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Spatial and temporal variation in sediment-associated microbial respiration in oil sands mine-affected wetlands of north-eastern Alberta, Canada.

by

Jesse M. Gardner Costa

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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APPROVED BY:

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_____
, Advisor

_____
, Chair of Defense

Declaration of Co-Authorship/Previous Publication

I hereby declare that this thesis incorporates material that is result of joint research, as follows: This thesis incorporates the outcome of a joint research undertaken in collaboration with Carsten Slama under the supervision of Dr. Jan Ciborowski. The collaboration is covered in Chapter 3 of the thesis. Carsten and I designed, sampled and wrote about the study detailed in chapter 3. In all cases, the key ideas, primary contributions, experimental designs, data analysis and interpretation, were equally divided by the co-authors.

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 my 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

We measured whether carbon loss in the form of sediment-associated microbial respiration differed between unvegetated sediments of recently constructed oil sands process-affected (OSPM) and reference wetlands. Constituents of OSPM-wetlands (increased salinity, conductivity) were expected to influence respiration, increasing gas (methane and carbon dioxide) flux and sediment oxygen demands (SOD) compared to reference wetlands. However, OSPM-wetlands released 1/10th the methane of reference wetland sediments but did not differ in CO_2 ebulition. Sediment oxygen demand (SOD) rates were twofold higher in OSPM than reference wetlands; chemical SOD exceeded biological SOD for both wetland classes (~90% of total SOD). OSPM-wetland sediments, likely have less microbial activity and more chemical oxidation than reference wetlands. Carbon accrual is necessary for reclaiming Alberta boreal wetlands. Low microbial activity may promote carbon sequestration within OSPM-wetlands but high chemical SOD may limit available oxygen for benthos respiration.

For Satan,
hopefully well worth the price.

For my parents,
for taking it all in stride.

Acknowledgements

Since this is a page of acknowledgements, I would first like to acknowledge that most people in their right mind would not read a thesis for the hell of it. That said, one of the few sections that one may peruse is this one, and as such I will try to either: 1) Thank as many people as I can fit and/or 2) Be entertaining whenever possible. There are so many people to thank that I will have to forgo their personal contributions. Besides, it would be inadequate to address their significance to my research and personal life in a 3 page acknowledgement. I will say that at some point all of these people had a hand in helping me with this project. I am aware that the immensity of this list may appear to dilute the contributions of these people; however, I hope to illustrate the large amount of time and work invested into this project (as well as any collaboration along the way) and that the efforts of the individuals mentioned below are anything but trivial.

It has been said it takes a village to raise a child. Over the course of my Master's I've found it takes a community to raise a researcher (coincidently I consider myself a man-child). So many people have been instrumental in the brainstorming, planning, execution, and analysis of this project that I must acknowledge everyone involved to minimize the risk of taking credit where the credit is not mine.

I would like to start with industry representatives, I would not have been able to perform my studies without: Wayne Tedder, Christine Daly (Suncor), Clara Qualizza, Terry Van Meer, Nadia Loubiri, Chris Beierling, Tara Penner, Lori Cyprien, Mike MacKinnon (Syncrude), Dr. Lee Foote (University of Alberta) and last but not least Dr. Jon Hornung (Petro-Canada, now Suncor). Highlights go to Christine, Chris and Lori as they've managed equipment, logistics and so much more for the entirety of my Master's.

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Although I've blabbered as long as I have now, I'll spare a few words for Joshua P. Martin. My "partner in crime", Josh and I started our program in 2007 as rock-star MSc. students taking the department by storm. Whether we've left our mark or not, only time will tell. Nevertheless, the time we've spent learning and mentoring each other or arguing, managing field crews and slacking off together are no doubt memorable; I consider myself lucky to have become your friend (Josh, you've no doubt noticed the dedication at the top and now owe me $20).

Last but not least, I would like to thank my family and friends (and pets), a motley crew (some of them anyways) from whom I draw strength and inspiration. I would

elaborate but I'll save it for something they may actually read (by no means do I intend offence; rather, a compliment to their good taste and well developed sense of self preservation). You know who you are, thank you for all that I am and hope to be…

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List of terms, abbreviations

There are a number of acronyms throughout this thesis, many of which are industry-specific. Here is a quick reference list:

OSPM = Oil sands process materials
MFT = Mature fine tailings (settled tailings material)
CT = Consolidated tailings (settled tailings material with gypsum to decrease water content and increase structural integrity compared to MFT)
SOD = Sediment Oxygen Demand
BSOD = Biological Sediment Oxygen Demand
CSOD = Chemical Sediment Oxygen Demand
ATP = Adenosine tri-phosphate (An energy molecule)
LOI = Loss on ignition (Using a muffle furnace to burn carbon from a sample calculating differences in weight between pre and post-burn)
PRS = Plant root simulators (membranes inserted into the sediment to absorb bio-available nutrients)

Chapter 1: General Introduction

The goal of this project was to study sediment-associated microbial respiration to characterize and quantify carbon flow in the substrates of vegetated and non-vegetated areas of constructed wetlands in the oil sands lease area of north-eastern Alberta. An additional objective was to determine the effects of oil sands mine process material (wastewater and tailings; OSPM) on microbial production and respiration.

1.1 Wetlands: structure and function

The definition of a 'wetland' is debated because it covers a broad range of physical and chemical characteristics. Wetlands are recognized as environmental quality indicators (Batzer & Sharitz 2006) and as such wetlands have been defined by North American governments to ensure the protection and maintenance of wetland systems. According to Canadian authorities,

> "a wetland is land where the water table is at, near or above the surface or which is saturated for a long enough period to promote such features as wet-altered soils and water tolerant vegetation. Wetlands include organic wetlands or "peatlands," and mineral wetlands or mineral soil areas that are influenced by excess water but produce little or no peat."
>
> (Federal Policy on Wetland Conservation: http://www.qc.ec.gc.ca/csl/fich/fich001_001_e.html

According the U.S. Fish and Wildlife Service (USFWS),

> "Wetlands are lands transitional between terrestrial and aquatic systems where the water table is usually at or near the surface or the land is covered by shallow water. Wetlands must have one or more of the following three attributes: 1) at least periodically, the land supports predominantly hydrophytes; 2) the substrate is predominately undrained hydric soil; 3) the substrate is non-soil and is saturated with water or covered by shallow water at some time during the growing season of each year."
>
> (p 1 Batzer & Sharitz 2006)

Conversion of natural land for agricultural uses, industrialization and increasing urban sprawl results in the loss of wetlands. With clear definitions, these areas can be characterized and studied, as well, policy can be created to protect and restore wetland systems.

Wetlands make up less than 10% of the biosphere (Kladec & Knight 1996) and are a central hub of interaction for organisms living within and around them (Zedler 2005). Wetlands are typically productive (Zedler 2005, Kladec & Knight 1996), supporting a high diversity of both transient and resident species ranging from bacteria to large mammals (Zedler 2005). Wetlands provide crucial ecological services from a number of perspectives (Batzer & Sharitz 2006). They control flooding events, regulate groundwater recharge/discharge and modify water quality, thereby affecting local community composition (Kladec & Knight 1996, Oil Sands Wetland Working Group (OSWWG) 2000). From cultural and economic standpoints wetlands support revered or economically useful organisms, which attract tourists, provide subsistence for aboriginal communities, and support the local economic community (Kladec & Knight 1996, OSWWG 2000). Wetlands are also crucial for nutrient cycling - the biogeochemical transfer of carbon, nitrogen, phosphorus etc. within a drainage basin (OSWWG 2000, Batzer & Sharitz 2006). The extent of cycling depends on a number of factors, determined by the wetland's location.

1.2 Oil sands region boreal wetlands

A unique opportunity to study carbon flow within wetlands comes from the Athabasca oil sands region near Fort McMurray, in north-eastern Alberta. This area lies within the boreal forest/peatlands, of which 20-40% is comprised of wetlands (OSWWG 2000, Zoltai & Vitt 1995). These wetlands are known as carbon sinks- carbon accumulating wetlands. Carbon accumulation occurs in a wetland when its production exceeds the rate of decomposition of organic matter. Thorman *et al.* (1999) found that production: decomposition ratios were negatively correlated with water depth, pH and Ca^{2+} concentrations. They speculated that high quality litter or high nutrient levels result in greater microbial biomass which promotes decomposition and reduces net carbon accumulation. As well, shallow water is more readily fully aerated, facilitating aerobic

respiration, which is more efficient than anaerobic respiration processes, leading to carbon loss. Shallow, alkaline, aerated, nutrient rich wetlands were less likely to accumulate carbon; these characteristics are associated more with fens than bogs (Zoltai & Vitt 1995). Thorman *et al.* (1999) found that bogs typically store more carbon than fens or mashes.

Climate also controls carbon accumulation. A shift (roughly 5,000 years ago in the present-day boreal area) from a warmer, dry climate towards a cooler, moist climate in the middle of the Holocene led to a rise in water table, providing the conditions for widespread peatland creation (Kuhry 1997). These peatlands and wetlands formed in topographical depressions where the water table rose. These areas started off as vegetated marshes, (noted as *Typha* dominated, but not necessarily) which led to an accumulation of sediments and organic matter (Kuhry 1997). The water table rose more quickly than organic matter accumulated, thus forming shallow ponds, or wetlands. Peatlands formed from the accumulation of partially decomposed mosses, which replaced *Typha* and other aquatic vegetation. As organic matter accumulated in ponds and wetlands, these systems re-terrestrialized and formed the boreal area we see today (Kuhry 1997). Consequently, both peatlands and wetlands can have millennia of accumulated carbon stored within them.

Bridgham *et al.* 2006 reviewed and estimated the accrual of organic matter for natural (pre-mining) wetlands in North America. They estimated accrual rates of 0.092 ± 0.085 cm/y for peatlands and 0.63 cm/y (range: 0.6-2.6 cm/y, no standard deviation provided) of sediment for wetlands. However, accumulation rates decrease with increasing latitude and are highly variable (Bridgham *et al.* 2006). Wetlands this far north (56 ^{o}N) accumulate organic matter slowly over the course of hundreds or thousands of years (Kuhry 1997) to eventually form thick biogenic layers, creating a substantial carbon pool (Bridgham *et al.* 2006). Their displacement by oil sands mining could potentially result in a large flux of carbon into the atmosphere as greenhouse gases.

1.3 Carbon: Forms and dynamics in wetlands

Wetlands can function as sinks or sources of carbon. A carbon sink is a system (wetland or body of water) that sequesters carbon in organic or inorganic forms (biomass,

detritus vs. dissolved in the water), while a carbon source is a system (wetland) that releases carbon (in our case: CH_4 and CO_2) into the atmosphere or out of the system (Bubier 1995, Girard 2005). A wetland may serve both as a sink or source of carbon, although their description as such typically refers to the net effect; a carbon source releases more carbon than it stores, and *vice versa* with carbon sinks. Typically, natural northern wetlands are known as CO_2 sinks and CH_4 sources (Bridgham *et al.* 2006).

In carbon's inorganic forms, carbon dioxide and methane move readily into and out of a wetland. These gases are produced biologically and are typically stored where biological activity is highest. In wetlands, sediments are often more productive than overlying waters (Batzer & Sharitz 2006) because gases are trapped within the sediments and are either stored or recycled by sediment microbial (bacterial and algae) communities. Carbon dioxide is readily available for uptake by plants in various forms; it may be dissolved in wetland water or pore water as gas or as bicarbonate (Blumenberg *et al* 2006; the formation of bicarbonate is outlined briefly in the discussion of Chapter 2). Methane is relatively insoluble compared to CO_2; consequently, most of the methane produced rises to the surface and diffuses into the atmosphere (Blumenberg *et al* 2006, Ding *et al* 2005).

Carbon dioxide and methane are produced from the metabolic pathways of organisms living not only within wetlands, but in a variety of ecosystems worldwide (del Giorgio & Williams 2005). These metabolic processes are collectively known as **respiration**: *the metabolic catalysis of organic molecules to produce energy*. Gaseous end products can be measured (in volume of gas produced per unit of biomass over time) and related to metabolic activity (del Giorgio & Williams 2005). Respiring carbon is a commonality shared by many organisms; however, methane is almost exclusively produced by microbes.

1.4 Microbial processes in wetlands

Microbes (micro-organisms) are a fundamental component in any ecosystem, regulating oxidation of organic matter and ultimately nutrient flow through a system (Batzer & Sharitz 2006, Legendre 1995, Ogrinc 2002). Microbes include viruses, bacteria, algae, protozoa, archaea, and fungi (Batzer & Sharitz 2006); throughout this

thesis the term 'microbes' will refer to bacteria and archaea. Microbes are thought to be the most abundant organisms and taxonomically diverse group in wetlands (Batzer & Sharitz 2006). High rates of biological activity in wetlands indicate relatively quick cycling of nutrients, which is predominantly achieved by microbial breakdown (Kladec & Knight 1996).

Within wetlands, gas flux is often measured within sediments (del Giorgio & Williams 2005, Batzer & Sharitz 2006, Bubier 1995). Throughout the literature it appears there is an implicit understanding that carbon flow is most important and abundant within the sediments, largely regulated by microbes. This assumption is valid for wetlands (particularly our boreal study wetlands) because: 1) overlying wetland water is usually shallow (from a few cm to 2 m depth), the volume of which may be less than the volume of sediments. These overlying waters are usually unproductive; wetland water is clear from a lack of photosynthetic production, which allows epibenthic production by biofilms to occur because light can penetrate to the bottom. 2) Organic carbon serves as an energy source for organisms that accumulate in the sediments; one would expect most biological activity where energy sources are abundant. 3) Within wetland sediments, there are a number of terminal electron acceptors (oxygen, iron, nitrate, sulphate, carbon dioxide) exploited by microbes (Batzer & Sharitz 2006). Microbes can use these acceptors when oxygen is depleted (typical of sediments). Methane is produced almost exclusively by microbes and only under anaerobic conditions; therefore any study of methane flux in wetlands is almost always limited to sediment microbial communities. 4) Microbes are found in densities unrivalled by any other organism in a wetland, with estimates in sediments around $10^{10}/cm^3$ (Batzer & Sharitz 2006). These are the justifications for focusing on carbon flux in wetland sediments.

Within sediments, carbon may be respired under two broad conditions: aerobic (presence of oxygen) and anaerobic (absence of oxygen). Under aerobic conditions carbon is respired as carbon dioxide by metabolizing carbon molecules as an energy source:

E.g., $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$ (Madigan *et al.* 2002)

Most organisms respire aerobically including mammals, invertebrates and some microbial groups. The above equation shows the oxidization of a low molecular weight carbon molecule by microbes (specifically methanotrophic bacteria).

In anaerobic conditions typical of sediments in wetlands and limited mostly to bacteria, methanogenic bacteria predominantly utilize, but are not limited to H_2 and CO_2 or acetate (seen in the example below as acetic acid) to produce methane or methane and carbon dioxide, respectively (Le Mer & Roger 2001):

E.g. $C_2H_4O_2 \rightarrow CH_4 + CO_2$ (Le Mer & Roger 2001)

Wetland sediments are not limited to methane production. .In fact this is one of the least effective ways to obtain energy, but methane release is directly related to carbon use and emissions within a wetland (Bridgham *et al.* 2006).

Respiration provides a measurable endpoint of energy used by an organism and so marked changes in respiration are expected to be an indicator of stress:

> "a state produced by an environmental or other factor which extends the adaptive responses of an animal beyond normal range *or* (emphasis added) which disturbs the normal functioning to such an extent that the chances of survival are significantly reduced"
>
> (Lugo 1978 cited in Fox 1993 p. 723)

Increased stress on an organism requires that energy be allocated to maintaining bodily functions that would otherwise be devoted to growth or reproduction. Thus, a stressed organism would respire (i.e., convert carbon biomass to CO_2) as much as or more than an unstressed individual of the same biomass.

In contrast, one would expect less stressed individuals to ultimately achieve more biomass or greater reproductive output, or if biomass was equal between the two individuals, an unstressed individual would respire less carbon dioxide or methane. Since microbes are expected to cycle a large fraction of carbon within the sediments of wetlands, measuring microbial respiration and changes in biomass (productivity) would serve as an indicator of wetland condition and function (source or sink of carbon).

1.5 Oil sands extraction

The oil sands region is so aptly named for its large subsurface deposits of oil sands; oxidized crude oil (termed bitumen) mixed with sand (del Rio *et al* 2006). Oil sands are open-pit mined and then refined to produce a crude oil used in a variety of everyday applications. The Athabasca oil sands constitute 25% of Canada's petroleum supply (del Rio *et al* 2006). They occur in deposits beneath a shallow (<80 m deep), fine-particle layer intermixed with sodic clay from Cretaceous marine, river and estuarine deposition (FTFC 1995). This layer is removed to expose the oil sands and stored for later use in landscape reclamation. The removal of these top layers results in the displacement of the biogenic layer, including wetlands and any other habitat (e.g. bogs, fens, meadows, forests) in the area. Oil sands are transported to refineries on the lease site by truck. There, bitumen is separated from the oil sands using Clark's extraction method using alkylated hot water (80°C) (FTFC 1995). Newer methods are now or will soon be extracting bitumen with lower water temperatures, thus reducing energy input and thermal pollution (Nadia Loubirri, Syncrude Canada, pers. comm.)

Once bitumen has been extracted from the oil sands, it is refined into lighter (shorter chain) hydrocarbons (FTFC 1995). The mixture of residual materials composed of mine process water, clay, and sand is known as tailings and must be stored to prevent release any of these materials into the environment. The components, termed herein as Oil Sands Process Water (OSPW) or Oil Sands Process Materials (OSPM; sediments), contain elevated concentrations of salts, naphthenic acids, ammonia, sulphate, and polyaromatic hydrocarbons (FTFC 1995, Peters *et al* 2007). Exposure to high concentrations of these materials can have negative consequences on the local flora and fauna (Ciborowski *et al.* 2006).

1.6 Implications for reclamation

Elevated salinity in oil sands process material-affected wetlands is expected to reduce freshwater wetland productivity at all or most trophic levels in the wetland. Crowe *et al.* (2001) (reported in Baker 2007), found that salinity of process-affected waters creates an osmotic stress for plants, which can lead to decreased photosynthesis.

The same may hold true for other organisms such as microbes, but little work has been done to evaluate these effects (Baldwin *et al* 2006).

High concentrations of sulphate promote the establishment of sulphur reducing bacteria, which may suppress activity of methanogenic bacteria; reducing methane outputs from wetlands (Blumenberg *et al* 2006, Dowrick *et al* 2006).

Ammonia in high concentrations is toxic to most biota (Hickey & Vickers 1994, Britto *et al* 2007), but can be oxidized by microbes (nitrifiers) in the water column (Vymazal 2007). Oxidation of ammonia can consume large amounts of dissolved oxygen (1:4 ratio of (mg) nitrate produced: (mg) oxygen consumed) and bicarbonate (1:8 ratio of (mg) nitrate produced: (mg) bicarbonate consumed), lowering oxygen levels, and limiting certain organisms from thriving in usually oxic conditions (Vymazal 2007).

If the constituents of constructed OSPM and OSPW negatively affect organisms living in a wetland, we would expect effects to be seen in their carbon assimilation efficiency. Within an organism we would expect more energy to be allocated to maintenance to cope with elevated concentrations of salt, ammonia, etc. It is expected that the microbial communities of wetlands subject to high salinity will exhibit greater bacterial respiration per unit biomass than wetlands unaffected by mine processing.

Under the Alberta Environmental Protection and Enhancement Act (AEPEA) the oil sands companies are prohibited from releasing any of the process materials into the environment uncontained or untreated (OSWWG 2000). They are obligated to re-establish "a diversity and abundance of wildlife habitat types and qualities consistent with pre-disturbance levels and restore the lease site back to original functionality" (p. 10, OSWWG 2000). The oil sands industry partners, Syncrude Ltd, Suncor Energy, Canadian Natural, Shell Canada, Imperial Oil, Total, and Petro-Canada are now collaborating with researchers to assess the efficacy of their reclamation methodologies and demonstration projects. This presents an opportunity to study ecosystem processes, including microbial respiration in newly formed and established wetlands varying in chemical and species composition. The specific goal of this thesis was to characterize and quantify carbon flow via sediment respiration in vegetated and non-vegetated areas of wetlands in north-eastern Alberta. Additionally I evaluated the effects of oil sands process material (OSPM, OSPW) on microbial production and respiration.

In collaboration with colleagues at several universities and the oil sands companies, my research will contribute to the assessment of the extent to which food web compartments and energy flow of constructed systems reflect their natural counterparts. This thesis is a component of the Carbon Dynamics, Food-web structure and Reclamation in Athabasca oil sands Wetlands (CFRAW) research group, which includes 5 universities and dozens of researchers. The projects within this research group include these research themes:

> "1) Carbon Dynamics: How are different forms of biomass (carbon sources) incorporated into the food web as constructed wetlands age?
>
> 2) The influences of oil sands process materials (OSPM) on biological community development and the ultimate productivity of fish and wildlife.
>
> 3) Predicting successional changes in constructed wetlands towards a reference condition, and recommending reclamation strategies that can speed up the process."
>
> (CFRAW website http://web2.uwindsor.ca/cfraw/index.htm)

We categorize wetlands based on their age, water/sediment source (using mine tailings or unaffected materials) and initial organic amendment (poor = no organic amendment, high = varying amounts of organic matter, often stockpiled peat from pre-mining peatlands) (figure 1.1). The CFRAW matrix was used as a template by which to select study wetlands so we may evaluate and contrast the effects of age classes, tailings materials, and organic amendment on any number of components within a wetland food-web. By measuring respiration in oil sands wetlands, I quantified the effects of using mine-derived materials in reclamation (OSPM) on wetland microbial community processes. This information adds to the growing pool of carbon dynamics research that enables us to understand wetland energy flow processes and ultimately help recommend strategies that will mitigate the effects of oil sands mining.

1.7 Overview and postulates of the thesis

In Chapter 2 I described the composition and proportion of wetland gas flux and report flux values of carbon dioxide ($\mu g/m^2$ d, including partial pressures of carbon dioxide (μatm)) and methane ($\mu g/m^2$ d) primarily from unvegetated, permanently inundated sediments of constructed (OSPM-affected, and reference) and natural wetlands for the summers of 2008 and 2009. I contrasted flux rates among wetland classes with wetland age (old – 8 years + and young - ≤ 7 years), sampling trial (spring vs. summer) and wetland zone (vegetated – emergent vegetation; or unvegetated – submerged or no vegetation). Sampling trial and sampling zone were compared in 2008. In 2009, studies were focused in unvegetated sediments so values could be used for sediment oxygen consumption estimates (chapter 3).

1) We expected, that gas flux would be higher in OSPM-affected wetlands than reference wetlands, as they would use more energy (and respire more methane or carbon dioxide) to cope with any negative effects associated with the constituents of oil sands tailings. Daly (2007) found bacterioplankton biomass to be two to three times greater in water of older OSPM-affected than in younger OSPM-affected and reference wetlands. However, this difference was not statistically significant. She also found that organic amendment had no effect on flux rates within these wetlands. Assuming equal bacterial biomass, we expected to observe lower carbon dioxide flux rates in reference than in OSPM-affected wetlands. Due to the higher concentrations of sulphate (~100-400 ppm) associated with OSPM-affected wetlands;

2) we expected methanogenesis to be inhibited in OSPM-affected wetlands and thus higher methane flux in reference wetlands.

3) Between sampling trials (2008 only) we expected flux rates for methane and carbon dioxide to be lower in the spring than in the summer due to colder water temperatures and thus lower rates of biological activity.

4) For wetland zone (2008 data only) we expected unvegetated sediments to release more gas than vegetated sediments, due to the potential gas transport of emergent

vegetation as well as the potential for plant roots to channelize sediments and oxygenate anoxic sediments, disrupting ideal conditions for methanogenic bacteria.

