hand is most partially derived from the addition of gypsum in tailings in order to help the solids settle at a quicker rate (Matthews *et al.* 2002). The development of techniques that no longer require consolidated tailings production would result in reduced sulphate concentration and ultimately the salinity. Such alternative methods of dewatering mine tailings are already being used (J. Hornung, Suncor Energy Inc., pers. comm.). Continued monitoring of receiving wetlands should soon indicate whether the reduced use of gypsum as a flocculant have affected the salinity of these wetlands and their respective submergent macrophyte populations.

Alternative strategies to facilitate macrophyte establishment could include the introduction of halophyte species tolerant to these saline conditions (Renault *et al.* 1998). However, this again is dependent on the type of community that is desired.

Implications

Wetland reclamation in mine lease areas is essential because the postmining landscape must be restored to equivalent function to that of pre-mining conditions (OSWWG 2000). Understanding how wetlands develop and function is vital in guiding reconstruction. The CFRAW initiative addresses this need by focusing on carbon flow and food web dynamics (Ciborowski *et al.* 2008). The effect of high SOD on wetland biota can include altered trophic structure (Breitburg *et al.* 1997) leading to reduced benthic invertebrate and microbe colonization rates and abundance. Gardner Costa (2010) found that microbial abundance was reduced in the sediments of OSPM-affected wetlands below a certain depth (anoxic layers) relative to the sediments of natural wetlands, reflecting a decreased rate of respiration. Because of this, decomposition of organic matter will slow (del Giorgio 2005, Thormann *et al.* 1999), and this could promote the net accumulation of benthic carbon. On the other hand, wetlands that contain few submergent macrophytes will have a reduced rate of organic matter accumulation on the sediment surface. Because macrophytes are a key contributor of carbon in natural wetlands, their presence is vital in determining whether these wetlands ultimately become carbon sinks or sources (Craft & Casey 2000, Bridgham *et al.* 2006). These wetlands have the potential to lack the carbon stores of natural wetlands and therefore are most likely heterotrophic and releasing carbon (del Giorgio 2005, Bridgham *et al.* 2006). However, the combination of slow decomposition rates and few submergent macrophytes for potential carbon accrual makes OSPM-affected wetlands good candidates for organic matter amendments, by which to support primary and secondary consumers and their production (Brett & Goldman 1997). As far as SOD rates are concerned, their impact on submergent macrophyte community seems to be minimal. In addition, the somewhat similar rates of oxygen demand between the wetland classes suggest that OSPM material has less of an impact on oxygen consumption than initially thought. This is not to say that these wetlands can be deemed reclaimed but rather that SOD rates are in accordance with natural wetlands. Our initial postulate of SOD affecting submergent macrophyte growth is in fact not the case. As a result, the flow chart presented in Chapter I needs to be altered. Although CSOD is still the most dominant factor affecting overall sediment oxygen demand rates, no indirect link between SOD and nutrient concentrations could be

demonstrated. Rather, salinity may be limiting the growth of submergent macrophytes (Figure 4.1).

This work not only pertains to reclaimed wetlands in the oil sands region, but can be applied to any body of water. Sediment oxygen demand rates have the potential to affect certain biochemical processes by altering the concentrations of dissolved oxygen. In shallow wetlands, the SOD is a large part of oxygen depletion but can be constantly aerated from the atmosphere. Increased sediment oxygen demand in lakes will undoubtedly contribute to anoxic conditions. As a result, SOD is a critical process in any water body. By understanding the impacts that it has on these natural systems, this knowledge can be applied to constructed wetlands and lakes in any region. Studying SOD contributions to ecosystem processes will not only allow one to understand complex aquatic process in natural systems but can be used to construct wetlands that mimic these processes as close as possible.

Wetland age must also be taken into consideration. Macrophyte colonization and organic matter accumulation are undoubtedly age-dependent (Hossler & Bouchard 2010). Our study wetlands, which are fairly young, may not have had enough time to fully develop a substantial macrophyte community. The age and structure of sediment layers is the most likely reason for the differences observed between the constructed wetlands (all 30 years old or less) and natural ones (potentially centuries old).