In Chapter 3 we measured 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 reclaimed wetlands (containing those materials). The objectives were to determine the sediment oxygen demand (SOD - rate of dissolved oxygen consumption at the water-sediment interface (Murphy & Hicks 1986)) for 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). We determined whether chemical or biological oxygen-consuming processes dominate within the study wetlands and the effect of oil sands process materials (OSPM) on oxygen demands within wetland sediments.

1) We expected chemical SOD (CSOD) to be higher in OSPM-affected wetlands than in reference wetlands; likely due to the increased chemical oxidation of ammonia, whose concentrations are elevated in OSPM-affected wetlands.

2) We expected the biological SOD (BSOD) to be lower in OSPM-affected than in reference wetlands. We observed lower gas flux in OSPM-affected than reference wetlands (Chapter 2) indicating less microbial activity, potentially limiting the oxygen available for biological consumption at the substrate level. Elevated salinities and conductivities (characteristic of OSPM-affected) were expected to further limit BSOD.

3) We 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 we expected higher CSOD in OSPM-affected wetland and therefore a higher overall SOD in OSPM-affected than in reference wetlands.

In Chapter 4, I estimated microbial sediment biomass using an ATP assay as a proxy specific to bacteria. I sampled collected sediment cores to a depth of 14 cm from the study wetlands and measured ATP concentration, particle size distribution, loss on

ignition carbon content, 1.0, 5.1 and 10.2 cm from the surface of each sediment core.

1) Since our wetlands fall under 'older' classification and Daly (2007) found higher abundances of heterotrophic bacterioplankton in 'older' OSPM-affected than in 'older' reference wetlands, likely due to water enriched carbon (from naphthenic acids > 20 mg/L) , I expected to find greater biomass in the sediments of OSPM-affected wetlands than in sediments of reference study wetlands as these sediments were also likely enriched with naphthenic acids and residual bitumen.

2) I expected to detect more bacterial biomass within the top layer (1-2 cm) of wetland sediments than deeper sediments. Microbial biomass tends to be higher in soils that contain greater organic content and higher concentrations of nutrients (carbon and nitrogen - Inubushi & Acquaye 2004). Microbial biomass decreases with increasing soil depth from aerobic surface layers to deeper anaerobic zones (Inubushi & Acquaye 2004). Furthermore, organic matter and nutrients accumulate at the sediment surface (Batzer & Sharitz 2006); fewer nutrients occur at greater depths, and consequently less microbial biomass is expected to occur there. Our study wetlands typically have shallow organic layers (1-5 cm), with clays or oil sands tailings below, reflecting the short periods of time since their construction. If microbial biomass is limited by the availability of nutrients and by soil type (loose organic layer vs. impermeable clay layer), then I would expect to find less microbial biomass in deeper sediments than in the surface sediment layer.

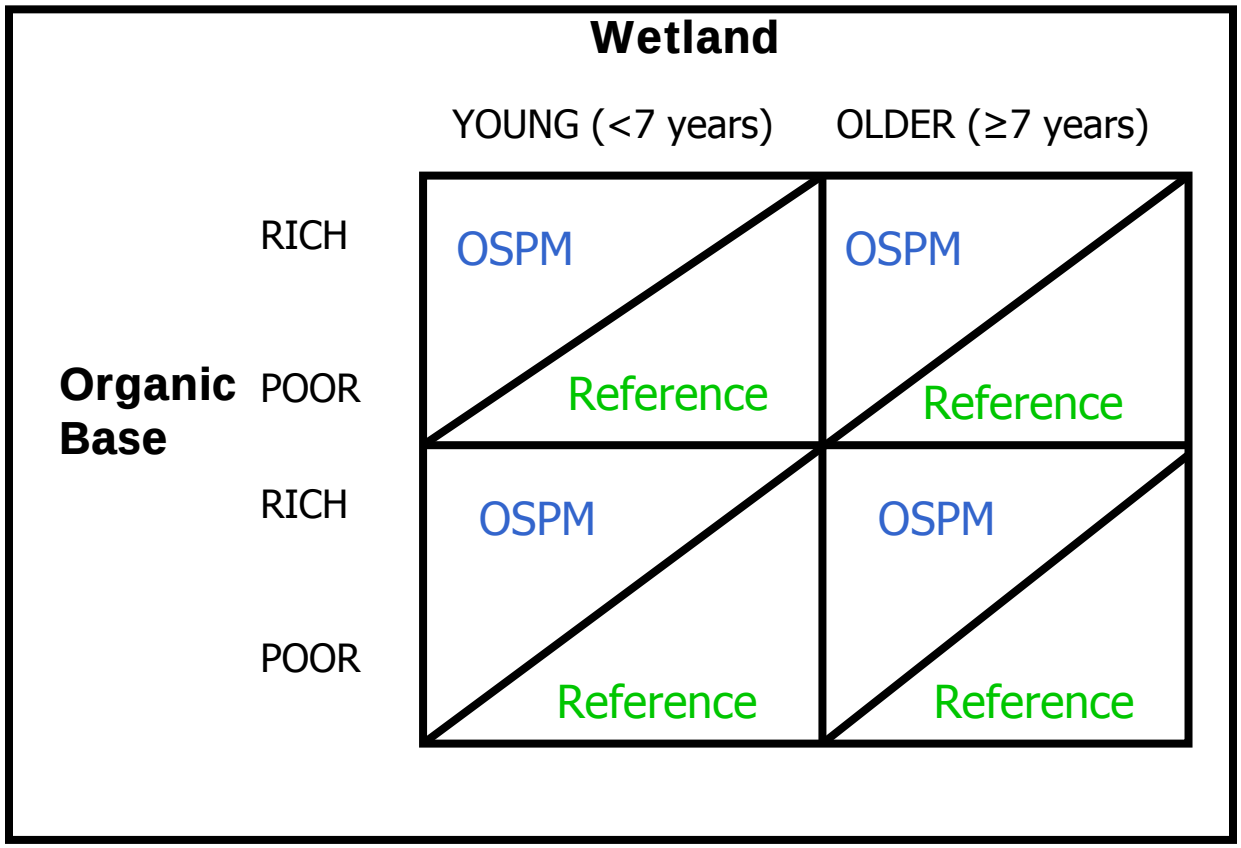

Figure 1.1. The CFRAW wetland matrix. This chart classifies wetlands based on: Age class (young ≥ 7 years, older > 7 years, based on Leonhardt 2003), mine material status (OSPM-affected – created using process affected water or sediment, or reference (created naturally or constructed, using fresh water and unaffected sediments) and organic amendment (poor = no organic amendment, rich = varying amounts of organic matter, often stockpiled peat from pre-mining peatlands). There are 60+ wetlands in the available CFRAW wetland inventory. Individual characteristics of the 11 wetlands studied in this thesis can be found in the methods of chapter 2.

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Chapter 2: Spatial variation of wetland sediment gas flux in constructed wetlands of north-eastern Alberta

2.1 Expectations

In this chapter I described the composition and proportion of wetland gas flux and report flux values of carbon dioxide (μg/m^2 d, including partial pressures of carbon dioxide (μatm)) and methane (μg/m^2 d) primarily from unvegetated, permanently inundated sediments of constructed (OSPM-affected, and reference) and natural wetlands for the summers of 2008 and 2009. I contrasted flux rates among wetland classes with wetland age (old – 8 years + and young - ≤ 7 years), sampling trial (spring vs. summer) and wetland zone (vegetated – emergent vegetation; or unvegetated – submerged or no vegetation). Sampling trial and sampling zone were compared in 2008. In 2009, studies were focused in unvegetated sediments so values could be used for sediment oxygen consumption estimates (chapter 3).

1) We expected, that gas flux would be higher in OSPM-affected wetlands than reference wetlands, as they would use more energy (and respire more methane or carbon dioxide) to cope with any negative effects associated with the constituents of oil sands tailings. Daly (2007) found bacterioplankton biomass to be two to three times greater in water of older OSPM-affected than in younger OSPM-affected and reference wetlands. However, this difference was not statistically significant. She also found that organic amendment had no effect on flux rates within these wetlands. Assuming equal bacterial biomass, we expected to observe lower carbon dioxide flux rates in reference than in OSPM-affected wetlands. Due to the higher concentrations of sulphate (~100-400 ppm) associated with OSPM-affected wetlands;

2) We expected methanogenesis to be inhibited in OSPM-affected wetlands and thus higher methane flux in reference wetlands.

3) Between sampling trials (2008 only) we expected flux rates for methane and carbon dioxide to be lower in the spring than in the summer due to colder water temperatures and thus lower rates of biological activity.

4) For wetland zone (2008 data only) we expected unvegetated sediments to release more gas than vegetated sediments, due to the potential gas transport of emergent vegetation as well as the potential for plant roots to channelize sediments and oxygenate anoxic sediments, disrupting ideal conditions for methanogenic bacteria.

2.2 Carbon

Carbon dioxide and methane are metabolic by-products of the biological breakdown of organic molecules to produce energy utilized by organisms living in a variety of ecosystems worldwide (Del Giorgio & Williams 2005). These carbon-based gases provide a common currency that can be expressed across all ecosystems as a function of the volume of gas produced per unit of biomass over time. Respiration has been studied across a wide variety of aquatic and terrestrial systems (Chanton *et al*. 1989, Del Giorgio & Williams 2005, Batzer & Sharitz 2006, Bubier 1995, Chanton *et al.* 1995 and references therein).

Under aerobic conditions carbon is respired as carbon dioxide through the metabolism of carbon-based molecules, to provide energy:

e.g., $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$ (Madigan *et al.* 2002)

The above equation shows the typical oxidization of a low molecular weight carbon molecule by microbes.

Organisms that reside primarily in anaerobic conditions (mostly bacteria, fungi and endoparasitic metazoans) must use alternative pathways. Aquatic sediments are almost always anaerobic, and resident biota are limited mostly to bacteria, which use various respiratory pathways (Madigan *et al.* 2002, Batzer & Sharitz 2006). Methanogenic bacteria predominantly utilize H_2 and either CO_2 to produce methane or acetate to produce methane and carbon dioxide, respectively (Le Mer & Roger 2001): e.g,

$C_2H_4O_2 \rightarrow CH_4 + CO_2$ (Le Mer & Roger 2001)

Wetland microbial sediment-communities are not limited to methane production; this is one of the least efficient ways to obtain energy (CO_2 is not as strong an oxidizing agent as oxygen, oxygen's redox reactions release the most energy), iron, nitrate or sulphate. Energy release is related to differences in reduction potential, the redox reaction of CO_2 /CH_4 has a lower difference in reduction potential than other oxidants paired with their respective reductants. Therefore, reactions producing methane release less energy (Batzer & Sharitz 2006). Despite this, methane release is directly related to carbon use and emissions within a wetland (Bridgham *et al.* 2006).

Because all organisms respire, the collection of ebulliated carbon-based gases from aquatic systems may theoretically permit one to quantify community-based metabolism by

measuring respiratory end products and allow one to compare rates of carbon flow through a food-web (del Giorgio *et al.* 1997). Although all organisms contribute to carbon flux, there is evidence suggesting that microbes are in many cases, the most important contributors (Legendre 1995, Ogrinc 2002, Batzer & Sharitz 2006).

Microbes are abundant in aquatic systems (~ 10^6 cells/mL; especially in wetland sediments; Kladec & Knight 1996) and they contribute significantly to carbon flow (reviewed in Del Giorgio & Williams 2005). They use a diverse range of electron acceptors (oxygen, nitrates, sulphates, iron oxides etc.), have quick turnover rates, and are ubiquitous in nature (Batzer and Sharitz 2006). They are major contributors to carbon flow (breakdown of organic matter, cycling of nutrients) and are most frequently the only group of organisms referred to in wetland respiration studies (Moore *et al.* 2002, Juutinen *et al.* 2003, Blodau *et al.* 2004, Del Giorgio & Williams 2005, Daly 2007, Repo *et al.* 2007, Jaatlen *et al.* 2008 and almost every study referenced in this chapter).

2.3 Respiration in wetlands

Wetlands can function as sinks or sources of carbon. A carbon sink is a system (wetland or body of water in our case) that sequesters carbon as organic/inorganic matter (biomass, detritus, dissolved in the water); a carbon source is a system (wetland) that releases carbon (CH_4 and CO_2) from the system, often into the atmosphere (Bubier 1995, Girard 2005). A wetland may serve both as a sink or source of carbon, although its description as such typically refers to the net effect; a carbon source releases more carbon than it stores, and *vice versa* with carbon sinks. Typically, natural northern wetlands are considered to be CO_2 sinks and CH_4 sources (Bridgham *et al.* 2006).

The exchange of gases between a body of water and the atmosphere is known as gas flux (Chanton *et al.* 1989). Gas flux occurs in one of three ways: diffusion, ebullition, or through transport in the tissues of emergent plants (Chanton 2005). Diffusion is the movement of dissolved molecules from high concentrations to lower, in this case from wetland waters to the atmosphere. Ebullition is the bubbling of gas, from wetland sediments in this case (especially methane due to its low solubility, Tokida *et al.* 2005). Emergent plants conduct gases from the roots through their tissues to their leaves, and eventually the atmosphere.

The wetlands in this study range from being heavily vegetated with emergent plants to supporting almost no vegetation, although emergent vascular vegetation cover is not homogenous in vegetated wetlands; there are areas with no macrophytes whatsoever. We recognize that plants can transport gas from wetland sediments to the atmosphere (discussed in Chanton 2005). This aspect is being quantified in other studies (J. Hornung, Suncor Energy, Inc., F. Mollard, University of Alberta, in prep.). We restricted our study to flux directly from sediments so that we could compare ebullition rates in vegetated and unvegetated sediments of wetlands.

Gas flux from microbial communities has been estimated by several methods. The most common method is to use *in situ* chambers, which capture gases produced in the sediments and water beneath the chambers (Chanton *et al.* 1989, Daly 2007). Often, flux chambers (microcosms) are used to trap gases and then measure gas concentrations or changes in concentration within the headspace (Clair *et al.* 2002, Bubier *et al.* 2003, Daly 2007). Transparent chambers may be used to trap the gases produced by both photosynthetic and heterotrophic processes during daylight hours. Carbon-based respiratory gases are trapped using opaque "dark" chambers, which inhibit photosynthesis – a carbon consuming process (Clair *et al.* 2002, Bubier *et al.* 2003, Daly 2007). This allows for estimates of respiratory gases (methane and carbon dioxide).

Eddy covariance towers are becoming more commonly used to measure regional gas flux – these are gas measuring towers that integrate gas fluxes over large areas (Werner *et al.* 2005, Bonneville *et al.* 2008). Werner *et al.* (2005 and studies referenced therein) used eddy covariance towers (regional scale flux measurements), essentially a tower equipped with infra-red gas analyzers. These towers measure vertical atmospheric turbulence (mixing) combined with infra-red detection of CO_2 and CH_4 to give rates of flux over an area.

Both gas measuring techniques have advantages and disadvantages, depending on the cost and scale of a study. We used microcosms rather than eddy covariance towers because:

1) Covariance towers are much more expensive than static chambers. Chambers are portable, allowing us to assess any wetland we choose; we would need several towers to perform this study.
2) Combined with gas chromatography we can measure a variety of organic and inorganic gases that the covariance tower may not detect, although this study focused on carbon based respiratory gases.

3) Microcosms trap gases right at the sediment surface, permitting estimates of gross release rates of carbon from the sediments. Whereas towers average values over the landscape, microcosms allow one to quantify carbon released only from our study wetlands. The wetland was our unit of replication and the focus of this study.

2.4 Athabasca oil sands wetlands

The quantity of CH_4 and CO_2 released from wetlands is particularly important in newly constructed wetlands in north-eastern Alberta. The Athabasca oil sands area lies within the boreal forest/peatlands, of which 20-40% by area is comprised of wetlands (Oil Sands Wetland Working Group 2000). Bridgham *et al.* 2006 reviewed and estimated the accrual of organic matter for natural (pre-mining) wetlands in North America. They estimated accrual rates of 0.092 ± 0.085 cm/y for peatlands and 0.63 cm/y (range: 0.6-2.6, no standard deviation provided) of sediment for wetlands. However, accumulation rates decrease with increasing latitude and are highly variable (Bridgham *et al.* 2006). Boreal wetlands accumulate organic matter slowly over the course of hundreds or thousands of years to eventually form thick biogenic layers, creating a substantial carbon pool (Bridgham *et al.* 2006).

Organic matter potentially accumulates faster under anoxic than aerobic conditions (Bridgham *et al.* 2006). Decomposition is slowed because oxygen is absent and only less energetic oxidants are available (Batzer & Sharitz 2006). Carbon gas flux has been shown to increase when anoxic organic matter (seen in wetlands) is exposed to oxic conditions (Batzer & Sharitz 2006). Therefore, the displacement of wetland sediments and their subsequent exposure to oxic conditions (via stockpiling) could potentially result in a large flux of carbon respired into the atmosphere.

2.5 Implications for wetlands

Elevated salinity in oil sands process material-affected wetlands is expected to reduce freshwater wetland productivity at all or most trophic levels in the wetland. Crowe *et al.* (2001; reported in Baker 2007), found that salinity of process-affected waters creates an osmotic stress for plants, which can lead to decreased photosynthesis. The same may hold true for other organisms such as microbes, but little work has been done to evaluate these effects (Baldwin *et al* 2006). High concentrations of sulphate promote the establishment of sulphur reducing bacteria,

which suppress activity of methanogenic bacteria, reducing methane outputs from wetlands (Blumenberg *et al* 2007, Dowrick *et al* 2006).

If the constituents of wetlands constructed using OSPM and OSPW negatively affect organisms living in a wetland we would expect effects to be seen in their efficiency of assimilating carbon. Within an organism, we would expect to observe energy allocated to maintenance to cope with elevated concentrations of salt, ammonia, etc., which would otherwise be used for growth or reproduction. It is expected that the microbial communities of wetlands subject to high salinity will exhibit greater bacterial respiration per unit biomass than wetlands unaffected by mine processing.

The role of gas flux, and ultimately the microbes that produce these gases may be crucial to the establishment of a rich biogenic layer in newly constructed wetlands. The rate at which microbes metabolize organic matter is manifested in the gas flux in the wetlands. Thus, high rates of metabolic activity or flux may indicate impeded rates of establishment of an organic layer, preventing peatland formation.

Methods

2.6 Study sites

The study wetlands were located in and near Syncrude Canada Ltd. and Suncor Energy, Inc. lease areas in north-eastern Alberta (Figure 2.1). Three categories of wetlands were studied: OSPM-affected, reference, and natural.

OSPM-affected wetlands are constructed wetlands (formed in the mined landscape and containing mine tailings materials (OSPM: solids and water). These wetlands exhibit elevated salinities and naphthenic acid concentrations and may contain trace quantities of bitumen (FTFC 1995).

Reference wetlands are either naturally formed or constructed wetlands found on mined sites unexposed to OSPM (solids or water). They do not contain mine-derived sediments or water. However, wetlands that have formed in areas with sodic substrate may have salinities that approach those found in OSPM-affected wetlands.

Natural Wetlands are naturally formed, unaffected by OSPM and are located in areas that have not been mined or cleared of vegetation – relatively undisturbed areas. These were used to

test whether reference wetlands, whose age and successional status were similar to those of OSPM-affected wetlands, differ in flux and production from their undisturbed, unconstructed, older counterparts.

Ten wetlands (4 OSPM (4-m CT had 2 zones that were treated separately - 'peat zone' and 'no peat zone'), 4 reference, 2 natural wetlands) were studied (Table 2.1). The chosen wetlands fit within the CFRAW matrix (chapter 1), allowing one to test for differences among wetlands differing in age class, initial organic content, and status (OSPM or reference or natural). They have been studied extensively and compared to other wetlands in the area. Each wetland has been mapped (area, bathymetry) and is subject to complementary, ongoing studies of food web processes at higher trophic levels (Ciborowski *et al.*2006). Those data will be used to relate carbon flow to this work.

2.7 Gas flux microcosms

Construction: Microcosms were built using black 5-cm inner diameter x 30 cm long PVC piping (figure 2.2) attached to a 10-cm ID coupling to form the base, which enclosed a surface area of 400 cm^2. The microcosm caps were built using inverted 5-cm diameter polyethylene funnels glued to a machine-cut 5-cm diameter PVC pipe cap. The microcosm caps (funnel tips) were fitted with 70 cm of 7.5 mm ID Tygon tubing, which was capped with a red rubber stopper or a 3-way stopcock to prevent gas from escaping the microcosm. (This length of tubing was used to distance the point of sampling from the microcosm to minimize disturbance).

Site Selection Within Wetlands: Microcosms (rockets) were deployed during spring (June 4-19) and summer (July 21 – August 8) 2008. Three to 4 wetlands could be sampled within a 5-d period (Table 2.2 for sampling protocol; Table 2.1 for wetland descriptions). Twelve units (1 unit = 1 pair of microcosms) were placed in each wetland in a stratified random fashion. Each wetland was divided into 6 radial sections. Two pairs of rockets were placed in each section of a

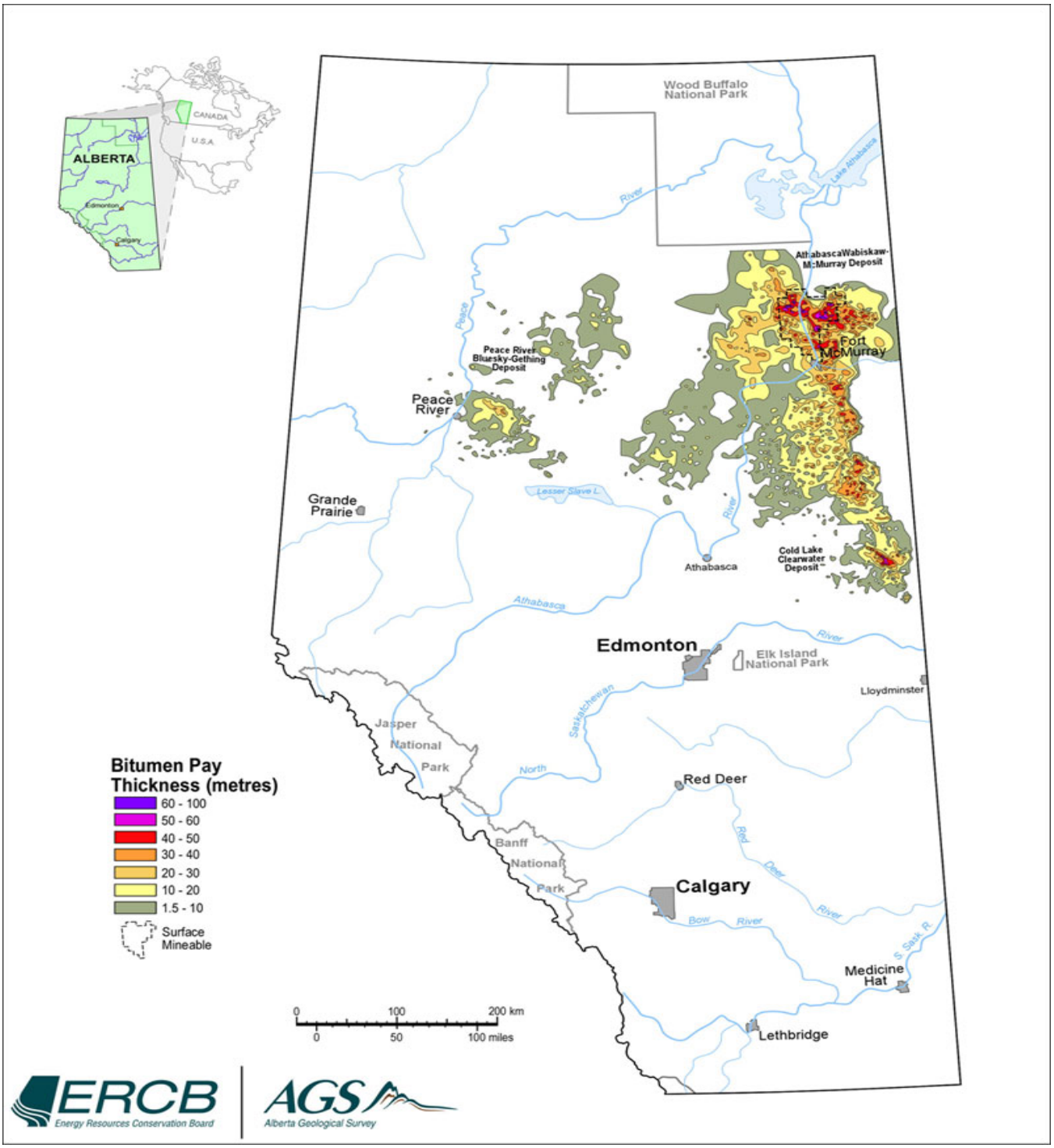


Figure 2.1. Location of Alberta oil sands deposits, which cover over 140,000 km^2. Map taken from: http://www.ags.gov.ab.ca/activities/CBM/alberta_oil_sands.html

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

Wetland Class	Original organic base	Wetland	Age in 2009	Water Source	Sediment type	Lease Area	Additional comments
Natural	Poor	South Beaver	5?	Fresh	Native undisturbed	Syncrude	Thin (< 5 cm) organic top layer, clay underneath
Natural	Rich	South West Sands	~25	Fresh	Native undisturbed	Syncrude	
OSPM	Poor	Test Pond 9	16	Process water	Clay lining	Syncrude	Clay lined – pockets of bitumen present within the clay
OSPM	Poor	Mike's Pond	17	Process water	Clay lining	Syncrude	Clay lined, deeper area (~3 m) has a halocline
OSPM	Rich	Natural Wetland	23	Process water	Post mining sand	Suncor	Sandy bottom with a > 15 cm of Muskeg on top
OSPM	Rich & Poor zones	4-m CT	12	Process water	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.
Reference	Rich	High Sulphate	25	Fresh	Post mining clay/sand mix	Suncor	Lined with sodic overburden, 15 cm of muskeg rests on top
Reference	Rich	Golden Pond	8	Fresh	Post mining clay/sand mix	Syncrude	Lined with Clay/loam (80 cm) with natural organic material on top
Reference	Rich	Peat Pond	8	Fresh	Post mining clay/sand mix	Syncrude	Lined with Clay/loam (80 cm) with peat/mineral mix (> 20 cm) on top
Reference	Poor	CNRL	5	Fresh	Sand	Canadian Natural	Sand lined bottom
Reference	Poor	Shallow Wetland	17	Fresh	Post mining clay/sand mix	Syncrude	1 m clay cap with sodic overburden,

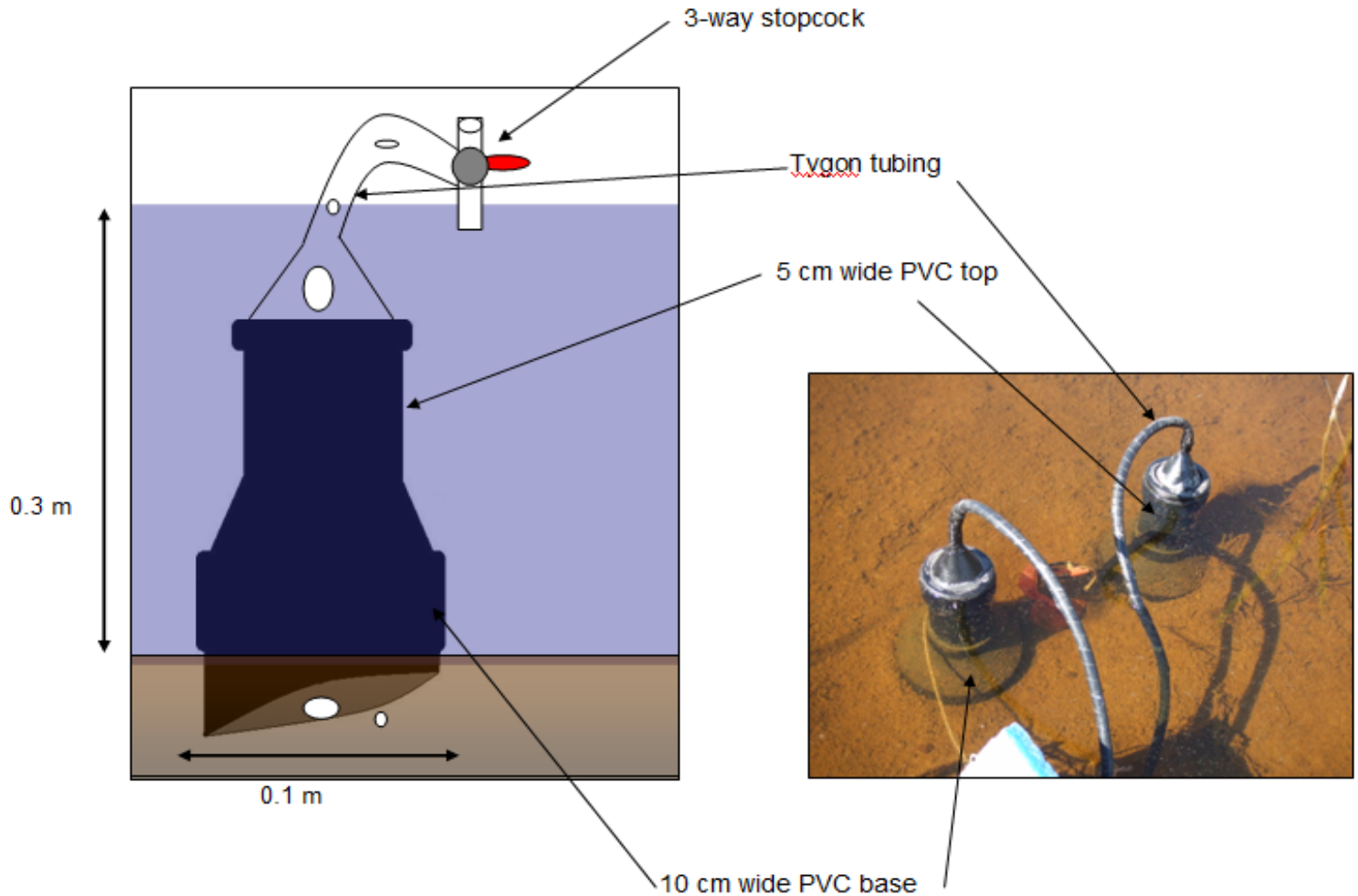


Figure 2.2. Gas flux microcosms; theoretical (left) and actual (right).

Table 2.2. Sampling schedule of summer 2008 field sampling for gas flux in 10 study wetlands.

Sample Component	Day 1	Day 2	Day 3	Day 5
Microcosm placement	- Place rocket base in wetlands, allow sediments to settle - Set out markers for uncapped rockets	- Cap microcosm	- Sample gas @ time = 24 h Run sample through GC	- Sample gas @ t = 72 h Run sample through Gas Chromatograph
Water chemistry measurements	- Measure: Dissolved oxygen, conductivity, salinity, temperature, pH, alkalinity		- Measure: Dissolved oxygen, Conductivity, salinity, temperature, pH - Alkalinity for t=24 h in rockets	- Measure: Dissolved oxygen, Conductivity, salinity, temperature, pH - Alkalinity for t = 72 h in rockets
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	Remove used rocket to be cleaned for next trial
ATP/ production analysis	18 cm core taken at microcosm site, stored @ 4 C^o			

wetland and the depth of water (cm) recorded. One pair was situated in an unvegetated area (containing no emergent vegetation and either little or no submerged vegetation). The other pair was placed in an adjacent vegetated area (supporting emergent and submerged macrophytes). Vegetated locations were situated 1 - 5 m away from paired unvegetated locations. The two members of a pair were situated within 20 cm of each other. One member of a pair was sampled after 24 h. The other member was sampled after 72 h to ensure samples were independent.

Because the study wetlands tended to have marsh-like (rather than fen-like) morphometry (Zoltai & Vitt 1995), the area that was unvegetated was greater than the area supporting emergent vegetation, especially the OSPM-affected wetlands. Typically, emergent vegetation was restricted to the wetland periphery, covering no more than one-tenth (estimated) of the surface area. So any respiration or gas transport by plants that we missed by our rocket design is likely only a small fraction of total gases released from each wetland. Estimates of plant biomass and density within our wetlands are currently under way (M.C. Roy, University of Alberta in prep.) and can be combined with plant respiration estimates (J. Hornung, Suncor Energy, Inc. unpubl.) and gas transport by plants (F. Mollard, University of Alberta, in prep.).

Our gas samples were taken at mean 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 in areas deeper than 1 m because of safety concerns and a lack of equipment needed to place rockets at deeper depths (a boat and/or SCUBA gear). Beyond the occasional beaver run, our study wetlands were typically < 1 m deep and we could walk across and sample the entire wetland. Test Pond 9, Mike's Pond and Peat Pond had inaccessible deep ends that we could not sample. However, flux in shallower areas is likely greater than in deeper waters because shallower waters are usually better oxygenated and are more likely to undergo 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).

Deployment: Each microcosm was inserted 5-8 cm into the sediment and allowed to sit undisturbed for 24 h. Each microcosm was then carefully capped so as not to jostle it or disturb the surrounding sediment. The caps were lubricated with petroleum jelly to ease with capping and seal the base and cap. Each rocket was gas-free at the start of the study. To cap a rocket, the top of the unit (cap and attached tubing) was filled with water and then submerged beneath the surface before being set into place on the shaft. A minority (20/240) of rockets placed in wetlands in 2008 were in areas too shallow to submerge the entire microcosm. In these cases, the units were water-filled by pouring water into the cap and immediately capping the microcosm. It was noted for later analyses if the head of a microcosm was sticking out of the water. However, the exact volumes of headspace were not estimated. These samples were later excluded from analyses. We did not encounter this problem in 2009.

2.8 Gas Collection

2008: Gas was typically sampled in the morning to mid afternoon (0900 - 1500 MDT), although most sampling occurred in the morning hours. Gas was sampled at 24 or 72 h (± 0.5 h) after capping to ensure consistent sampling times. One microcosm at each microcosm site (1 vegetated/unvegetated pair) was sampled at 24 h, and then removed. The other microcosm of the pair was sampled and removed after 72 h, ensuring that microcosms were sampled independently of each other.

Gas samples were taken using a 60-mL airtight syringe fitted with a size 21G hypodermic needle. The needle was inserted into the stopcock or rubber stopper just below the water surface. The plunger of the syringe was slowly pulled to create negative pressure, sucking both gas and water from the tubing and inside the microcosm. The gas collected was immediately placed into an evacuated vial (10 mL) with a 1 cm (thick) rubber septum for later analysis using gas chromatography. Additionally, atmospheric gas samples and wetland water samples (for water chemistry) were taken during 24 and 72-h (1 sample for each wetland for each sampling time) sampling periods for each wetland. Total gas volumes were recorded in the field (using graduated syringes) and averaged for vegetated and unvegetated zones within each wetland. Gas samples were stored in the evacuated vials for no longer than 4 weeks prior to analysis.

2009: We followed the same protocol used in 2008. However, we collected gas at 72 h, but not 24 h and only worked in unvegetated (no emergent vegetation) areas of the wetlands. We also ran samples immediately after returning from a wetland, we kept samples within the sampling syringe (by capping the needle hub) instead of moving gas into an evacuated vial – this minimized transfers and the potential for contamination.

In addition, we collected large funnel or 'stirred sediment' samples; gas taken using an inverted Vinter's funnel (4,000 cm^2 surface area) by stirring sediment and trapping any released bubbles. This method was used to collect large enough volumes of gas to permit us to survey the types of gas released from the sediments as well as determine whether our microcosms allowed atmospheric air to leak in; we immediately trapped and measured gases in the large funnel samples; therefore, there was no chance for atmospheric contamination.

2.9 Water Collection

Water samples were collected contemporaneously with each gas sample inside the 60-mL syringes. Once all the gas had been taken from a microcosm, 50 mL of microcosm water was collected and placed in a 50-mL centrifuge tube. Samples were then analyzed for alkalinity no more than 24 h after collection. All of our study wetlands were slightly basic (pH 7.57-9.55) so we assumed most of the carbon dioxide produced would be buffered by the overlying waters and shift to become bicarbonate (Stumm & Morgan 1996):

$$H_2CO_3 + 2\ H_2O \leftrightharpoons \mathbf{HCO_3^-} + H_3O^+ + H_2O \leftrightharpoons CO_3^{2-} + 2\ H_3O^+$$

At a pH range of 8-10, bicarbonate is the dominant ion, so any dissolved carbon dioxide would become bicarbonate (Stumm & Morgan 1996). We had suspected that differences in CO_2 produced were masked by dissolved bicarbonate so we investigated the alkalinity of the surrounding water to estimate the amount of CO_2 dissolved within a wetland. We measured the microcosm water alkalinity with a precision of ± 5 ppm $CaCO_3$ (= 1.25×10^5 $\mu g/m^2$ d). We compared changes in alkalinity between 24 h to 72 h samples within a wetland; however, because our detection limit was much larger (~ 10^3) than flux values

we were measuring, we could not confidently detect changes in alkalinity among microcosms within a wetland.

Water was collected (1 L of water collected 10 cm below the water's surface) from each wetland at the time of the 24 h sampling period to determine alkalinity and pH in the lab. The water portion of each sample was taken to test alkalinity using the THERMO© Total Alkalinity kit. In addition, water was collected once from each wetland in July and shipped to the Syncrude Canada Ltd. analytical laboratory (Edmonton, AB) for full water chemistry analysis (Syncrude 1995).

2.10 Water chemistry

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 $SO_4^=$) and ammonium (NH_4^+) concentrations were determined by ion chromatography (Dionex Corporation, Sunnyvale, CA, USA Model DI-300 IC). Alkalinity (HCO_3^- and $CO_3^=$) 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 1745 cm^{-1} were measured with a Thermo Instruments (Canada) Inc. Nicolet Model 8700 FT-IR spectrometer.

2.11 Partial pressure of CO_2

The partial pressure of carbon dioxide indicates whether water contains more, less or an equilibrial amount of dissolved CO_2 compared to the atmosphere. It may be used to calculate relative rates of reactions in water, such as weathering (dissolving limestone) but in this study it gives a measure of the likelihood of CO_2 release in the study wetlands. Partial pressures of carbon dioxide are calculated by: concentrations of H+

and HCO_3 measured in moles per litre. $H+$ is given by 10^{-pH} moles/litre, while the bicarbonate concentration is obtained by multiplying the alkalinity (expressed in $CaCO_3$ mg/L) by (16.38×10^6). The logarithm of the pCO_2 is then calculated. If the log pCO_2 is < -3.5, then the partial pressure is lower than one would expect for water in equilibrium with the atmosphere, i.e. there is less CO_2 in solution than one would expect, and one would expect the wetland to act as a CO_2 sink. If it is > - 3.5, the partial pressure is higher than equilibrium with the atmosphere and it is expected that the wetland is a source of CO_2.

2.12 Gas analyses

Gas samples were analyzed using an Agilent 3000A micro gas chromatograph (GC) equipped with Plot Q (10 m x 0.32 mm column, 2 µL injection volume, helium carrier gas, sample column temperature 60 ^oC) and Molsieve (10 m x 0.32 mm column, 2 µL injection volume, helium carrier gas, sample column temperature 100 ^oC) columns, a variable sample injector and a thermal conductivity detector (TCD). The GC was calibrated using a Sulpeco 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; catalogue # A-19792). Total gas volumes were recorded in the field.

We sampled one wetland per day, and analyzed all gas samples taken from a wetland on the day of sampling. 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 within the lab) as a blank. We also measured atmospheric samples taken over each wetland, but these were treated in the same fashion as the microcosm samples.

Concentrations of CO_2, CH_4, O_2 and N_2 were obtained from gas chromatograph (GC) sample elution profiles. The amounts (µg) 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 per m^2 per day (moles/L m^2 day). 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:

Example. 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) = 3.56 x10^{-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:

(Mole CH_4 Sample / Mole CH_4 Std) = (Area CH_4 sample / Area CH_4 std)

Rearranged: Mole CH_4 Sample = Mole CH_4 Std * (Area CH_4 sample / Area CH_4 std)

2.13 Statistical Analyses

Statistical analyses were performed using Statistica version 7.0 (Statsoft, Inc., Tulsa, OK). We wished to test whether gas flux of CO_2 and CH_4 differed among OSPM-affected, reference and natural wetlands with respect to age class (young ≤ 7 years, older > 7 years, based on Leonhardt 2003), trial (late spring vs. summer), and wetland zone (emergent macrophytes vs. no emergent macrophytes). Before analyzing our data, we $Log_{10}(Y+1)$ transformed all of our flux values. For the 2008 data, we had samples for 24 and 72 h, the 72 h samples were divided by 3 (3 days) to get an average 24 h estimate over the 3 days of study – after the conversion all values were and averaged for vegetated and unvegetated zones within each wetland. In 2009 we only worked in unvegetated zones of wetlands at 72 h; all values within a wetland for each zone were averaged and converted to gas released per day per m^2. Our data were analyzed using a randomized

block ANOVA design. Within a wetland, mean gas concentrations from vegetated zones were subtracted from mean concentrations from adjacent unvegetated zones and tested to see whether the difference was significantly different from 0. Significance levels were set at $p<0.05$ (using 2-tailed hypotheses) with wetland as the unit of replication (n= 4 OSPM-affected n= 4 reference, n= 2 natural). We used planned comparisons in 2009 to test whether constructed wetlands differed from natural wetlands and whether reference wetlands differed from OSPM-affected constructed wetlands. We only sampled in unvegetated areas of wetlands and we had 9 study wetlands (n= 4 OSPM-affected, n= 3 reference, n= 2 natural).

To test for differences in gas released between 2008 and 2009 sampling seasons we used a 2 x 3 factorial ANOVA (with each wetland considered a block) to compare the amount of gas released (ug/m^2 d, Log_{10}+1 transformed) for each gas (CH_4, CO_2, N_2 and O_2) with wetland class (natural, reference or OSPM-affected) and sample type (i.e., the source of the sample). The 2008 and 2009 gas samples were compared to: 1) test year to year variability of amount of gas released, 2) estimate atmospheric contamination, if any, in 2008 gas samples. The comparisons were:

1) Atmosphere 2008 vs. Atmosphere 2009 (comparison of atmospheric samples from 2008 and 2009 respectively)
2) Stirred sediment 2009 vs. unvegetated 2009 (gas collected after stirring up unvegetated sediments and trapping gases released compared with gas sampled from microcosms in unvegetated areas left over 72 h to collect gas)
3) Stirred sediment 2009 vs. unvegetated 2008 (the same comparison above, but with gas collected from unvegetated sediments in 2008)
4) Unvegetated sediment 2009 vs. unvegetated 2008 (comparing samples taken from microcosms in unvegetated sediments from 2008 and 2009, respectively.

Results & Discussion

2.14 Water chemistry

There were no consistent differences in salinity and conductivity between reference and OSPM-affected wetlands in 2008 (Table 2.3). Reference and natural wetlands showed a

larger range of conductivities and salinities (256-2,780 µs/cm^2, 0.1-1.4 ppt) than OSPM-affected wetlands (ranges 1,166-2,070 µs/cm^2, 0.9-1.1 ppt). Conductivity and salinity in reference and natural wetlands were bimodal; Shallow, Beaver south, and CNRL were all fresh (<400 µs/cm^2) while Southwest Sands Beaver, High Sulphate and Peat Pond were more saline (≥ 1200 µs/cm^2) and approached (or surpassed- High Sulphate) conductivities in OSPM-affected wetlands. Higher conductivities likely reflect the underlying substrate in which the wetland formed; High Sulphate and Peat Pond were formed on sodic overburden and Southwest Sands Beaver's water level has been dropping since beavers abandoned the pond in 2007, concentrating ions in the water as a result. The pH in OSPM-affected wetlands was typically higher than reference (8-9.55, 7.57-9.32, respectively) although pH in some reference wetlands (CNRL, and Peat Pond) fell within range of the OSPM-affected wetlands. Naphthenic acid concentrations were 10X higher than reference on average in OSPM-affected wetlands, with the exception High Sulphate which had naphthenic concentrations (18 ppm) approaching those of OSPM-affected wetlands. Water temperature (taken at the time of sampling) in all the wetlands ranged from 17-27 ^{o}C. Most wetlands had water temperatures of around 20 ^{o}C. Where dissolved oxygen (DO) was measured, all wetlands were well oxygenated (saturation at 20 ^{o}C is about 9 mg/L D.O. – our range for all wetlands was 7.3-11.7 mg/L). Water chemistry parameters for natural wetlands fell within range of the reference wetlands.

Table 2.3. Summary of selected water chemistry parameters for each study wetland measured during at time of gas sampling period in summer 2008. Temperature, pH, conductivity and dissolved oxygen were all measured in situ. Salinity was not measured in 2008 and was estimated from conductivity values (Lewis 1980) by multiplying conductivity by 0.51 and converting the values to parts per thousand (ppm) Ion and compound concentrations were analyzed from water samples by the Syncrude Analytical Laboratory, Edmonton, AB. The Syncrude-derived values are only a subset of total water chemistry analyses. BDL: below detectable limit. NR: no record available

Wetland Class	Wetland	Temperature °C	pH	Conductivity (µs/cm^2)	YSI Dissolved Oxygen (mg/L)	Salinity (ppt)	SO_4 (mg/L)	Iron (ppm)	NH^{4+} (ppm)	Naphthenic acids (ppm)
OSPM	4 m CT	20.6	8.00	1756	4.9	0.9	499	0.2	0.15	24.9
OSPM	Natural	27.4	9.55	1166	11.7	1.0	239	1.7	0.57	46.5
OSPM	Test Pond 9	21.2	9.38	2070	8.2	1.1	115	0.2	0.33	20.4
Reference	CNRL	21.7	9.32	256	8	0.1	NR	NR	NR	NR
Reference	Shallow	23.1	7.57	360	8.0	0.2	8.7	0.1	<0.01	2.3
Reference	High Sulphate	22.2	7.93	2780	8.2	1.4	1630	BDL	<0.01	18.0
Reference	Peat Pond	19.9	8.23	1200	7.5	0.6	368	BDL	0.12	0.1
Reference	Golden	21.7	8.14	1745	7.0	0.9	783	BDL	0.34	3.7
Natural	South Beaver	19.1	7.57	345	NR	0.2	5.1	0.9	<0.01	3.2
Natural	Southwest Sands Beaver	16.7	8.04	1270	7.3	0.6	386	BDL	0.17	0.9

Water chemistry 2009: There were no consistent differences in salinity and conductivity among natural, reference and OSPM-affected wetlands 2009 (Table 2.4). Water chemistry in 2009 was very similar to measurements taken in 2008. Reference and natural wetlands showed a range of salinities and conductivities (0.2-1.5 ppt, 313-2980 $\mu s/cm^3$, respectively) and so on average these wetlands weren't different from OSPM-affected wetlands. The same spread of salinity seen in 2008 reference wetlands was also present in 2009 reference wetlands. The pH in OSPM-affected wetlands was typically higher than in reference wetlands (7.97-8.87, 7.35-8.52, respectively). Naphthenic acid concentrations were 10X higher on average in OSPM-affected wetlands. The temperature in all the wetlands ranged from 14-24 ^{o}C, slightly lower than 2008. All wetlands were well oxygenated (6.68-12.5 mg/L), with the exception of Beaver South (natural) (3.01 mg/L).