Future Work

This research was the first of its kind to address sediment oxygen demand in the oil sands region by evaluating both biological and chemical components. Although working *in situ* was expected to give realistic estimates, field assessment was difficult and gave variable results; complementary SOD trials could be conducted in the lab. This would permit use of chemical inhibitors on the sediment that would allow one to quantify the true chemical sediment oxygen demand in these systems. In addition, conducting trials under controlled laboratory conditions would help reduce environmental variability. A broad array of sediments could be studied under controlled conditions with the results being directly indicative of sediment process rather than other factors in the wetland. Net SOD measurements in the field could be obtained by modifying (enlarging) the chamber

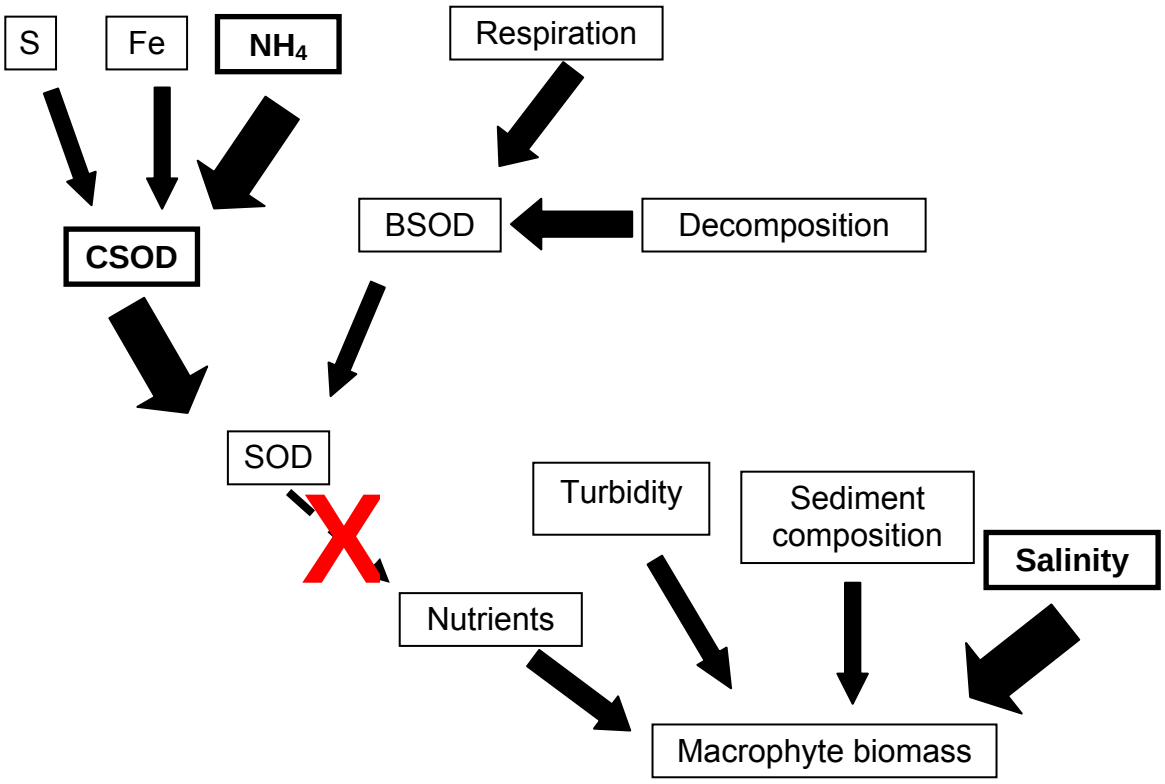

Figure 4.1 Changes in flow chart following the study. Although ammonium is still the largest contributing factor to CSOD, and CSOD is still the largest component of SOD, no link was found between SOD and the concentrations in nutrients. In addition, the concentrations of nutrients could not be used to explain the biomass of submergent macrophytes and so the most important limiting factor shifts to salinity.

design and deployment method. Other studies should especially focus on relating salinity levels to macrophyte growth. Categorizing wetlands according to salinity gradients as well as by oil sands process materials would permit one to determine whether this factor is the major cause of the reduced macrophyte population seen in OSPM-affected wetlands.

This thesis has laid the groundwork for future studies of sediment oxygen demand. By understanding how the sediments interact with both the overlying water and macrophytes, a greater deal of understanding can be achieved into the underlying biochemical processes. Oil sands companies have options in selecting a substrate that produces a wetland that will develop into a sustainable ecosystem. Understanding the processes occurring in and around the sediment are key aspects of the reclamation process. Substrates clearly influence oxygen consumption, which ultimately leads to complicated ecosystem community dynamics. Knowledge of sediment processes should be incorporated into the reclamation process by selecting a substrate material that will foster submergent macrophytes establishment and provide the foundation of a healthy and functioning food web. This study will help to assess the policy of reclamation strategies on the oil sands lease sites, and by contributing to other studies underway in the CFRAW project, will lead to a better understanding of carbon dynamic and food web structure in both natural and constructed wetlands.

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