2.15 Gas collection

Gas collected in both stirred sediment and microcosm samples in 2008 and 2009 was composed of nitrogen, oxygen, carbon dioxide and methane gas. Nitrogen was found in the highest proportions (38-83 %), followed by oxygen (6-32 %), methane (0-48 %) and carbon dioxide (0.1-8 %) (Table 2.7). Values reported for microcosm samples are reported per m^2 d to give estimates of flux. A study in estuaries by Chanton *et al.* (1989) showed wetland gases to be primarily made of CH_4 (12-89 %), CO_2 (2-6 %), N_2 (3-84 %) and almost no O_2 (0.3-2.4 %). Higher values of nitrogen and oxygen and lower values of carbon dioxide and methane were seen in vegetated than in unvegetated areas. The presence of oxygen was regarded as a contaminant as the sediments were assumed to be anoxic. The researchers note that oxygen-contaminated samples with atmospheric air would also contaminate nitrogen estimates; however, methane and carbon dioxide values are presumed to be untouched. A study in Minnesota peatlands also showed that gas collected from wetlands was composed of CH_4 31.2 ± 3.2 %, CO_2 6.0 ± 1.1 %, N_2 57.0 ± 3.2 %, O_2 5.8 ± 0.2 % (Williams & Crawford 1984). The presence of oxygen likely indicates that these samples must have had some contamination; however, this is not addressed as the analyses in this chapter only focused on CH_4 and CO_2.

Our values of oxygen and nitrogen are much higher than those reported in Chanton *et al.*1989. Our values are likely contaminated by air at the transfer of a sample from syringe into the GC. We suspect that transfer of a sample from syringe to sampling vial (2008 protocol) introduced air into the sample; by removing this transfer in 2009 oxygen % dropped from ~20 % to 9 %. In some wetlands, particularly vegetated zones of a wetland, the proportion of oxygen reaches 30+ %, suggesting oxygen bubbles from either roots or submerged macrophytes may be trapped within the microcosms. Overall, the general trends for methane and carbon dioxide (discussed below) appear to be the same between 2008 and 2009, suggesting they were largely unaffected by atmospheric contamination.

The amounts of carbon dioxide, nitrogen and oxygen in atmospheric samples did not differ between 2008 and 2009 (factorial ANOVA, $p>0.05$). We did not expect differences but these samples served as controls for our wetland samples to calibrate our GC and look for any signs of air contamination in our samples. Similar levels of oxygen and nitrogen among atmospheric samples and our wetland samples suggested atmospheric contamination, meaning our estimates of oxygen and nitrogen may be unreliable.

Trace amounts of methane were found in some of the atmospheric samples, and the amount of carbon dioxide was lower than concentrations found in wetlands samples.

Table 2.4. Summary of selected water chemistry parameters for each study wetland measured during at time of gas sampling period in summer 2009. Temperature, pH, conductivity and dissolved oxygen were all measured in situ. Ion and compound concentrations were analyzed from water samples by the Syncrude Analytical Laboratory, Edmonton, AB. The Syncrude-derived values are only a subset of total water chemistry analyses. BDL: below detectable limit. NR: no record available

Wetland Class	Wetland	Temperature °C	pH	Conductivity (**µS/cm^2**)	YSI Dissolved Oxygen (mg/L)	YSI Salinity (ppt)	NO_3 (mg/L)	SO_4 (mg/L)	Iron (ppm)	NH^{4+} (ppm)	Naphthenic acids (ppm)	Oxidation Reduction Potential
OSPM	Mike's Pond	16.0	8.45	4550	NR	2.3	BDL	565	BDL	0.137	26	106
OSPM	Natural	17.7	8.85	1090	8.27	0.5	27	134	0.499	1.08	33	87
OSPM	4 m CT	15	7.97	2300	6.68	1	1.4	335	0.04	BDL	30	124
OSPM	Test Pond 9	23.9	8.87	2050	12.08	1	BDL	97.8	BDL	BDL	19	79
Reference	Shallow Wetland	18	7.53	391	7.2	0.2	1.4	6.58	0.036	BDL	<1	113
Reference	Golden	19.9	8.52	1737	12.5	0.9	2.3	825	BDL	BDL	2	99
Reference	High Sulphate	15.2	7.35	2980	NR	1.5	2.2	1650	BDL	0.166	10	124
Natural	South-west sands Beaver	13.8	7.92	1252	9.82	0.6	BDL	437	BDL	BDL	<1	119
Natural	South Beaver	15.9	7.18	313	3.01	0.1	0.47	2.84	0.894	BDL	<1	79

Methane was typically present in wetlands that released methane but not present when atmospheric samples were taken over land, or non-gas producing wetlands. Shallow Wetland (reference) had a large percentage of methane in its atmospheric sample (29%). While taking an atmospheric sample in the wetland we must have captured methane coming out of the wetland, this skewed the average percentage (9%) when combined with the other reference wetlands; these wetlands had less than 1% methane within their samples.

Furthermore, we calculated the coefficient of variation for our calibration gas sample and atmospheric samples - taken in Windsor and Fort McMurray. Repeated measurements of our calibration gases showed high precision (coefficient of variation 3%), while our atmospheric samples were slightly more variable (coefficient of variation 15%). Calibration gases are fed into the gas chromatograph straight from a pressurized gas tank, delivering gas at constant pressure. Hand injection of samples might have caused an increase of variability in gas concentrations. It is difficult to ensure samples are delivered at a constant speed at constant pressure, especially if samples are small (< 10 mL). Switching our protocol in 2009 by leaving rockets in the wetland longer to collect more gas ensured we collected enough sample to run 2-3 times, flushing the column with sample and then averaging concentrations for a single sample. Both 2008 and 2009 show similar patterns in gas flux albeit with higher gas flux in 2009 than 2008, we are confident these patterns reflect true variation and not computer error.

Gas collected in both stirred sediment and microcosm samples in 2008 and 2009 were composed of nitrogen, oxygen, methane and carbon dioxide gas. Nitrogen was found in the highest concentrations (~2000 $\mu g/m^2$ day), followed by oxygen (~600 $\mu g/m^2$ day) and then methane (~200 $\mu g/m^2$ day) when it was detected in a wetland) and finally carbon dioxide (~50 $\mu g/m^2$ day) (Appendix figures 2.1-4). We did not observe any hydrogen sulphide (H_2S), likely because it wasn't in high enough concentration to detect. We suspect H_2S was present at least in trace amounts in some of our samples because: Sulphur-reducing bacteria (SRB) were identified in oil sands wetlands (Sobolewksi 1999), sulphate (the electron acceptor used by SRBs) is present in almost all of our wetlands (Table 2.3-4) and we could occasionally smell the distinctive rotten egg smell of hydrogen sulphide in some of the wetlands.

We compared the concentrations of 'stirred sediment' collections of gas in 2009 with microcosm samples of gas taken from 2008 and 2009. Carbon dioxide release was ~10X higher in natural than in either reference or OSPM-affected wetlands for unvegetated 2009 samples. Carbon dioxide was not apparently different among wetland class for stirred 2009 samples. The relative concentration of carbon dioxide was not significantly higher (7.9 vs. 0.7 vs. 1.7 % for natural, reference, and OSPM, respectively) in 2009 microcosm samples than in stirred sediment samples from 2009 for any wetland class (0.9 vs. 2.6 vs. 0.7 % for natural, reference and OSPM, respectively Table 2.5) (ANOVA, d.f. 11*, $F=0.29$, $p>0.05$). * Note when combining the 2008 and 2009 data, there are 12 unique wetlands, which is why degrees of freedom for these tests are 11.

Despite the large differences in methane between unvegetated 2009 samples (47.8 vs. 1.6 vs. 23.2 % for natural, reference and OSPM, respectively) and stirred sediment samples from 2009 (7.7 vs. 11.8 vs. 2.6 % for natural, reference and OSPM, respectively Table 2.5), these differences were not statistically significant owing to large among-wetland variability (ANOVA, d.f. 11, $F=1.86$, $p>0.05$).

The amount of oxygen varied among wetlands, comprising 5.7-28.5 % of total gas within our samples, vegetated areas of wetlands (2008 data) generally had the most oxygen, while unvegetated sediment samples (2009 data) had the lowest (Appendix figure 2.3). We found that oxygen concentration was significantly lower in unvegetated 2009 samples compared with stirred, unvegetated 2008 and vegetated 2009 samples (ANOVA, d.f. 11, $F= 13.9$, $p<0.01$). In 2008, we sampled at 24 and 72 h after microcosm deployment but only at 72 h in 2009. Our enclosed, opaque chambers would trap oxygen, which would be consumed over time (oxygen consumption rates for sediments are in chapter 3). Our 2008 data averages all gases for the 72 and 24 hour periods, but when looked at separately, the concentration of oxygen found is higher in 24 h samples than 72 h samples. This suggests that some of the oxygen I've measured is not entirely atmospheric contamination and in fact some oxygen may be present around the sediments, perhaps produced by submerged macrophytes.

The amount of nitrogen varied among wetlands comprising 25-83 % of total gas within our samples. Like oxygen, nitrogen was highest in vegetated areas of wetlands (2008 data) while unvegetated sediment samples (2009 data) had the lowest nitrogen

values (Appendix figure 2.4.) We found that nitrogen was significantly lower in unvegetated 2009 samples compared with vegetated 2008 samples, but not significantly different from stirred and unvegetated 2008 samples (ANOVA, d.f. 11, $F= 5.9$, $p<0.05$).

We took stirred sediment samples to probe the wetlands for gas as well as to serve as a check for signs of atmospheric contamination; collecting stirred samples immediately ensured we had collected gas only from the wetland sediments. There was no difference in composition of gases between stirred and microcosm samples; composition we are confident that atmospheric contamination did not occur during gas collection. Had the concentrations of carbon dioxide and methane in microcosm samples been significantly lower than the stirred samples we would have evidence to believe our microcosms leaked gas and air was seeping in. Though it is likely that our samples are contaminated, it appears this contamination did not occur in the field.

There was apparently more carbon dioxide and methane and less nitrogen and oxygen released in 2009 than 2008. The differences between sample types in these comparisons may either reflect true, biological differences or possible contamination/sample loss. We altered our protocol in 2009 because we suspected we may have lost some of the sample gas using storage tubes in 2008. If this is the case, the fluxes of methane and carbon dioxide were underestimated for 2008. Our 2009 protocol has mitigated this potential problem.

Table 2.5. Mean (± S.D.) relative concentrations (%) of carbon dioxide, methane, nitrogen and oxygen found in natural, OSPM-affected and reference wetlands in 2008 and 2009. Sample type refers to how the gas was collected: Atmosphere= atmospheric sample; Stirred sediment = gas samples taken by disturbing the sediment and catching gas bubbles, collecting them immediately; Unvegetated = samples taken from microcosms in unvegetated areas of wetlands as described in the methods. Vegetated = samples taken from microcosms in vegetated areas of wetlands as described in the methods. NR = no record available

Wetland class	Sample type	% CO_2 ± S.D.	n	% CH_4 ± S.D	n	% N_2 ± S.D	n	% O_2 ± S.D.	n
Natural	Atmosphere 08	0.23 ± 0.02	2	0.03 ± 0.04	2	66.83 ± 10.57	2	32.90 ± 10.54	2
OSPM	Atmosphere 08	0.28 ± 0.10	4	1.42 ± 2.82	4	70.32 ± 5.33	4	27.99 ± 4.21	4
Reference	Atmosphere 08	0.31 ± 0.11	4	0.27 ± 0.50	4	83.08 ± 11.79	4	16.35 ± 12.27	4
Natural	Atmosphere 09	NR		NR		NR		NR	
OSPM	Atmosphere 09	0.13 ± 0.00	3	0.00 ± 0.00	3	74.48 ± 4.23	3	25.39 ± 4.23	3
Reference	Atmosphere 09	1.00 ± 1.54	3	9.80 ± 16.96	3	64.70 ± 14.28	3	24.50 ± 4.21	3
Natural	vegetated 08	1.01 ± 0.22	2	2.65 ± 1.00	2	72.99 ± 0.48	2	23.35 ± 1.26	2
OSPM	vegetated 08	0.46 ± 0.12	4	0.58 ± 0.98	4	75.96 ± 2.25	4	22.99 ± 3.16	4
Reference	vegetated 08	0.65 ± 0.50	4	1.59 ± 2.59	4	72.22 ± 4.74	4	25.53 ± 1.82	4
Natural	unvegetated 08	1.98 ± 0.11	2	5.98 ± 2.50	2	69.72 ± 1.64	2	22.33 ± 0.98	2
OSPM	unvegetated 08	0.65 ± 0.36	5	0.98 ± 2.03	5	74.75 ± 1.10	5	23.62 ± 1.01	5
Reference	unvegetated 08	1.57 ± 0.26	2	10.17 ± 9.89	2	74.03 ± 18.47	2	14.23 ± 8.31	2
Natural	unvegetated 09	7.91 ± 5.89	2	47.81 ± 4.66	2	38.58 ± 14.08	2	5.70 ± 3.53	2
OSPM	unvegetated 09	1.71 ± 1.91	2	23.18 ± 11.43	2	68.25 ± 14.62	2	6.86 ± 5.10	2
Reference	unvegetated 09	0.65. ± 1.1	1	1.57 ± 2.73	1	25.00 ± 43.22	1	6.15 ± 0.65	1
Natural	Stirred sediment 09	0.92 ± 0.93	2	7.73 ± 10.78	2	62.87 ± 11.12	2	28.47 ± 0.59	2
OSPM	Stirred sediment 09	0.71 ± 0.89	3	2.57 ± 3.67	3	73.52 ± 0.39	3	23.20 ± 4.94	3
Reference	Stirred sediment 09	2.55 ± 2.91	3	11.85 ± 17.54	3	65.59 ± 11.28	3	20.01 ± 9.19	3

2.16 Volume of gas

The mean volume of gas collected for each wetland class in vegetated and unvegetated zones of wetlands for the summer of 2008 & 2009 (only unvegetated sediments) can be found in Table 2.6. Volumes of gas were used to calculate the concentrations of CH_4, CO_2 in gas samples collected (Table 2.7). Volumes of gas measured in 2008 averaged no more than 10 mL per microcosm/24 h. Volumes recorded in 2009 for reference and natural wetlands were 2-3 times higher than volumes recorded for the same wetland types in 2008. There was high variability of the volumes of gas collected among microcosms within each wetland for both years (coefficients of variation typically ranged from 50-100%), suggesting great spatial heterogeneity of gas production in these wetlands. The higher volumes were observed in 2009 most likely because we sampled microcosms only after 72 h instead of 24 h sampling (we did both in 2008), allowing sediment-derived gas to build up within each microcosm, and reducing the potential relative contribution of residual gas (oxygen) that may have been associated with the sediment surface at the start of each trial.

We normalized volumes and concentrations of gas to 24 h; ebullition of wetland gas is typically variable (reviewed in Segers 1998, Roehm 2005, Chanton 2005) and so leaving microcosms in sediments longer makes it more likely for measurable quantities of gas to bubble up from the sediments. Higher volumes permitted more accurate estimation of gas concentrations because we could run samples 2-3 times, allowing the gas chromatograph to purge the column with sample gas. Gas ebullition, specifically carbon dioxide and methane gas flux was "highly variable" in the OSPM-affected and reference wetlands studied by Daly (2007).

In 2008, there were no significant differences in the overall volume of gas released with respect to wetland class, wetland zone (vegetated vs. unvegetated), trial, or age (randomized block ANOVA, $p>0.05$). In 2009, we only measured gas within unvegetated sediments and found no significant differences among wetland classes (planned comparison ANOVA, d.f. = 7, F= 0.6 for natural vs. constructed (reference +

Table 2.6. Mean (S.D) volume of gas (mL/microcosm/24 h) released from OSPM-affected, reference and natural wetlands in 2008 and 2009. Averages are calculated for "Unvegetated"- gas volumes taken from unvegetated sediments (some submerged macrophytes may have been present), and "Vegetated"- gas volumes taken from zones with emergent vegetation. Trial 1 and 2 denote late spring and mid summer, respectively. In 2009, only one trial was conducted and only within "Unvegetated" zones.

Status	Zone	Trial	Mean± S.D. volume of gas (mL)	n
		2008		
OSPM	Unvegetated	1	5.6 ± 3.9	4
OSPM	Unvegetated	2	2.9 ± 4.3	4
OSPM	Vegetated	1	8.0 ± 6.3	4
OSPM	Vegetated	2	9.7 ± 6.6	4
Reference	Unvegetated	1	5.8 ± 4.4	4
Reference	Unvegetated	2	4.0 ± 4.7	4
Reference	Vegetated	1	3.9 ± 4.7	4
Reference	Vegetated	2	3.2 ± 3.4	4
Natural	Unvegetated	1	2.5 ± 1.5	2
Natural	Unvegetated	2	2.6 ±1.5	2
Natural	Vegetated	1	4.4 ± 2.1	2
Natural	Vegetated	2	3.2 ± 2.6	2
		2009		
OSPM	Unvegetated	1	3.5 ± 1.75	4
Reference	Unvegetated	1	16.3± 17.4	3
Natural	Unvegetated	1	10 ± 13.6	2

Table 2.7. Mean µg /m^2/d ± S.D. concentrations of methane, carbon dioxide, nitrogen and oxygen gas collected after 24 h of microcosm deployment in OSPM-affected, reference and natural wetlands in 2008 and 2009. Averages are calculated for each wetland and wetland zone -"Unvegetated"= gas volumes taken from unvegetated sediments (some submerged macrophytes may have been present). 'Vegetated' = samples taken in areas of a wetland with emergent vegetation. BDL = below detectable limit – less than 3 mL of gas were collected (usually no gas at all) so we could not analyze the sample and it is assumed as zero gas flux. NR denotes no record available – the data is missing

Wetland class	Wetland	Sample type	CH_4 µg /m^2/d ± S.D.	CO_2 µg /m^2/d ± S.D	N_2 µg /m^2/d ± S.D	O_2 µg /m^2/d ± S.D
OSPM	4 m CT	Unvegetated 2009	36.4 ± 45.3	61.9 ± 67.4	NR	271.8 ± 150.3
OSPM	Mike's Pond	Unvegetated 2009	BDL	BDL	BDL	BDL
OSPM	Natural Wetland	Unvegetated 2009	109.6 ± 63.1	22.2 ± 20.5	570.7 ± 380.2	23.6 ± 13.3
OSPM	Test Pond 9	Unvegetated 2009	260.0 ± 97.9	3.0 ± 3.4	481.8 ± 218.1	87.1 ± 28.9
Reference	Golden Pond	Unvegetated 2009	BDL	BDL	BDL	BDL
Reference	High Sulphate	Unvegetated 2009	BDL	BDL	BDL	BDL
Reference	Shallow Wetland	Unvegetated 2009	276.8 ± 261.0	114.3 ± 114.0	4384.9 ± 3234.4	1080.0 ± 1127.3
Natural	Beaver South	Unvegetated 2009	516.6 ± 178.1	43.5 ± 11.3	563.2 ± 173.8	37.2 ± 16.1
Natural	Southwest Sands Beaver	Unvegetated 2009	747.3 ± 797.3	176.5 ± 244.8	418.6 ± 327.8	119.9 ± 73.1
OSPM	4 m CT	Unvegetated 2008	4.4 ± 8.5	41.1 ± 46.9	3691.6 ± 4079.7	1146.5 ± 1272.6
OSPM	4 m CT (peat zone)	Unvegetated 2008	0.9 ± 1.3	15.8 ± 17.5	2428.9 ± 2732.7	787.9 ± 911.0
OSPM	Natural Wetland	Unvegetated 2008	2.1 ± 1.3	13.9 ± 18.4	907.4 ± 433.8	291.2 ± 139.1
OSPM	Test Pond 9	Unvegetated 2008	64.1 ± 46.7	7.9 ± 5.7	1011.7 ± 427.6	304.6 ± 170.6
Reference	Canadian Natural CNRL	Unvegetated 2008	BDL	BDL	BDL	BDL
Reference	High Sulphate	Unvegetated 2009	BDL	BDL	BDL	BDL
Reference	Peat Pond	Unvegetated 2008	184.5 ± 322.7	18.9 ± 23.8	655.6 ± 248.8	216.2 ± 84.0
Reference	Shallow Wetland	Unvegetated 2008	53.6 ± 72.9	23.4 ± 29.3	1471.3 ± 1527.3	141.1 ± 52.6
Natural	Beaver South	Unvegetated 2008	55.1 ± 57.7	24.9 ± 32.1	928.4 ± 576.7	301.5 ± 218.3
Natural	Southwest Sands Beaver	Unvegetated 2008	115.7 ± 168.9	30.7 ± 51.3	1024.0 ± 478.2	323.2 ± 137.1
OSPM	4 m CT	Vegetated 2008	1.0 ± 1.2	37.4 ± 27.4	4316.2 ± 3580.5	1457.7 ± 1190.8
OSPM	4 m CT (peat zone)	Vegetated 2008	1.5 ± 1.8	29.4 ± 30.0	5256.0 ± 4839.9	1719.4 ± 1619.3
OSPM	Natural Wetland	Vegetated 2008	6.2 ± 6.5	9.3 ± 11.4	1828.5 ± 1321.5	584.5 ± 418.6

OSPM	Test Pond 9	Vegetated 2008	71.3 ± 80.1	13.9 ± 20.6	2769.7 ± 3364.6	638.9 ± 623.5
Reference	Canadian Natural CNRL	Vegetated 2008	0.9	4.2	2516.4	844.3
Reference	High Sulphate	Vegetated 2008	0.4 ± 0.2	19.6 ± 15.3	1684.5 ± 1135.1	540.5 ± 368.8
Reference	Peat Pond	Vegetated 2008	21.2 ± 47.9	9.1 ± 7.0	1765.2 ± 1874.7	591.7 ± 670.9
Reference	Shallow Wetland	Vegetated 2008	72.7 ± 81.3	16.6 ± 12.8	873.3 ± 637.0	377.8 ± 418.4
Natural	Beaver South	Vegetated 2008	71.2 ± 78.8	18.2 ± 25.0	1554.8 ± 1314.3	476.1 ± 494.5
Natural	Southwest Sands Beaver	Vegetated 2008	83.7 ± 174.0	50.2 ± 44.4	3126.5 ± 3080.3	1043.2 ± 1115.2

OSPM), F= 0.4 for reference vs. OSPM, $p>0.05$). We could not test for differences between sampling trials (due to equipment failure) and age; all wetlands were considered "old" by our criteria.

Volumes of gas (all gases combined) were on average higher in vegetated areas of wetlands (2008 data) than in unvegetated areas of wetlands (Table 2.6). However, this difference was not significant (randomized block ANOVA, $p>0.05$). We had expected more gas to be released from unvegetated than vegetated sediments because plants transport gas through their roots to the atmosphere (Chanton 2005), making it less likely for gas ebullition to occur in vegetated areas. We did observe more oxygen in vegetated than unvegetated areas of wetlands. This would suggest that a source of oxygen was coming from plants (either produced or trapped and eventually captured in our microcosms. Oxygen would make up a larger fraction of total gas in vegetated areas and would partially explain why we observed higher gas volumes in vegetated sediments but higher concentrations of greenhouse gases in unvegetated areas.

2.17 Carbon dioxide flux

Carbon dioxide flux values for 2008 ranged from 0-50 $\mu g/m^2/d$ of CO_2 for wetland classes in vegetated and unvegetated zones. Southwest sands Beaver (natural, vegetated zone) released the most CO_2, followed by 4-m CT (OSPM, unvegetated zone) with 50 and 41 $\mu g/m^2/d$ CO_2, respectively. CNRL (reference) released the lowest amount of CO_2, 0 and 4 $\mu g/m^2/d$ in the unvegetated and vegetated zones of the wetland, respectively. Carbon dioxide release from the unvegetated sediments of reference wetlands (43-176 $\mu g/m^2/d$) was higher than OSPM-affected (3-62 $\mu g/m^2/d$) wetlands in 2009 (unvegetated sites were not sampled). Southwest sands (natural; oldest wetland in the study) released the most CO_2 while Test Pond 9 (OSPM) released the least CO_2. There was a difference of two orders of magnitude between these two wetlands (Table 2.7). The range of CO_2 release illustrates the high variability among OSPM-affected, reference and natural wetland classes.

We failed to collect any gas Mike's Pond, High Sulphate and Golden Pond and so these were included as zero in the analyses. If ebullition events are episodic and irregular (del Giorgio & Williams 2005), we may have missed gas release events over the

3 days of sampling for each wetland. However, stirred sediment trials also failed to release gas from these wetlands. We never noticed gas ebullition from these wetlands over many sampling events, we are confident that flux values for these wetlands are close to zero.

Carbon dioxide flux within our study wetlands was 2-4 orders of magnitude less than CO_2 flux values reported from other Canadian boreal wetlands (Table 2.9). Our values were also lower (1.7×10^{-6} vs. 1.6×10^{-4} mol C $m^{-2}d^{-1}$) than the flux reported by Daly (2007, Table 2.8) from some of the same wetlands. However, Daly's 2005 study did not directly measure gas volumes. Instead, literature values were used to convert concentrations of gas into mass of carbon released. Overall, carbon dioxide flux was usually lower than methane flux (for 2009 data: 1.7×10^{-6} CO_2 vs. 2.0×10^{-5} CH_4 mol C $m^{-2}d^{-1}$) from all methane-producing wetlands. Carbon dioxide fluxes were generally not different among wetland class, between age classes, between wetland zones or between sampling trials.

The amount of carbon dioxide released in 2008 did not differ among wetland classes, between age classes, between trials or between zones (vegetated and unvegetated). OSPM-affected, reference and natural wetlands were not significantly different from each other (randomized block ANOVA, $p>0.05$).

The partial pressure of carbon dioxide (pCO_2) in 2008 (Table 2.9) was highly variable among wetlands, ranging from 61 to 6665 µatm. Partial pressures of CO_2 for most wetlands in 2008 were saturated compared with the atmosphere at the time of sampling, and were predicted to be sources of carbon (Table 2.9). Two of the OSPM-affected wetlands, Natural Wetland and Test Pond 9 were in approximate equilibrium with the atmosphere and so these wetlands could either be sinks or sources. Natural Wetland and Test Pond 9 released the least amount of CO_2 of wetlands that released measureable quantities of CO_2 (Table 2.7). With the exception of High Sulphate and CNRL, all reference wetlands were predicted to be CO_2 sources. Indeed, measurable quantities of CO_2 were captured in microcosms. High Sulphate was slightly saturated (406 µatm) and expected to be a small source of CO_2 in trial 1. In trial 2, High Sulphate was under-saturated compared with the atmosphere (126 µatm) and expected to be a sink;

Table 2.8 Estimated methane and CO_2 respiration from freshwater wetlands (Mean value with ranges in brackets). Reproduced and modified from Roehm 2005 (Chapter 6 in del Giorgio & Williams 2005, p. 88). 2008 and 2009 values are taken from this study, as well; values are taken from the literature to illustrate the range of flux in wetlands.

Type	**Boreal flux (mmol C $m^{-2}d^{-1}$)**	**Oil sands area flux (mmol C $m^{-2}d^{-1}$)**	**Literature values of northern wetlands (mmol C $m^{-2}d^{-1}$)**	**Temperate flux (mmol C $m^{-2}d^{-1}$)**	**Tropical flux (mmol C $m^{-2}d^{-1}$)**
(a) Methane: Marsh and swamp	0.56 (0.40-6.4)	**2005**: 1.1 [b] **2008**: 3.4 x10^{-4} (5.2 x10^{-5}- 0.012) **2009**: 0.020 (2.3 x10^{-3}-0.047)	(0.069 – 7.5) [c] (2.2–4.2) [d] (3.4-5.7) [e]	6 (0.01-40)	11.80 (2.3-35)
Total (Tmol a^{-1})	0.24[a] (0.10-3.5)			0.01[a] (0.00-0.06)	10.50[a] (1.8-36)
Type	**Boreal flux (mol C $m^{-2}d^{-1}$)**	**Oil sands area flux (mol C $m^{-2}d^{-1}$)**	**Literature values of northern wetlands (mol C $m^{-2}d^{-1}$)**	**Temperate flux (mol C $m^{-2}d^{-1}$)**	**Tropical flux (mol C $m^{-2}d^{-1}$)**
(b) CO_2: Marsh and swamp	0.21 (0.04-0.54)	**2005**: 1.6 x10^{-4} [b] **2008**: 3.1 x10^{-7} (2.2x10^{-9}– 9.3x10^{-7}) **2009**: 1.7 x10^{-6} (6.8x10^{-8}-4.0 x10^{-6})	(0-0.070) [c] (0.0022-0.12) [f] (0.0061-0.0085) [d] (0.020-0.26) [g] (0.031-0.049) [h]	0.21 (0.04-0.54)	0.11 (0.02-0.87)
Total (Tmol a^{-1})	83[a] (9.5-296)			0.31[a] (0.06-0.79)	98[a] (15-889)

[a] These numbers represent the calculated total aerial fluxes (means and ranges) for each wetland type and region. The lower and upper ranges are calculated by multiplying the lowest flux by the lowest aerial coverage and the highest flux by the highest aerial coverage.
[b]= Daly 2007 [c] = Repo *et al.* 2007 [d] = Blodau *et al.* 2004 [e] = Juutinen *et al.* 2003 [f] = Bonneville *et al.* 2008 [g] = Moore *et al.* 2002 [h] Jaatinen *et al.* 2008

Table 2.9. Partial pressures of CO_2 for 2008 data for trials 1 and 2. The last column indicates whether a wetland should be storing or releasing CO_2. When any acid (carbonic) produced is neutralized we expect a potential CO_2 sink; when more acid is produced than can be neutralized we expect a system to be a source of CO_2; and equilibrium indicates the wetland could be either a CO_2 source or a sink. The wetland names and characteristics are described in the methods under 'study site'. NR = no record available

Wetland class	Wetland	Trial	Date	Alkalinity $CaCO_3$ (mol/L)	pH	Salinity (mol/L)	Temp ^oC	pCO_2 (µatm)	log pCO_2	Carbon sink or source
OSPM	Natural	T1	06/06/2008	138	8.66	NR	18.0	392.5	-3.4	source
OSPM	Test Pond 9	T1	18/06/2008	798	9.52	1	17.1	313.3	-3.5	equilibrium
OSPM	4 m CT	T1	11/06/2008	953	8.17	1.1	17.5	8376.0	-2.1	source
OSPM	4 m CT (peat zone)	T1	11/06/2008	953	8.17	1.1	17.5	8376.0	-2.1	source
Reference	High Sulphate	T1	08/06/2008	18	7.76	NR	17.0	406.6	-3.4	source
Reference	Shallow	T1	17/06/2008	153	8.06	0.2	16.1	1732.3	-2.8	source
Reference	CNRL	T1	11/06/2008	23	8.46	0.1	20.3	103.7	-4.0	sink
Reference	Peat Pond	T1	06/06/2008	188	8.31	NR	18.0	1197.0	-3.0	source
Natural	Southwest Sands Beaver	T1	18/06/2008	258	7.91	0.6	17.3	4126.3	-2.4	source
Natural	Beaver South	T1	18/06/2008	138	7.43	0.1	16.7	6665.3	-2.2	source
OSPM	Natural	T2	01/07/2008	349	9.55	1166	27.4	127.8	-3.9	sink
OSPM	Test Pond 9	T2	04/08/2008	652	9.38	NR	21.2	353.3	-3.4	source
OSPM	4 m CT	T2	29/07/2008	551	8.27	NR	24.4	3845.5	-2.4	source
Reference	High Sulphate	T2	23/07/2008	104	9.03	NR	23.0	126.3	-3.9	sink
Reference	Shallow	T2	04/08/2008	199	7.57	NR	23.1	6969.3	-2.2	source
Reference	CNRL	T2	01/07/2008	98	9.32	256	21.7	61.2	-4.2	sink
Reference	Peat Pond	T2	23/07/2008	NR	NR	NR	NR	NR	NR	NR
Natural	Southwest Sands Beaver	T2	04/08/2008	319	8.04	1270	16.7	3780.4	-2.4	source
Natural	Beaver South	T2	31/07/2008	189	7.57	345	19.1	6625.1	-2.2	source

almost no gas was collected from this wetland in either trial. CNRL released no gas in unvegetated areas and only 4 mL in vegetated areas during either trial; it was most likely a carbon sink. Overall, calculated pCO_2 were consistent with our field observations of CO_2 release.

Within unvegetated sediments of our study wetlands in 2009, more carbon dioxide was released in reference (43-176 $\mu g/m^2/d$) than OSPM-affected (3-62 $\mu g/m^2/d$) wetlands. Significantly more carbon dioxide was released in reference than in OSPM-affected wetlands of unvegetated sediments (Figure 2.3; 1-way ANOVA $p>0.05$, $F=5.82$). The pCO_2 in 2009 indicated that all study wetlands were saturated with CO_2 compared to the atmosphere (Table 2.9); the wetlands were expected to be carbon sources.

It is unclear why we saw these differences in 2009 and not 2008; any number of factors may have influenced this. Water depth and temperature for our study wetlands were not significantly different between years. However, wetland areas sampled tended to be deeper and colder in 2009 (2008= 39.3 cm & 21.4 ^oC, 2009= 44.3 cm & 17.4 ^oC) in 2009 (Table 2.3, 2.4 & Appendix Table 2.1.). We sampled a few weeks earlier in 2009 (beginning of June) than in 2008 (middle of June and July), when water temperatures are usually lower and water levels higher earlier in the season. Shallower wetland sediments exposed to air due to drying increase aerobic respiration and release more gas than deeper, anaerobic wetland sediments (Roehm 2005) and warmer waters usually have higher biological activity (Winkler *et al*. 1996, Pringault *et al.* 2007). It is perplexing to observe higher gas flux in 2009 than 2008; we observed high variability within individual wetlands. Perhaps it is not unreasonable to expect high year-year variability. We have not collected enough data to assess how gas flux changes as these wetlands mature. However, the differences paralleled the among-year variation in methane; more gas in 2009 was likely due to our change in methodology to prevent atmospheric contamination. If the differences are truly biological and there were higher levels of biological activity producing more gas in 2009 than 2008, then we would need gas released per unit of biomass. This data as well as biomass data from chapter 4 can be combined with microbial production estimates (change in biomass over time) to give estimates of gas released per unit of biomass.

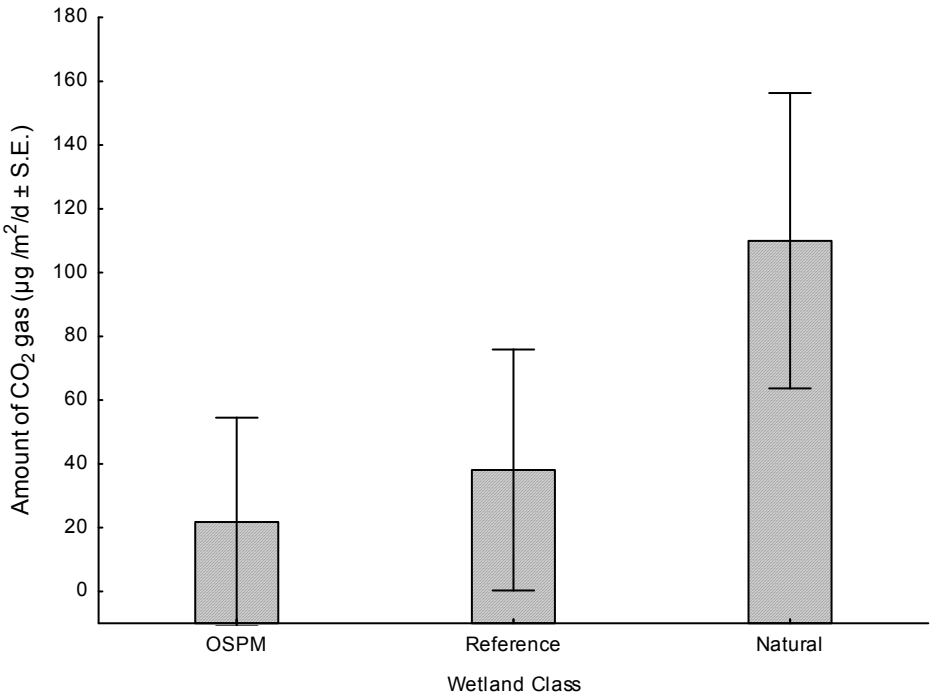


Figure 2.3. Differences of CO_2 (µg /m^2/d ± S.E.) released from unvegetated sediments of natural, reference and OSPM-affected wetlands from 2009.

Table 2.10. Partial pressures of CO_2 for 2009 data. The last column indicates whether its respective wetland should be storing or releasing CO_2. When any acid (carbonic) produced is neutralized we expect a potential CO_2 sink; when more acid is produced than can be neutralized we expect a system to be a source of CO_2; and equilibrium indicates the wetland could be either a CO_2 source or a sink.

Wetland Class	Wetland	Date dd/mm/yyyy	Alkalinity (expressed in CaCO3)(mg/L)	pH	Salinity(mol/L)	Temperature ^{o}C	pCO2 (µatm)	log pCO2	Carbon source or sink?
OSPM	Mike's Pond	04/06/2009	344	8.45	n/a	n/a	1586.7	-2.80	source
OSPM	Natural	06/06/2009	464	8.85	0.5	17.7	852.0	-3.07	source
OSPM	4 m CT	09/06/2009	1210	7.97	1	15	16855.0	-1.77	source
OSPM	Test Pond 9	02/06/2009	626	8.87	1	23.9	1097.8	-2.96	source
Reference	Shallow Wetland	07/06/2009	248	7.53	0.2	18	9514.7	-2.02	source
Reference	Golden	05/06/2009	151	8.52	0.9	19.9	592.8	-3.23	source
Reference	High Sulphate	03/06/2009	291	7.35	1.5	15.2	16898.0	-1.77	source
Natural	South Beaver	01/06/2009	201	7.18	0.1	15.9	17263.9	-1.76	source
Natural	South-west sands Beaver	08/06/2009	298	7.92	0.6	13.8	4657.6	-2.33	source

Partial pressures of CO_2 calculated for all 2009 study wetlands were saturated compared with the atmosphere, indicating that they were expected to be carbon sources of CO_2 (Table 2.10). Six of the 9 study wetlands did release CO_2. High Sulphate, Golden Pond (reference wetlands) and Mike's Pond (OSPW-affected) did not release any measureable amounts of CO_2. Yet, High Sulphate had one of the highest pCO_2 (16898 µatm) for our 2009 study and it was a carbon sink in 2008. Bicarbonate is the dominant ion at pH 8-10 (Stumm & Morgan 1996) so we would expect CO_2 solubility to drop as pH drops. At neutral or lower pH any wetland with measurable alkalinity would calculate pCO_2 as predicted carbon sources. In the case of High Sulphate (pH 7.35) predicted pCO_2 does not match the flux we measured (no flux at all). The pH of this wetland has shown a range from almost neutral to basic (pH 7.35-9.05 Table 2.9-10), it is unclear why there is such disconnect between predicted pCO_2 and measured flux values. Gas flux was variable in this study as well as in the literature (Table 2.8, Roehm 2005) we would expect wetlands to function as either sinks or sources of carbon given the right conditions. In fact, wetlands can simultaneously act as a carbon source or sink depending on the scale at which one measures gas flux (Roehm 2005).

Mike's Pond and Golden Pond were also saturated with CO_2 (1586 and 596 µatm, respectively) but the microcosms did not detect any gas. These two wetlands have almost no vegetation, ruling out vegetative gas transport (Chanton 2005). Mike's Pond supports an active biofilm layer at the sediment water interface, but it is essentially a clay-lined pool and likely supports little or no subsurface biological activity. The CO_2 saturation in this wetland is likely due to the relatively high pH (8.45 - due to the OSPM water used to fill it) driving any dissolved CO_2 into bicarbonate (Morgan & Stumm 1996) and trapping it in the water. The alkalinity (a measure of bicarbonate) was 344 mg/L, higher than all reference wetlands – this likely why the wetland was saturated with CO_2. We expected that Golden Pond, a reference wetland with a thick layer of organic matter (>10 cm deep, in some areas) would be a large gas producer similar to Peat Pond in 2008. Perhaps our sampling sites in Golden Pond did not produce any gas by chance, and we must attribute our observations to spatial variability. However, sediment stirring trials in Golden Pond also failed to release gas bubbles so we are confident we observed true measurements.

Our study wetlands were slightly or moderately basic (pH 7.57-9.55). Consequently, most of the carbon dioxide produced should have been buffered by the overlying waters to become bicarbonate (Stumm & Morgan 1996).

$$H_2CO_3 + 2\ H_2O \leftrightharpoons \mathbf{HCO_3^-} + H_3O^+ + H_2O \leftrightharpoons CO_3^{2-} + 2\ H_3O^+$$

At a pH range of 8-10, bicarbonate is the dominant ion, so any dissolved carbon dioxide (not as gas bubbles) would become bicarbonate (Stumm & Morgan 1996). Bubbles would be less likely to dissolve into the water; the diffusivity coefficient for CO_2 is 1.6 x 10^{-5} cm/s at 15 ^{o}C (Tamimi *et al.* 1994). The molar amounts of CO_2 in our samples range from 10^{-9}-10^{-11} moles. It only takes a few seconds for a bubble in these shallow wetlands to reach the surface so it is unlikely an appreciable amount of gas will diffuse into the wetland. Diffusion from the atmosphere and bubbles trapped in sediments may be the source of high alkalinity in these systems. Bubbling gases likely don't contribute.

We measured the microcosm water alkalinity with a precision of (± 5 ppm $CaCO_3$ or 1.25x10^5 µg/m^2 d). We compared changes in alkalinity between 24 h and 72 h samples within a wetland. However, because our detection limit was much larger (~ x10^3) than flux values we were measuring, we could not confidently detect changes in alkalinity among microcosms within a wetland. In addition, alkalinity for 0, 24 and 72-h time points were sampled independently (from different microcosms) the microcosm-to-microcosm variability also masked our ability to detect changes in alkalinity over time. If there were no differences in CO_2 gas flux between OSPM-affected and reference wetlands then either respiration did not differ between wetland classes *or* respiration rates were different, but the amount and turnover of carbon was higher in reference wetlands so that the net (observed) effect was equal among wetlands. We must conclude the former, but there is indirect evidence for the latter explanation.

Daly (2007) measured bacterial production rates within the water column of OSPM-affected and study wetlands. She found production rates to be 5X higher in reference wetlands and suggested that OSPM-affected wetland bacterioplankton must cycle carbon more slowly than bacteria in reference wetlands. If bacteria are reproducing faster within the sediments of reference wetlands, we would expect more respiration and more CO_2. As it stands, respiration did not differ in 2008 between wetland classes. Carbon dioxide flux was higher in natural than in reference and OSPM-affected wetlands

in 2009. However, bacterial biomass within the sediments was not different (Chapter 4). Microbes within natural wetlands may have quicker turnover rates and so we see more carbon dioxide respired despite the lack of a difference in sediment biomass.

2.18 Methane flux

Methane flux within our study wetlands was 2-5 orders of magnitude less than CH_4 flux values reported from other Canadian boreal wetlands (Table 2.8). Our values were also lower (2.0×10^{-5} vs. 1.1×10^{-3} mol C $m^{-2}d^{-1}$) compared with flux reported by Daly (2007, Table 2.8) for wetlands the same area, including some evaluated in the present study. However, Daly's 2005 study did not directly measure gas volumes. Instead, literature values were used to convert concentrations of gas into mass of carbon released.

Overall, more methane was released in natural than in reference than in OSPM-affected wetlands. More methane was released in unvegetated than vegetated zones of both wetland classes. Methane fluxes were generally not different between wetland age classes or between sampling trials.

Methane flux 2008 – Reference wetlands released more methane than OSPM-affected wetlands in 2008, ranging from 53-184 $\mu g/m^2/d$ and 0.8-64 $\mu g/m^2/d$, respectively (Table 2.7). Within reference wetlands, unvegetated areas released 2-5 times more methane than vegetated areas. Methane release did not differ between vegetated and unvegetated areas within OSPM-affected wetlands. Peat Pond – a reference wetland that was amended with peat was the largest producer of methane. The two natural wetlands, Southwest Sands Beaver and Beaver South released 116 and 55 $\mu g /m^2/d$, respectively (Table 2.7). The 2 natural wetlands - Southwest Sands Beaver released the 2nd highest amount of methane in 2008, whereas Beaver South Wetland released one of the lowest amounts of methane. This is counter-intuitive, generally wetlands with higher conductivities and sulphate concentrations (Southwest Sands Beaver) release less methane than `fresher` (Beaver South) wetlands (Appendix figure 2.5, Batzer & Sharitz 2006). There appears to be a threshold in which no methane is released once the concentration of sulphate is greater than 400 ppm (approximate, marked on Appendix figure 2.5 with a horizontal black line). Southwest sands is older and has a thicker organic layer (a source of carbon for microbes) than Beaver South, perhaps these factors are more important as wetland heterogeneity

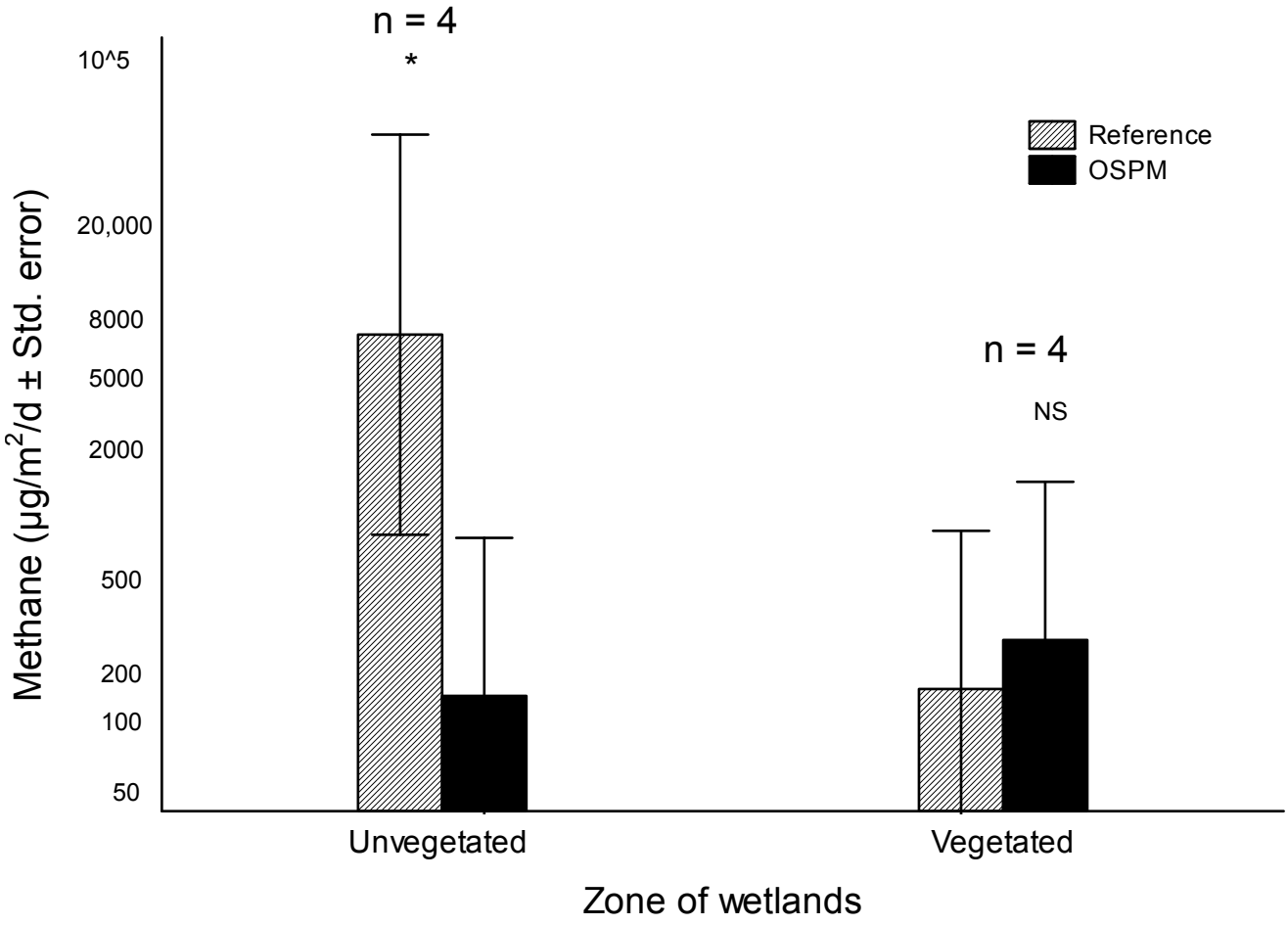


Figure 2.4. Mean methane (µg /m^2/d ± S.E.) released in 2008 reference and OSPM wetlands (natural wetlands excluded) of vegetated or unvegetated zones of those wetlands. Note the log scale. * denotes $p<0.05$ significance using a factorial ANOVA, NS denotes no significant difference.

may produce sulphate poor pockets (Fedorak *et al.* 2003) within sediments where methanogens may thrive and produce methane.

The amount of methane released was significantly higher in reference wetlands than in OSPM-affected wetlands (Figure 2.4; ANOVA d.f.=7, F= 4.59, $p<0.05$). There was a significant interaction between wetland zone (vegetated and unvegetated) and wetland status ($p<0.05$, F=6.48). Unvegetated areas of reference wetlands released more methane than vegetated reference areas of a wetland. Methane flux was not different between young (≤7 y) and older (>8 y) wetland classes or between trials (spring and summer) ($p>0.05$).

Methane (CH_4) flux 2009 – The amount of methane released was higher in natural than reference or OSPM-affected wetlands, ranging from 516-747, 0-276 and 0-260 $\mu g/m^2$ d, respectively in unvegetated sediments in 2009. Flux was not measured within vegetated sediments in 2009. The amount of methane released from natural wetlands was significantly higher than constructed (reference + OSPM) wetlands (planned comparison ANOVA d.f.= 7, F= 22.6, $p<0.01$). Methane flux between reference and OSPM-wetlands were not different (planned comparison ANOVA d.f.= 7, F=0.007 22.6, $p>0.05$). We likely did not detect any differences between reference and OSPM because two reference wetlands, Golden Pond and High Sulphate did not release any gas. We tried collecting gases from these wetland multiple times but we observed no flux, and so we considered flux for these wetlands close to zero and included them in our analyses, which brought down the average of the other reference wetland (Shallow- 276 µg of CH_4 /m^2 d). As discussed below, water chemistry (particularly salinity and sulphate) may be better predictors of methane flux than wetland class.

Wetland methane flux overall was significantly (2-5 times) higher in 2009 than in the 2008 sampling season ($p<0.05$). The slightly higher volumes of gas collected in 2009 are insufficient to explain the large between-year differences in gas release; water temperature and depth don't appear to be significant influencing factors.

Differences in water chemistry between OSPM-affected and reference wetlands likely play a larger role than organic amendment in determining the type and amount of respiration within wetlands. Wetlands differed in concentrations of sulphate, salinity and

ammonium in 2008 and 2009 not only among wetland classes but also among reference wetlands (Tables 2.3 and 2.4, respectively). The presence of sulphate promotes growth of sulphate reducing bacteria (SRB) (Batzer & Sharitz 2006). In turn, SRBs suppress growth of methanogenic bacteria, usually by taking up electron acceptors (H_2) more efficiently than methanogens (Batzer and Sharitz 2006, Dowrick 2006). In general, reference wetlands showed much lower levels of sulphate and more methane release than OSPM-affected wetlands. High Sulphate (reference) and Southwest sands Beaver (natural) are exceptions to this observation. Sulphate concentrations in High Sulphate were among the highest of all our study wetlands - we observed no gas released. Southwest sands released the most methane (747 $\mu g/m^2$ d, the closest to this value was ~ 500 $\mu g/m^2$ d (Shallow Wetland)) but sulphate concentrations fell around the median for all wetlands. Ion concentrations within this wetland have likely increased with declining water table since beavers have stopped damming the area.

Previous studies have identified the presence of SRB in OSPM-affected wetlands (Sobolewski 1999); suppression of methanogenesis by SRBs is likely the cause of differences in methane flux among wetlands. Methane, however, has been found in tailings (Fedorak *et al.* 2003), meaning methanogens and SRB's are not mutually exclusive, and can co-exist (Chris Weisener, U of Windsor, pers. comm.). Fedorak *et al.* 2003 suggested that methane could be produced at micro-sites depleted of sulphate in consolidated tailings (CT). If there is heterogeneity in a relatively homogeneous sediment such as CT, then we would expect heterogeneity of sulphate concentrations within wetland sediments (which are mixed or layered with clays, sand and organic matter), to release methane. Generally, OSPM-affected and reference wetlands with lower sulphate concentrations released more methane than OSPM-affected and reference wetlands with higher sulphate concentrations (Appendix figure 2.5).

High salinity is a general stressor (usually due to osmotic stress) to a number of organisms, including plants and invertebrates (Batzer & Sharitz 2006). Wetlands with higher salinities are typically less species rich (Leonhardt 2003, Batzer & Sharitz 2006, M.C. Roy, University of Alberta in prep.). Abril & Iverson (2002) observed a negative correlation between methane production and wetland salinity. Salinity effects on methane have often been observed in tidal wetlands; sulphate is a component of sea water (7.7%

Kladec & Knight 1996). It is difficult to determine whether sulphate or salts influence methanogenesis in these cases, although both factors will have some negative effect on methane production (Denier van der Gon & Neue 1995b (reported in Daly 2007), Abril & Iverson 2002). In all wetland classes, methane release decreased with increasing salinity, however, this trend was not as strong as the drop in methane release with increasing sulphate concentration (Appendix figure 2.6). Daly (2007) reported a similar autocorrelation between sulphate concentration and overall salinity.

Denier van der Gon & Neue 1995b (reported in Daly 2007) contrasted the effect of sulphate and salinity in rice-paddy fields and found that gypsum-amended ($CaSO_4$, a major source of sulphate in OSPM-affected wetlands) rice fields reduced methane emissions more than salt-amended fields. There was a negative but non-significant relationship (multiple regression $p>0.05$) between methane release and salt and sulphate concentrations within our study wetlands (d.f.=10*, $F=0.78$, $p>0.05$. * wetlands were pooled for 2008 and 2009 of which there were 12 unique wetlands). Southwest Sands Beaver Wetland (natural) was an exception to this as it released the most methane but fell within the middle of salinity and sulphate concentrations observed in our wetlands. Wetlands with the highest concentrations of sulphate and salt (High Sulphate – reference wetland = 1.5 ppt, 1650 ppm and Mike's Pond – OSPM-affected wetland = 1.4, 565 ppm) released no methane at all. Lab based microcosm studies measuring microbial communities (M. Chen, University of Windsor in prep.) in which one can control salt and sulphate concentrations could be undertaken to resolve the individual and additive effects of salt and sulphate on microbes.

In addition to sulphate and salt, the presence of ammonium within wetlands (particularly in OSPM-affected wetlands, C. Slama, University of Windsor, unpubl.) affects nitrogen cycling, which may also influence the flux of carbon dioxide (consumed by nitrifying bacteria, Belser 1979, Batzer & Sharitz 2006).

$$NH_3 + CO_2 + 1.5\ O_2 + \textit{Nitrosomonas} \rightarrow NO_2^- + H_2O + H^+$$
$$NO_2^- + CO_2 + 0.5\ O_2 + \textit{Nitrobacter} \rightarrow NO_3^-$$

(Belser 1979)

Sobolewski (1999) identified nitrifying bacteria within constructed OSPM-affected wetlands and found that nitrifiers were the least numerous bacteria compared with decomposers and naphthenic acid degraders. He noted that the abundance of nitrifiers were "comparable to healthy ecosystems" (Sobolewski 1999, p. 2) – the healthy ecosystem being Mildred Lake, a freshwater lake adjacent to oil sands operations. Ammonia is potentially toxic to plants, fish, invertebrates and algae (Health Canada 1999). Current studies are looking at the effect of nutrients (nitrogen and phosphorus) on primary production (H. Chen, University of Waterloo, in prep.) Microbial studies to date in the in oil sands region have not addressed nitrogen cycling by bacteria. Future studies should address nitrifying and denitrifying bacteria and their role in nitrogen cycling.

Methane flux and vegetation - High methane flux observed in reference wetlands was due to its production only in unvegetated reference sediments. There were no differences in methane flux from vegetated areas of OSPM-affected and reference wetlands. Within reference wetlands, methane flux was significantly higher in unvegetated sediments than in the vegetated areas.

Emergent vegetation (*Scirpus spp., Typha spp.,* and *Phragmities spp.*), can transport methane from sediments to the atmosphere via molecular diffusion - movement of molecules down a gradient from higher to lower concentration, or convective ventilation – the transport of gas within lacunae (open spaces within plants), where methane moves from the sediment to the roots to the atmosphere and oxygen (used for respiration) is moved (actively or passively) from the atmosphere to the roots (Boon & Sorrell 1995, Segers 1998, reviewed in Chanton 2005). Gas ventilation by plants can reduce sediment concentrations of methane and thus reduce its flux through ebullition and diffusion. Van der Nat *et al.* 1998 have shown that when present, plant transport is the dominant mode of gas flux in a wetland - up to 10 times more gas transport than unvegetated areas (reported by Chanton 2005). In contrast, the transport of atmospheric oxygen into plant roots may create aerobic patches in the surrounding sediment creating conditions for methane oxidation (Segers 1998, Ding *et al.* 2005), thereby serving an opposing function to gas channelization of methane (and carbon dioxide) through plant tissues and thereby into the atmosphere.

Methane is relatively insoluble (60% or more of the methane produced typically leaves a wetland before being dissolved, (Tokida *et al.* 2005)) and a number of studies report transport of gases by emergent vegetation (Van der Nat *et al.* 1998, Chanton 2005, Roehm 2005). Consequently, we suspect that vegetative gas transport accounts for the lower carbon flux observed from vegetated sediments to the atmosphere in the microcosms than from unvegetated sediments. Regardless of the mechanisms (plant transport of methane from sediments vs. in situ oxidation), the net effect is the same and less gas flux occurs from vegetated wetland sediments. However, the release of carbon from plants is an important component of carbon mass balances. This topic should be addressed in the future to estimate whether wetlands are sinks or sources of carbon.

2.19 Evaluation of technique

Our 'rocket' microcosm was a variation of the static chamber model that is often used to measure ebullition rates in aquatic systems (e.g., Chanton 1989, Roehm 2005). Because the rockets were constructed from black PVC they were opaque, preventing photosynthesis (a carbon dioxide consuming process) to ensure accurate measurement of net respiration rates within a wetland.

The *in situ* design provided several advantages over gas flux measurement techniques reported in the literature. The rockets were light yet sturdy, easy to disassemble and small enough to place within vegetated areas of wetlands without greatly disturbing the vegetation. The chambers covered a small area (400 cm^2), compared with 2000 cm^2 (Rask *et al.* 2002) and 3600 cm^2 (Maljanen *et al.* 2007) but we compensated by putting out more rockets – the other studies cited used 3 sampling stations, which were not moved around within their sampling areas. Our results showed that even over a small scale (cm^2) these wetlands show considerable variation and heterogeneity.

Other methods are commonly used to determine gas flux rates in aquatic systems. These can include calculating partial pressures (Werner *et al.* 2005) or using eddy covariance towers (which may use partial pressures or infrared detection of carbon dioxide; Roehm 2005, Werner *et al.* 2005, Bonneville *et al.* 2008). Both techniques work at a large scale (meters, hectares) and average flux values at a whole-wetland (or larger) scale, easily overlooking the variability we observed in our study wetlands. Partial

pressures are typically only calculated for (but not limited to) CO_2, whereas eddy covariance towers usually only measure CO_2 and CH_4 and are costly to deploy in the field.

Our microcosms are relatively cheap, portable, and trap any gases released. Therefore, we can analyze a sample for a variety of gases (oxygen, nitrous oxide, hydrogen sulphide, volatile hydrocarbons etc.), although we focused on methane and carbon dioxide. The microcosms trap gases right at the sediment surface, allowing us to estimate the total amount of carbon (provided that a large enough volume of gas is collected) being released from the sediments. Working at a smaller scale at the sediment interface has allowed us to speculate on the flow of carbon from bacterial communities through wetland food-webs. However, with smaller microcosms we had trouble obtaining enough sample to run through our gas chromatograph. This led to high variation in our reported flux values as well as potential errors since a smaller sample is more easily contaminated or diluted than a large volume of marsh gas. The microcosm method used in this thesis as well as Daly (2007) would likely improve by increasing the surface area covered over the sediment. Using chambers that cover anywhere from 0.5-1 m^2 as seen in Chanton *et al.* (1989) would serve as useful guidelines. Microcosms at this size may prove cumbersome or costly and it would be best to use microcosms with enough surface area to consistently collect 20 mL (or more) of gas per day (or 3 days in our case). Overall, each method has its advantages and drawbacks; the scale and objective of a study should be considered when deciding which method is appropriate.

2.20 Global wetland flux: a comparison

Table 2.8 lists averages and ranges of gas flux taken from boreal (including our oil-sands-region wetlands), temperate and tropical wetlands. Of all the values listed; our study wetland data from 2008 and 2009 represent the lower end of a continuum of methane and carbon dioxide flux values. Compared with temperate and tropical wetlands we would expect flux rates to increase the further south one studies, where temperatures are higher and consequently, biological activity is greater (Winkler *et al.* 1996, Pringault *et al.* 2007). This appears to be the general trend for methane, but not for carbon dioxide. It is important to note that flux values taken from our study include only permanently inundated areas of a wetland. Frequent wetting and drying of wetland soils usually

increases microbial respiration due to oxygen flushes caused by drying (Batzer & Sharitz 2006). This is likely why our estimates are less than those reported in the literature, however, studies in the area will address vegetation and wet meadow respiration (F. Mollard, University of Alberta). In addition to temperature and seasonal variability, the presence of sulphate at high concentrations is known to suppress methanogenesis (Batzer and Sharitz 2006, Dowrick 2006). Sulphate-rich wetlands, such as High Sulphate (it released no methane) are expected to be smaller sources of methane than less saline, warmer sites.

Carbon dioxide flux values are relatively similar across all spatial zones (boreal, temperate and tropical). The values from our study are lower than any other zone or study. There are two reasonable, non-exclusive explanations for this trend:

1) Many of study wetlands are relatively young (7-20 years old), and unamended wetlands have thin (< 5 cm) organic layers. This layer serves as a carbon source for organisms to metabolize and from which to eventually produce methane or CO_2. These wetlands likely don't have the carbon stores characteristic of natural wetlands (Bruland *et al.* 2009, Hossler & Bouchard 2010). Hossler & Bouchard (2010) suggest constructed wetlands may take anywhere from 70-300 years to accumulate carbon comparable to natural wetlands (assuming the wetlands are on trajectory to accumulate carbon).

Bruland *et al.* (2009) observed linear increases (although with only 4-5 replicates) in microbial biomass in wet marshes amended with organic matter (varying amounts of compost added to the soils). When compared with wetlands that are decades or centuries old, and which have thick (cm-m thickness), undisturbed organic layers one would expect a greater potential for carbon release in natural or organic-amended wetlands over younger, constructed wetlands. Our study design classifies wetland age classes based on the work of Leonhardt (2003), who reported that the number of invertebrate families reached an asymptote in wetlands after about 7 years after construction. This classification may not be appropriate in terms of carbon and methane flux values. However, at the time that the study design was developed, no other operational definition or pilot work was available.

2) Due to the mining extraction process, the oils sands process water in our OSPM-affected study wetlands is alkaline, which buffers these systems. Indeed, even the

reference and natural wetlands had well-buffered water. Carbon dioxide will dissolve into alkaline water and become bicarbonate, effectively limiting the amount of CO_2 that can leave a wetland in gas form (Stumm & Morgan 1996). This is likely the reason why we see more ebullition of methane than of CO_2. Although there may have been substantial conversion of respired CO_2 to bicarbonate relative to the gas flux rates that we measured,

Of the studies reviewed, our alkaline marsh wetlands released the least amount of carbon across boreal wetlands (table 2.8). A number of these studies (Blodau *et al.* 2004 Moore *et al.* 2002 and studies referenced therein) were conducted in boreal bogs, which tend to be acidic (Zoltai & Vitt 1995), meaning dissolved carbon dioxide is favoured over bicarbonate ions and thus more likely to be released from a wetland via flux (Stumm & Morgan 1996). Alkalinity in our wetlands must play a role in these flux values. Although we attempted to measure changes in alkalinity over the course of the flux studies, our alkalinity assays lacked the sensitivity to detect changes of less than 5 ppm, We observed no increase in alkalinity within microcosms over the duration of the studies. Comparisons to these wetland studies (table 2.8- of the studies listed, Blodau *et al.* 2004, Bonneville *et al.* 2008 include flux from vegetated areas of wetland, the others either work in unvegetated sediments or do not explicitly state which zone gas is measured) will be more accurate when estimates of gas transport through plants (F. Mollard, University of Alberta, in prep.) are combined with the ebullition rates of this study. The present study compares only a single component of carbon flux. We do not yet know the extent to which gas transport via wetland plants contributes to total wetland carbon flux.

The values reported here are still ~10^2-10^4 times lower than those previously reported for other wetlands in the area (Daly 2007). Our sampling protocols were nearly identical with the exception that Daly (2007) left headspace within the microcosms and determined changes in headspace over 24 h and 72 h. Our 'rockets' had no headspace and thus captured gas from the sediments, unmixed with the atmosphere. We directly measured ebullition from sediments whereas Daly (2007) estimated exchange rates between the wetland and air. Headspace would allow diffusion of dissolved gases into the headspace air, as well as catching bubbles coming from the sediments. Perhaps this is why she observed higher flux rates than this study.

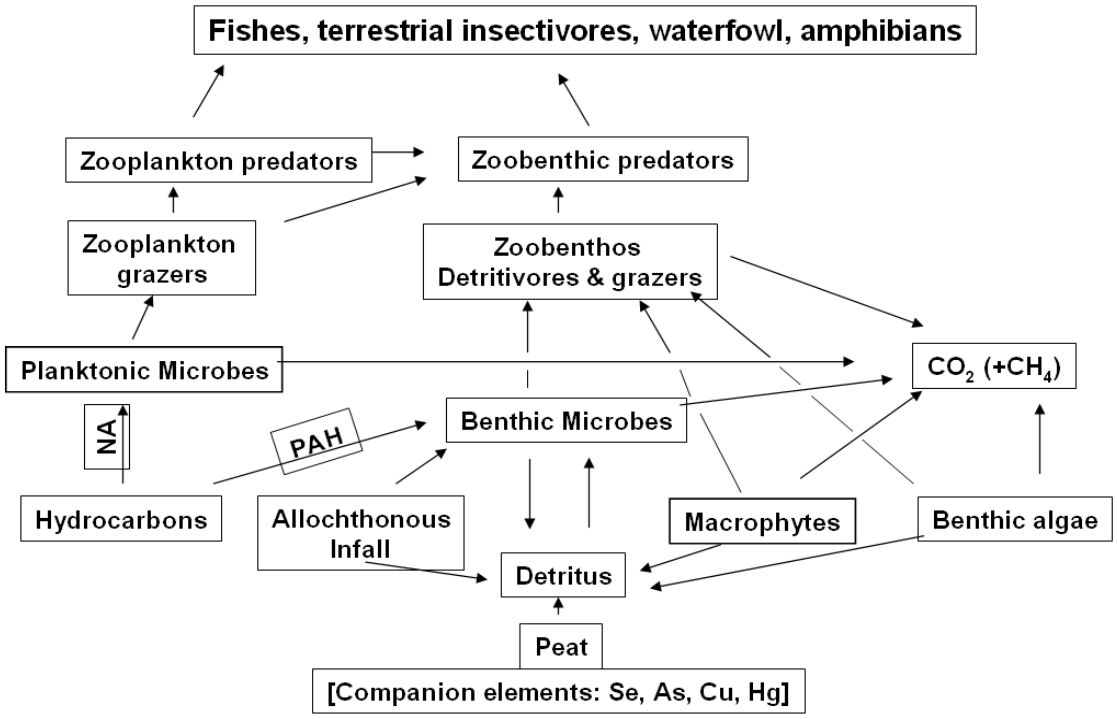


Figure 2.5. Hypothetical oil sands-affected wetland food web, which serves as a simplified conceptual model for the CFRAW research project (Ciborowski *et al.* 2006). The boxes denote the quantities of carbon listed (expressed as biomass or numerical abundance as most appropriate). The arrows denote the flow of carbon from one box to the next.

2.21 Implications

Overall, the study wetlands appear to release relatively little gas compared to other studies - a trend that is consistent for all 3 years of observations (1 year of sampling from Daly 2007). This study aimed to determine the variability of gas flux in constructed wetlands, and how OSPM-affected wetlands differed from reference and natural wetlands. Overall, these wetland systems are variable, and natural wetlands tend to release more gas (methane and carbon dioxide) than either reference or OSPM-affected wetlands from unvegetated sediments.

Our data suggest that most of the wetlands studied in 2008 and 2009 (natural, reference and OSPM-affected) were sources of carbon during the periods of study, releasing both carbon dioxide and methane. If observations of this study are any indication, I would predict these systems to be heterotrophic and allocthonous carbon inputs will be required to accumulate organic layers. This research is a single component of the CFRAW research group and will be used to estimate the rate in which carbon (ultimately energy) flows throughout a wetland food-web (figure 2.5). These data will eventually be used to determine overall patterns of total carbon input/output (Kovalenko *et al.* 2010) in a wetland and to estimate whether these systems are net sinks or sources of carbon. In terms of reclamation, knowing what affects carbon flow and how the carbon is transferred among components will help to formulate guidelines on how to re-establish boreal wetlands of equivalent function compared with pre-mining operations.

2.22 Future Studies and Conclusions

How does gas flux change from year to year? If we wished to measure long term gas flux and changes from year to year, the design and sampling method of this study would need modification. If the goal is to estimate carbon flux at the wetlands scale, gas flux should be monitored using eddy covariance towers that can integrate flux estimates over large areas (Bonneville *et al.* 2008). Perhaps we could modify a microcosm that can be left in a wetland from year to year and sampled periodically, something similar to piezometers used in ground water monitoring (similar to a design used in Juutinen *et al.* 2008). This could be coupled to yearly organic matter accrual estimates for each wetland to give estimate mass balances for carbon storage in constructed wetlands (e.g., M. Trites,

University of Alberta, in prep). A study such as this will be useful to evaluate: 1) the efficacy of reclamation strategies such as organic amendment towards recreating the boreal wetland landscape 2) the "trajectory" that reclaimed wetlands are following and whether they can be predicted to become similar to the conditions of natural wetlands.

How does gas flux vary seasonally? This is the next logical step to build upon this thesis, and it is important since the microbial community is one of the few groups of organisms (and one that has the largest biomass) that remain active throughout the year in a wetland (Maljanen *et al.* 2007) (fish are typically not present in our wetlands). As mentioned before, our design was not meant to test for differences in gas flux over time. Indeed, no consistent differences were found between spring and summer sampling trials. One would expect gas flux to vary with temperature (Del Giorgio & Williams 2005, Kladec and Knight 1996) throughout the year if most of the gas is biologically produced. High variability in these systems may mask differences within a season, but there is no doubt that we would detect differences in gas flux between the warm and cold seasons.

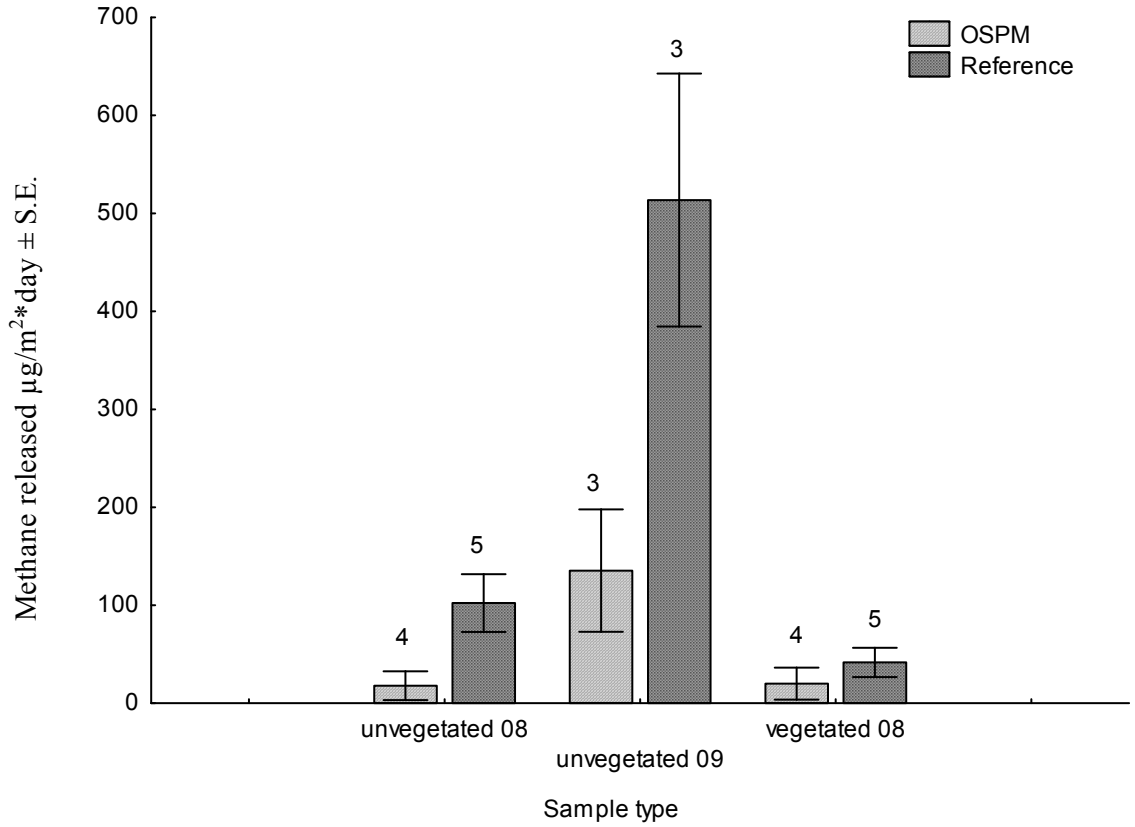
Fort McMurray is typically only warm for 5 months of the year, and the temperature in wetlands reaches 10+ ^{o}C in only 3 of those months. Wetland microbes stay active in the winter months; some may be even more active in the winter when they don't compete with summer-active organisms (G. Ferris, University of Toronto, pers. comm.). In terms of gas flux, gases usually build under the ice, and wetlands can experience large, rapid releases of gas during spring thaw (Maljanen *et al.* 2007). As long as the water doesn't freeze all the way to the sediment, microbes will continue to respire carbon.

Temperature loggers placed at the bottom of each study wetland indicate that all of the wetlands reach 0 ^{o}C (but not lower) over the winter months. Ice and snow act as barriers that insulate the bottoms of wetlands, allowing for some of the water to stay unfrozen (Maljanen *et al.* 2007). Therefore, some gas production is to be expected in the winter months in these wetlands. Ice cover and ultimately gas production will largely be controlled by the depth and salinity of the water as well as by snow fall and ambient temperatures in the area. Deeper, saltier wetlands are likely to freeze more slowly. This implies that OSPM-affected wetlands, with characteristically high salinities may be producing gas past the summer months. Despite the fact that reference wetlands release

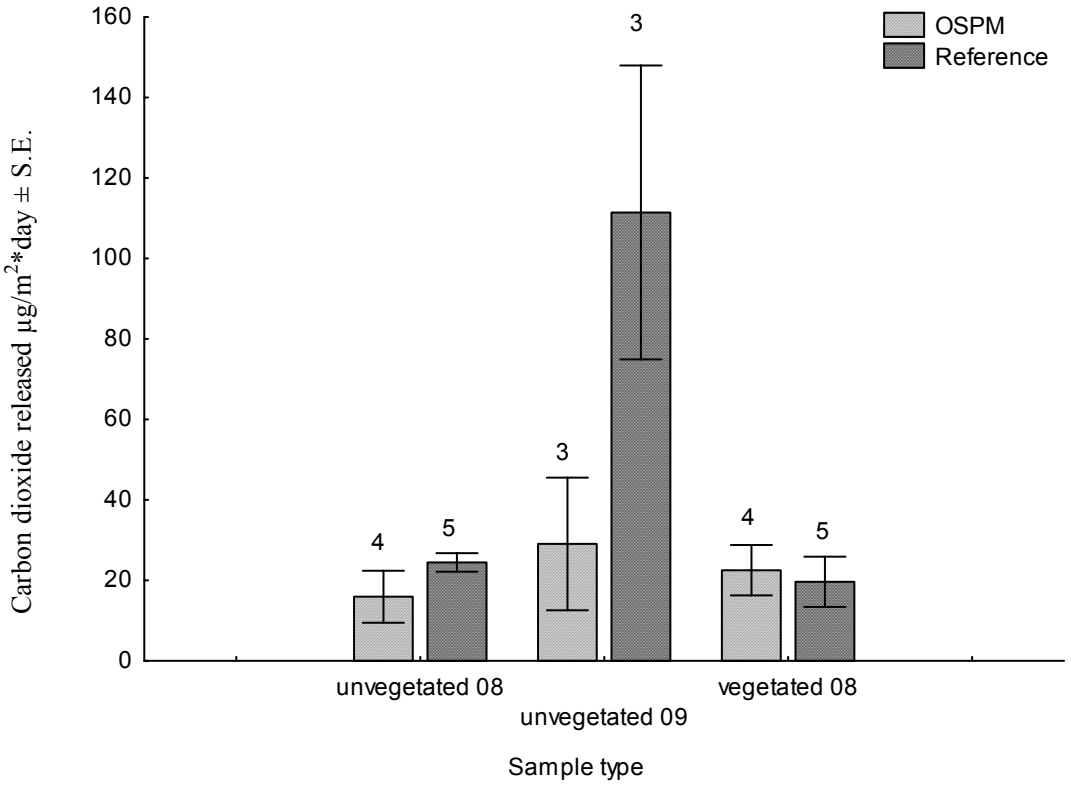
more gas in the summer than OSPM-affected, saltier OSPM-affected wetlands may release equal or greater amounts of carbon dioxide and methane over the course of a year (Kladec & Knight 1996, Gutknecht & Goodman 2006). Future studies should look at how gas flux varies seasonally and to determine the effect, if any, on carbon cycling and carbon import/export from these wetlands.

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.

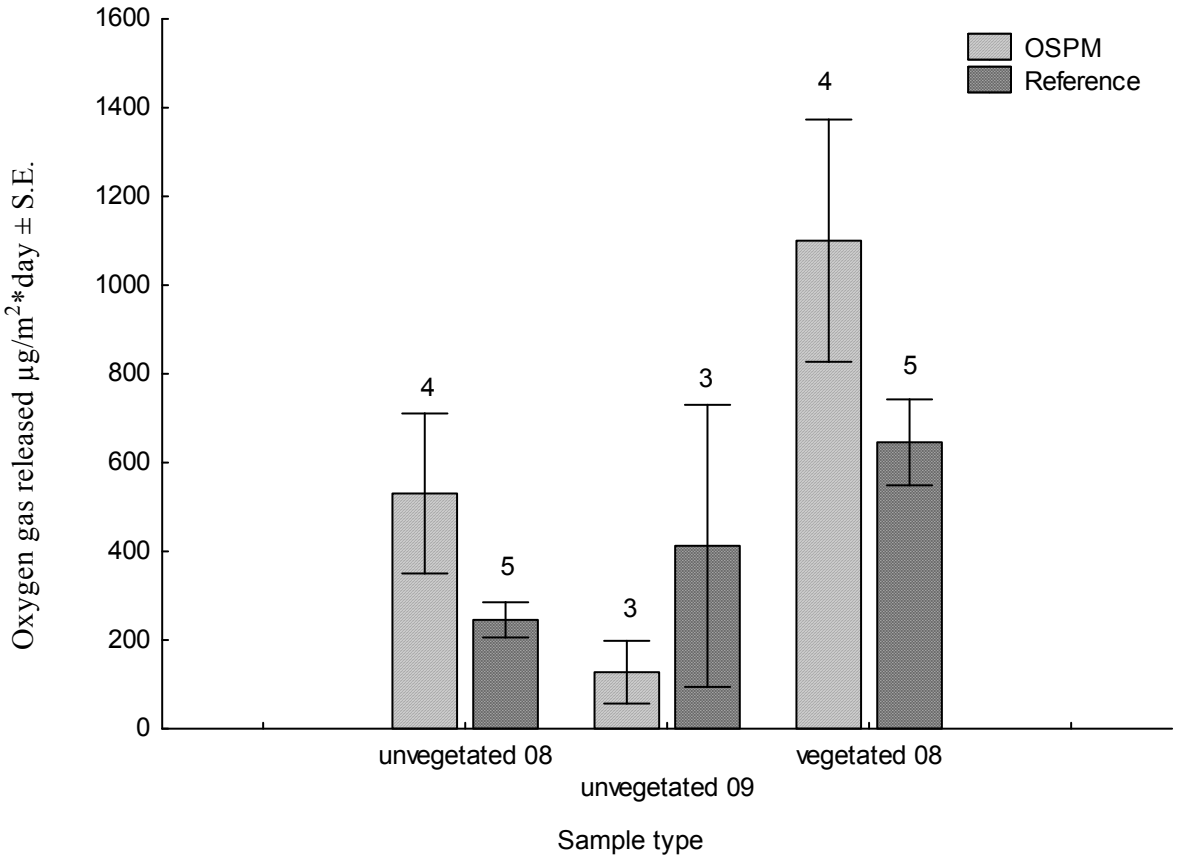
Year	Wetland class	Wetland	Wetland zone	Mean water depth (cm ± S.D.)
2008	OSPM	4 m CT	unvegetated	21.0 ± 8.6
			vegetated	19.7 ± 7.0
		Natural	unvegetated	37.3 ± 9.2
			vegetated	24.5 ± 5.1
		Test Pond 9	unvegetated	55.4 ± 7.2
			vegetated	34.8 ± 6.6
	Reference	High Sulphate	unvegetated	55.6 ± 7.9
			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
	Natural	Beaver South	unvegetated	48.9 ± 6.8
			vegetated	42.5 ± 6.7
		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
	Natural	South Beaver	unvegetated	52.9 ± 8.0
		Southwest sands Beaver	unvegetated	36.7 ±11.0



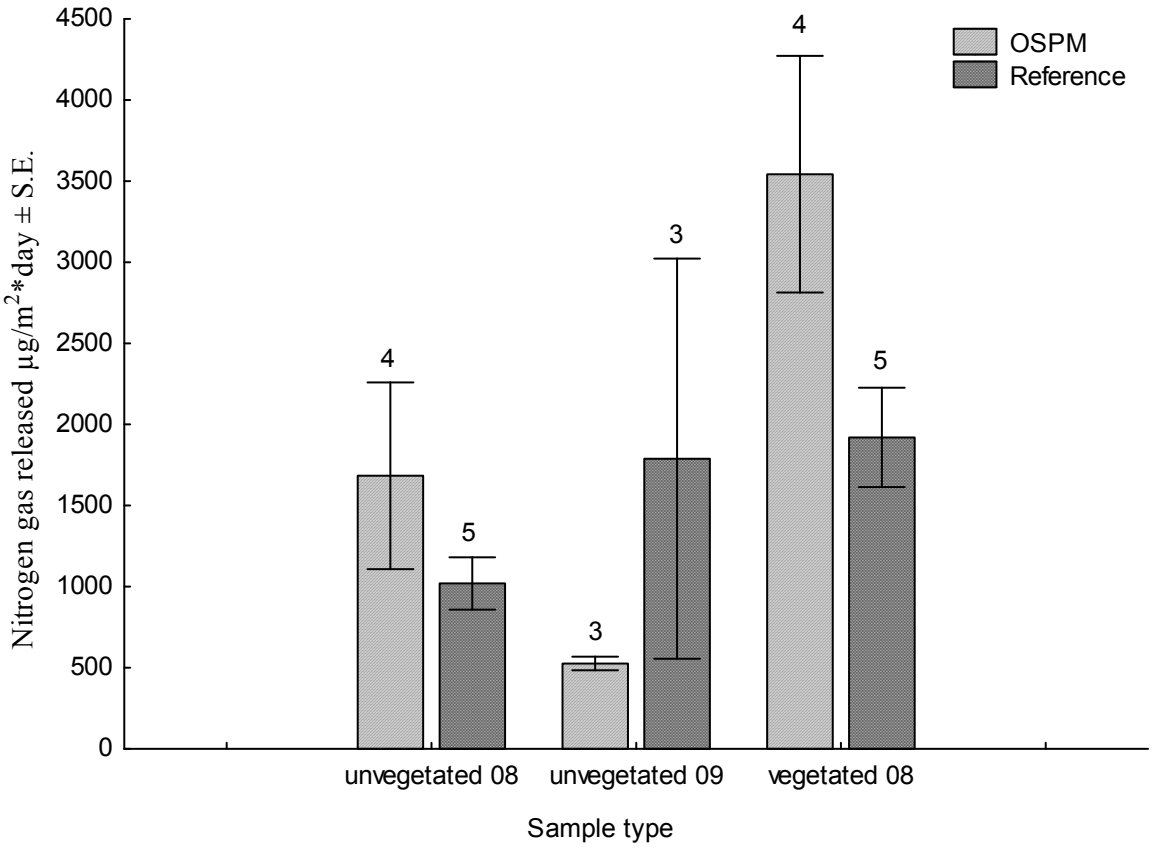
Appendix figure 2.1. Amount of methane released µg/m^2 day ± S.E. in 2008 and 2009 in OSPM-affected and reference wetlands using microcosm static chambers. Natural and reference wetlands were pooled for simplicity; these wetland classes were not found to be different from each other. Numbers over the bars represent the number of wetlands from which gas was collected.



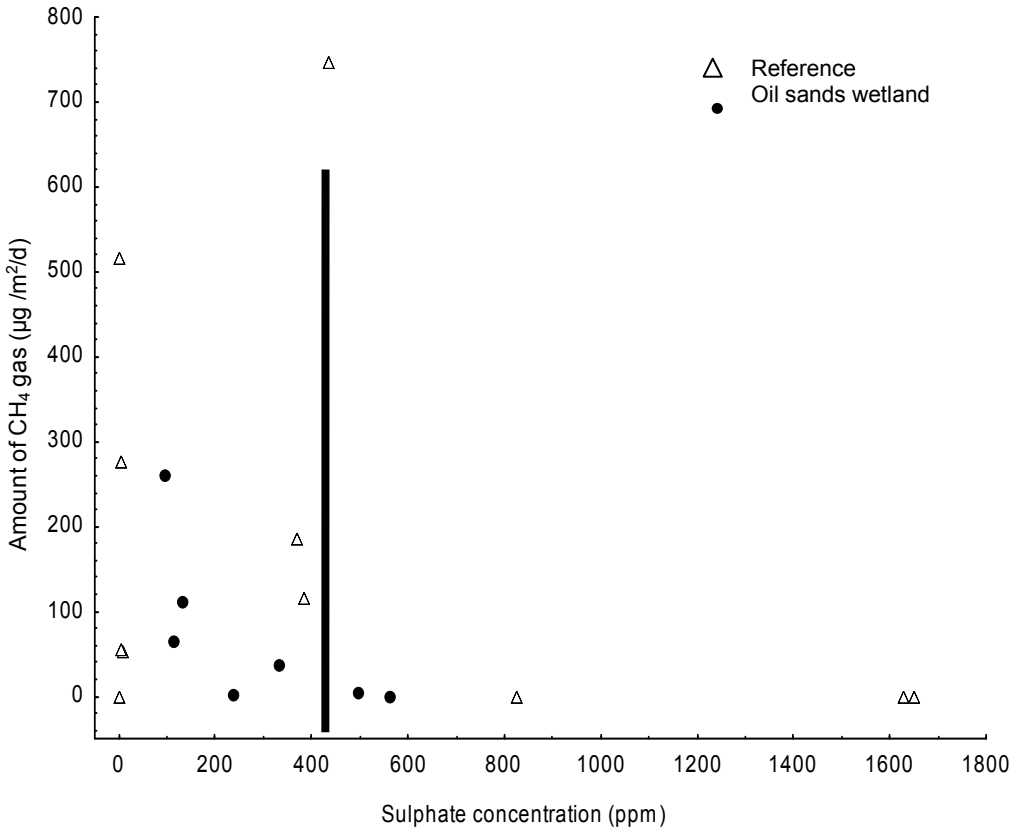
Appendix figure 2.2. Amount of carbon dioxide released µg/m^2 day ± S.E. in 2008 and 2009 in OSPM-affected and reference wetlands using microcosm static chambers. Natural and reference wetlands were pooled for simplicity; these wetland classes were not found to be different from each other. Numbers over the bars represent the number of wetlands from which gas was collected.



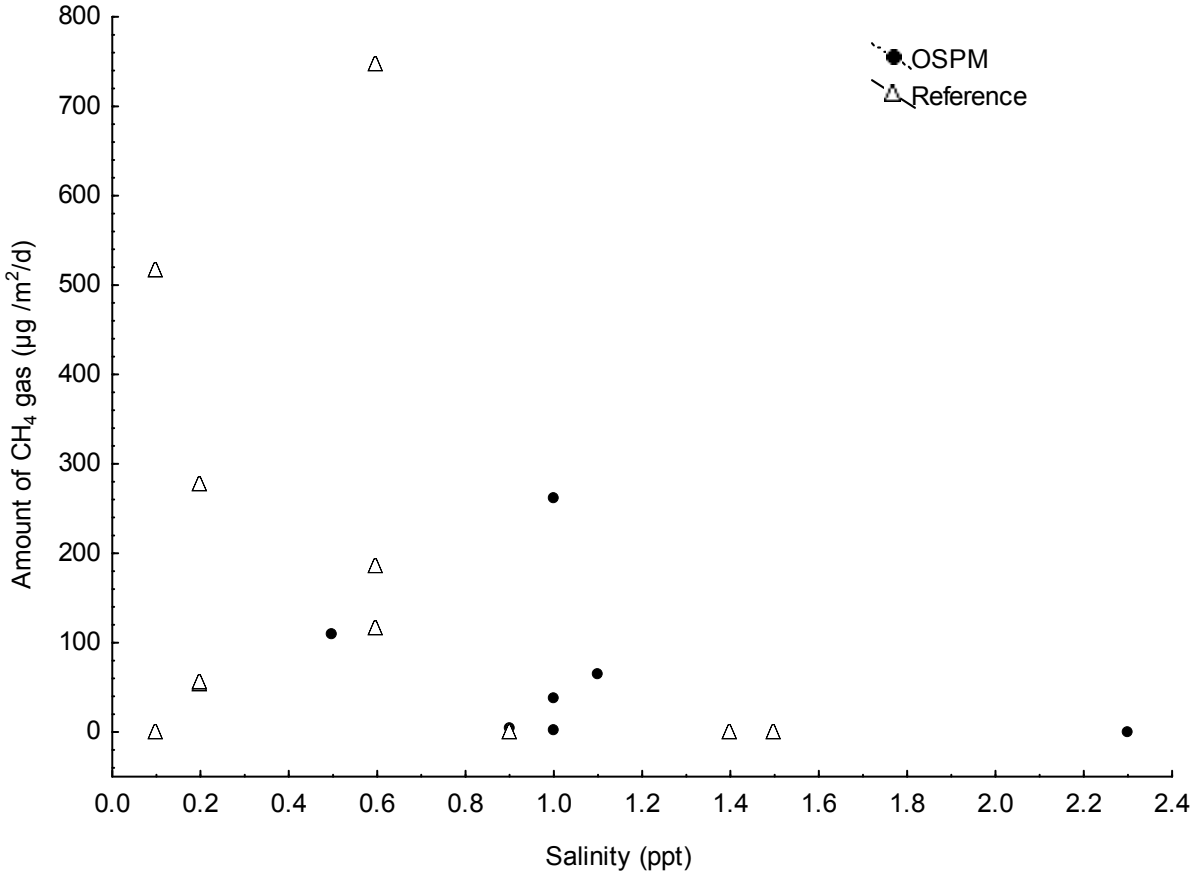
Appendix figure 2.3. Amount of oxygen observed µg/m^2 day ± S.E. in 2008 and 2009 in OSPM-affected and reference wetlands using microcosm static chambers. Natural and reference wetlands were pooled for simplicity; these wetland classes were not found to be different from each other. Numbers over the bars represent the number of wetlands gas was collected from.



Appendix figure 2.4. Amount of nitrogen released µg/m^2 day ± S.E. in 2008 and 2009 in OSPM-affected and reference wetlands using microcosm static chambers. Natural and reference wetlands were pooled for simplicity; these wetland classes were not found to be different from each other. Numbers over the bars represent the number of wetlands gas was collected from.



Appendix figure 2.5. Scatter-plot of methane released (µg /m^2/d) from our study wetlands in 2008 and 2009 vs. Sulphate concentration (ppm). Black circles denote OSPM-affected, white triangles are reference and natural wetlands; they were pooled because we only had 2 natural wetland values. Methane release drops with increasing sulphate concentrations in all wetland classes. Line fitted by eye to estimate a threshold value for methane production.



Appendix figure 2.6. Scatter-plot of methane released (µg /m^2/d) from our study wetlands in 2008 and 2009 vs. Salinity (parts per thousand). Black circles denote OSPM-affected, white triangles are reference and natural wetlands; they were pooled because we only had 2 natural wetland values. Methane release drops with increasing salinity in all wetland classes, however, the trend is not as strong as seen with increasing sulphate.

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Chapter 3: Using gas flux to estimate biological and chemical sediment oxygen demand in oil sands-affected wetlands – Wetland Oxygen Oscillations, Trapping Anaerobic Gases (WOOTAnG)

Collaboration by Jesse Gardner Costa (M.Sc. Candidate) and Carsten Slama (M.Sc. Candidate) from the Ciborowski lab, University of Windsor

3.1 Expectations

This 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 reclaimed wetlands (containing those materials). The objectives were to determine the sediment oxygen demand (SOD - rate of dissolved oxygen consumption at the water-sediment interface (Murphy & Hicks 1986)) for 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). We determined whether chemical or biological oxygen-consuming processes dominate within the study wetlands and the effect of oil sands process materials (OSPM) on oxygen demands within wetland sediments.

1) We expected chemical SOD (CSOD) to be higher in OSPM-affected wetlands than in reference wetlands; likely due to the increased chemical oxidation of ammonia, whose concentrations are elevated in OSPM-affected wetlands.

2) We expected the biological SOD (BSOD) to be lower in OSPM-affected than in reference wetlands. We observed lower gas flux in OSPM-affected than reference wetlands (Chapter 2) indicating less microbial activity, potentially limiting the oxygen available for biological consumption at the substrate level. Elevated salinities and conductivities (characteristic of OSPM-affected) were expected to further limit BSOD.

3) We 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 we expected higher CSOD in OSPM-affected wetland and therefore a higher overall SOD in OSPM-affected than in reference wetlands.

3.2 Introduction

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 biochemical processes, ultimately affecting the community composition (of all aerobically respiring organisms, Dauer *et al.* 1992). As a result of water's limited capacity to hold dissolved oxygen, the importance of sediment-associated oxygen-consuming processes increases as the volume of water overlying the sediment is reduced. Therefore, processes that occur near the substrate surface are major contributors to DO depletion in shallow water bodies (Sweerts *et al.* 1991). Respiration by benthic invertebrates, microbial-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 proportion 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 atoms. During oxidation, an element losses its electrons and chemically binds to one or more oxygen atoms by sharing their electrons. This binding of formerly free oxygen molecules (e.g., sulphates, nitrates), produces the demand for molecular oxygen. In 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 aerobic 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 summation of biological and chemical oxygen-consuming components yields overall sediment oxygen demand (Wang & Reed 1984).

There is uncertainty as to whether biological or abiotic chemical factors play a larger role within 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. Consequently, knowledge of the relative contributions of biological vs. chemical dynamics to the overall sediment oxygen demand is crucial to understanding the role these processes have on overall wetland 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. The congruent value for chemical reactions, specifically ammonia oxidation is approximately 2 for every 10 ^{o}C increase in temperature between 10-30 ^{o}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 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 light into energy and produce oxygen. At night, photosynthesis ceases and dissolved oxygen concentrations steadily decline as respiration and chemical oxidation continues. Reaeration, however, is a constant process and the most dominant factor in replenishing dissolved oxygen (Jain *et al.* 2005). This is dependent 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 used in this experiment measure oxygen consumption in the water column only and subtracted from our chambers that measure sediment and water column oxygen demand, giving us a measure of oxygen consumed solely within the sediments.

When the balance of 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 severely affect organism survival, community structure and ecosystem sustainability (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 may show impacts at 3-4 mg/L, whereas some krill species may not be affected until levels 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 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 quicker than it can be replenished leading to hypoxic conditions (Batzer & Sharitz 2006).

In the oil sands region of northeastern Alberta (Fig. 3.1), constructed 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 both provides

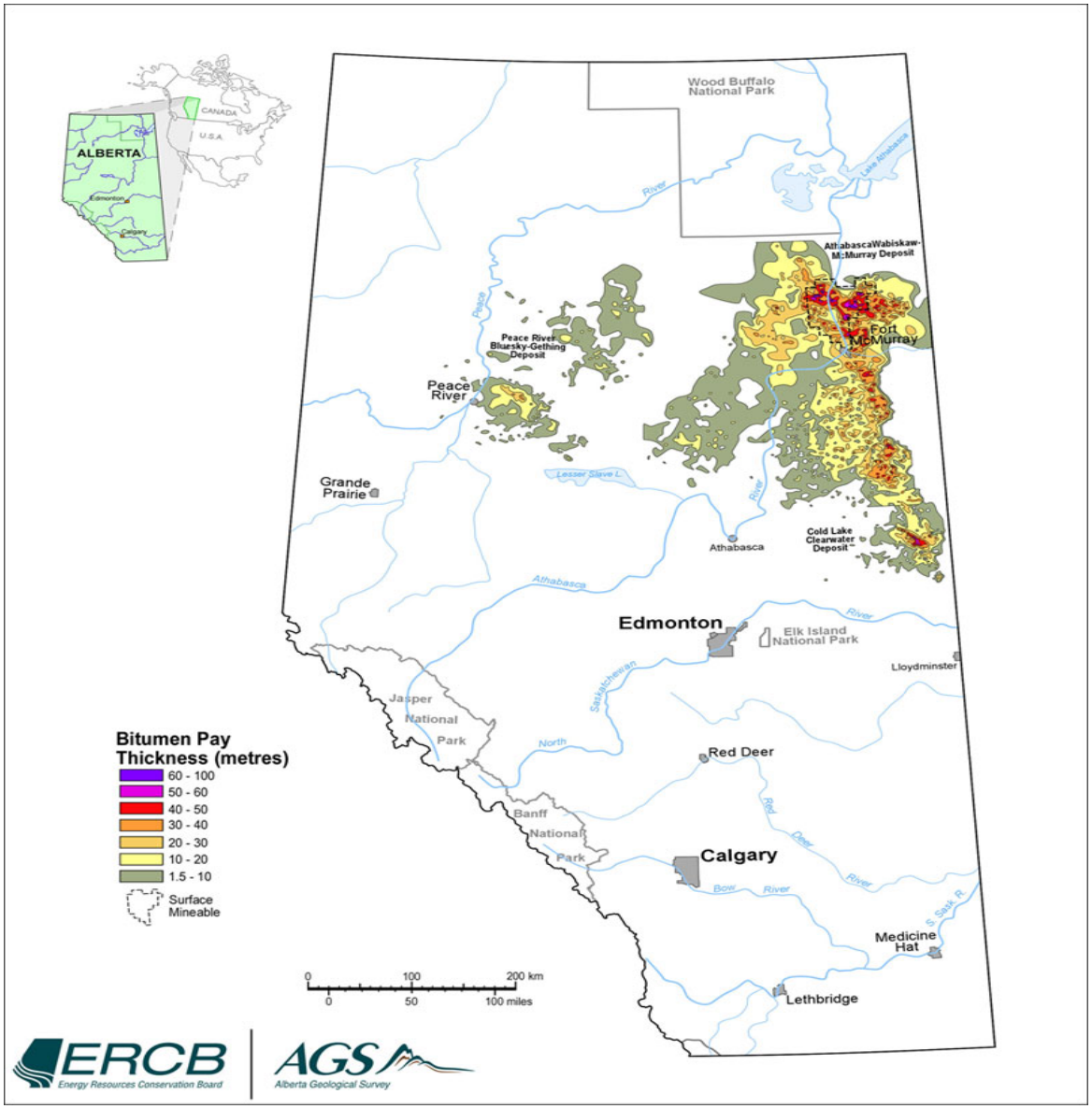

Figure 3.1. Location of Alberta oil sands deposits which cover over 140,000 km^2. Map taken from: http://www.ags.gov.ab.ca/activities/CBM/alberta_oil_sands.html

construction materials and serves as a means of disposing of tailings materials. However, oxygen consumption of OSPM-derived sediments in constructed wetlands has not been well studied. 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 reduction. 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 (ancient ocean beds) 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 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 (chapter 4) we expect 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 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 two classes of constructed wetlands – those built using OSPM and reference wetlands constructed or formed without OSPM.

Materials and Methods

3.3 Study sites

The study wetlands were located within or near Syncrude Canada Ltd. and Suncor Energy lease areas in north-eastern Alberta, Canada (57.0298870, 111.585388, Figure 3.1). We studied three categories of wetlands; OSPM-affected, reference, and natural.

OSPM-affected wetlands are wetlands constructed in the mined landscape using mine tailings materials (OSPM: solids and water) as demonstration projects by Suncor Energy, Inc. and Syncrude Canada, Ltd. 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 3.1).

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 years old (we aren't sure of the age of the natural wetlands. Beaver South is 'young' (less than 5 years old), and Southwest sands Beaver is at least 18 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), our study wetlands can be considered relatively young (usually no older than 30 years). We used an empirical approach to classify wetland age; Leonhardt (2003) reported that the number of invertebrate families reached an asymptote in wetlands after about 7 years after construction. The number of invertebrate families was not statistically different between an 8 or 25 year old wetland and so we set old as 8+ and young as ≤ 7.

Table 3.1. Wetland descriptions as of 2009. NR denotes 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	NR	Fresh	Native undisturbed	Syncrude	Thin (< 5 cm) organic top layer, clay underneath
Natural	South West Sands	Rich	NR	Fresh	Native undisturbed	Syncrude	
OSPM	Test Pond 9	Poor	16	Process water	Clay lining	Syncrude	Clay lined – pockets of bitumen present within the clay
OSPM	Mike's Pond	Poor	17	Process water	Clay lining	Syncrude	Clay lined, deeper area (~3 m) has a halocline
OSPM	Natural Wetland	Rich	23	Process water	Post mining sand	Suncor	Sandy bottom with a > 15 cm of Muskeg on top
OSPM	4 m CT	Rich & Poor	12	Process water	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.
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	Rich	8	Fresh	Post mining	Syncrude	Lined with Clay/loam

	Pond				clay/sand mix		(80 cm) with natural organic material on top
Reference	Peat Pond	Rich	8	Fresh	Post mining clay/sand mix	Syncrude	Lined with Clay/loam (80 cm) with peat/mineral mix (> 20 cm) on top
Reference	CNRL	Poor	5	Fresh	Sand	Canadian Natural	Sand lined bottom
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

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 statuses are similar to those of OSPM-affected wetlands, differed in flux and production from their undisturbed, unconstructed, older counterparts.

Nine wetlands (4 OSPM, 3 reference, 2 natural wetlands were studied (Table 3.1). The chosen wetlands fit within the CFRAW matrix (Chapter 1), 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.* 2004). Those data will be used to relate carbon flow to this work.

3.4 Rationale

Studies of gas flux and SOD were initially two independent projects, measuring biologically released gases from wetland sediments (Chapter 2) 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). In our study, 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.* 2006; Kovalenko *et al.* 2010).

Within a 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 (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 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 ends 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 encapsulate any vegetation. During early pilot work, we could not create a tight seal between the chamber and the sediment in vegetated areas. Because 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), we elected to sample similar areas found within a wetland between wetland classes to reduce variability. In addition, 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.

3.5 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 3.1L) 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® 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 polyethelyne 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. 3.2). 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 fiberglass screening

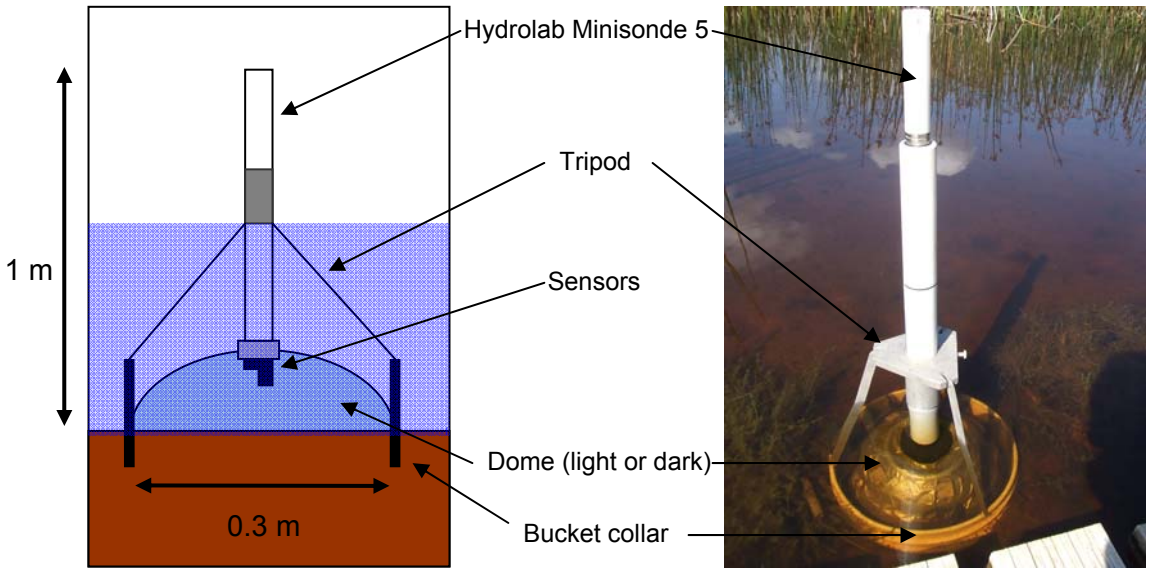

Figure 3.2. Sediment oxygen demand chamber setup; schematic (left) and actual (right).

permitted circulation between the substrate and water inside the dome but prevented 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. 3.2).

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.

3.6 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). Collars and domes were removed and placed in a new location between the two sampling periods.

3.7 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. 3.3) 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

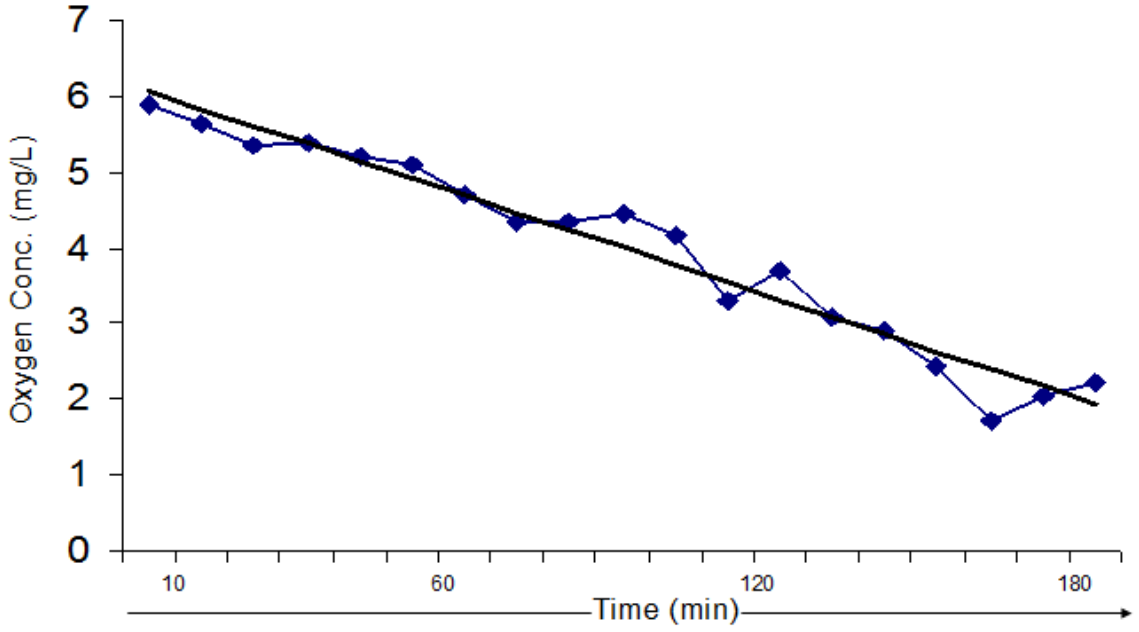

Figure 3.3. Dissolved oxygen depletion curve. Data points were collected every 10 min over a 3 h period. The slope of the linear regression analysis was used to calculate sediment oxygen demand. The correlation coefficient was always above 0.7.

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). We assumed that nighttime depletion rates were no different than daytime rates.

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

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

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 $g/m^2/d$.

3.8 Estimation of biological oxygen demand: Gas flux measurements

Microcosms were built using black 5 cm ID x 30 cm long PVC piping (Fig. 3.4) 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

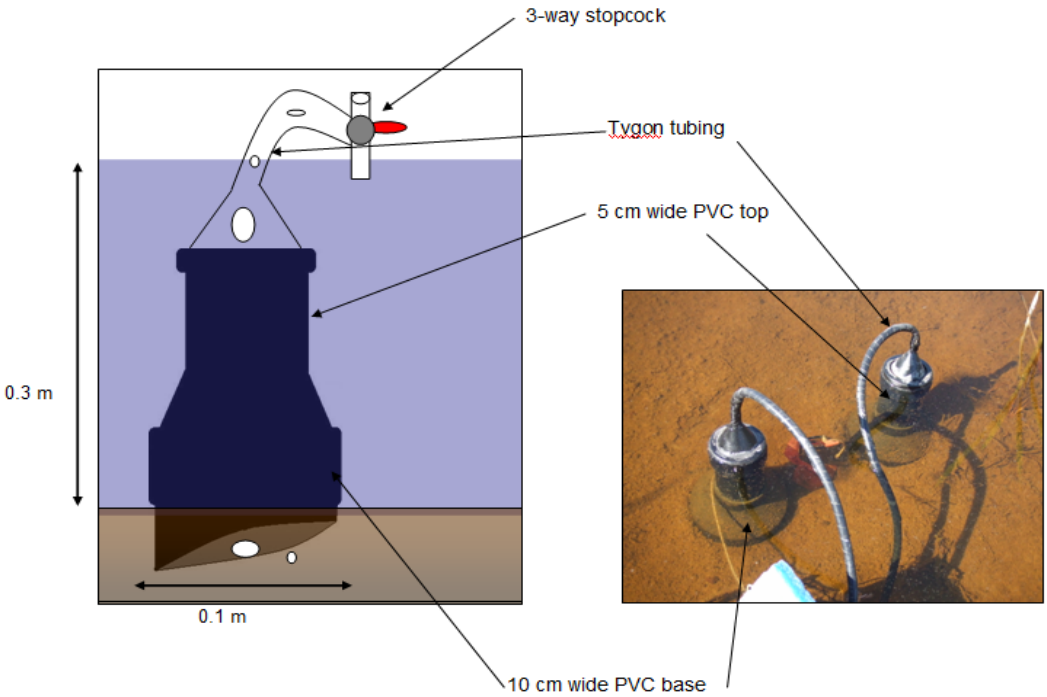

Figure 3.4. Gas flux microcosms; schematic (left) and actual (right).

(this length was used to distance sampling from the microcosm to prevent disturbance) of ID 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. One wetland (Table 3.1 for wetland descriptions) was sampled on each of the 9 days (see the schedule in Table 3.2). 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.

3.9 Gas collection

Gas sampling was typically done in the morning to mid afternoon (0900- 1500 h 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.

Table 3.2. Sampling schedule of summer 2009 field sampling for Sediment Oxygen Demand (SOD) and gas flux measurements in our 9 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	Seven inch 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

3.10 Water chemistry

Water quality measurements (dissolved oxygen concentration, conductivity, salinity, and temperature) were taken using an YSI model 85 meter at each wetland during gas collection and SOD sampling (see Table 3.2). 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 0.45-µm 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.

3.11 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) attachment. Cores were taken along to minimize disturbances and allow the probe to reach the sediment surface. The probe was placed flat on the sediment surface until readings stabilized.

3.12 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 (Supelco 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^2 d). 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:

$4.008\%\ CH_4 \times 2 \times 10^{-6}\ L = 8 \times 10^{-8}\ L\ CH_4$

Using the ideal gas law (PV=nRT),

n(moles) =PV/RT → n= 1 atm x (8×10^{-8} L) / (8.233† K*atm*L/mole x 273.15 K)

= 3.56×10^{-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 Sample / Mole CH_4 Std) = (Area CH_4 sample / Area CH_4 std)

Rearranged: Mole CH_4 Sample = Mole CH_4 Std * (Area CH_4 sample / Area CH_4 std)

3.13 Determining Biological Sediment Oxygen Demand (BSOD)

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).

3.14 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

3.15 Statistical analyses

Statistical analyses were performed using STATISTICA version 7.0 (Statsoft, Inc., Tulsa, OK). Statistical significance of differences among the classes of wetlands

(OSPM-affected, reference constructed, and natural wetlands) with respect to SOD, BSOD and CSOD rates were tested using planned-comparison ANOVA of log(Y+1)-transformed data. 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= 4 OSPM-affected n= 3 reference, n=2 natural).

Results

3.16 Wetland water chemistry

A summary of wetland water chemistry can be found in Table 3.3. There were no consistent differences in salinity and conductivity among natural, reference and OSPM-affected wetlands. Reference and natural wetlands showed a wide range of salinities and conductivities (0.2-1.5 ppt, 313-2,980 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 (7.97-8.87 vs. 7.18-8.52, respectively). Naphthenic acid concentrations were 10 times higher on average in OSPM-affected compared to other classes. All wetlands were well oxygenated (6.68-12.5 mg/L), with the exception of Beaver South (a natural wetland - 3.01 mg/L).

Table 3.3. 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°	pH	Cond. (µS)	YSI DO (mg/L)	YSI Salinity (ppt)	NO_3 (ppm)	SO_4 (ppm)	Iron (ppm)	NH^{4+} (ppm)	Naphthenic acids (ppm)	ORP (mV)	Sediment Redox Potential (mV)
OSPM	Mike's Pond	NR	8.45	4550	NR	NR	BDL	565	BDL	0.137	26	106	-188.6
OSPM	Natural	17.7	8.85	1090	8.27	0.5	27	134	0.499	1.08	33	87	-206.0
OSPM	4 m CT	15	7.97	2300	6.68	1	1.4	335	0.04	BDL	30	124	-214.1
OSPM	Test Pond 9	23.9	8.87	2050	12.08	1	BDL	97.8	BDL	BDL	19	79	-220.5
Reference	Shallow Wetland	18	7.53	391	7.2	0.2	1.4	6.58	0.036	BDL	<1	113	-151.0
	South-west sands	13.8	7.92	1252	9.82	0.6	BDL	437	BDL	BDL	<1	119	-204.3
Natural Reference	Beaver Golden	19.9	8.52	1737	12.5	0.9	2.3	825	BDL	BDL	2	99	-249.8
Reference	High Sulfate	15.2	7.35	2980		1.5	2.2	1650	BDL	0.166	10	124	-350.8
Natural Reference	South Beaver Pond 5	15.9	7.18	313	3.01	0.1	0.47	2.84	0.894	BDL	<1	79	-128.6
		NR	7.90	1260	8.95	0.6	NR	NR	NR	NR	NR	66	-151.7

3.17 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 in the OSPM class (Table 3.3).

3.18 Sediment Oxygen Demands

Values of SOD (O_2 g/m^2/d) can be found in Table 3.4. 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.

Sediment oxygen demand averages were 738 ± 0.194 O_2 g/m^2/d in reference wetlands, 0.904 ± 0.003 O_2 g/m^2/day in natural wetlands and 1.664 ± 0.519 O_2 g/m^2/d in OSPM affected wetlands. The linear regression coefficients of determination for the oxygen depletion curves were always 0.7 or higher. Mean sediment oxygen consumption for dark chamber domes in natural wetlands was not significantly different than the mean for constructed (reference and OSPM affected) wetlands (Table 3.5, Figure 3.5, planned comparison ANOVA, $F= 0.126$, $n=10$, $p>0.05$). Although SOD in OSPM-affected wetlands was higher than reference wetlands, this was a non significant trend (Table 3.5, Figure 3.5, planned comparison ANOVA, $F=3.331$, $n=10$, $p>0.05$).

3.19 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. However, the difference was not statistically significant (1-way ANOVA, $p>0.05$) owing to 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 - OSPM-affected and Southwest Sands Beaver - natural, respectively). Although the BSOD in natural wetlands was higher than in constructed wetlands (Table 3.6, Figure 3.6. planned comparison ANOVA, $F=2.715$, $n=9$, $p>0.05$), the data were highly variable, and the difference was not statistically significant. No difference was seen between reference and OSPM-affected wetlands (Table 3.6,

Figure 3.6, planned comparison ANOVA, F=0.138, n=9, p>0.05). The BSOD values in reference wetlands were more variable than values taken from OSPM wetlands.

3. 20 Chemical Sediment Oxygen Demands

By subtracting the BSOD from the SOD we were able to estimate oxygen consumption by chemical processes. CSOD ranged from ranged from 0.703 to 2.996 g O_2 /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 3.7, Figure 3.7. planned comparison ANOVA, F=0.294, n=9 p>0.05) or in reference wetlands compared to OSPM-affected wetlands (Table 3.7, Figure 3.7, planned comparison ANOVA, F=2.801, n=9, p>0.05). More variability in CSOD was seen in OSPM wetlands than in reference ones.

Table 3.4. Mean±SE SOD, BSOD and CSOD values ± S.E. for study wetlands in 2009. BDL - blow detectable limits (<5 mL 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
	Mean	**0.904 ± 0.003**	**0.124 ± 0.075**	**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	0.721	NR	NR
	Mean	**0.738 ± 0.194**	**0.045 ± 0.045**	**0.699 ± 0.231**
Test Pond 9	OSPM	1.440	0.003	1.437
Mike's Pond	OSPM	0.508	BDL	0.508
Natural Wetland	OSPM	3.022	0.026	2.996
4-m CT	OSPM	1.687	0.072	1.615
	Mean	**1.664 ± 0.519**	**0.025 ± 0.017**	**1.639 ± 0.513**

Table 3.5 Planned comparison table for SOD.

Source of Variation	SS	d.f	MS	F	p
Treatments	0.058	2	0.029	1.729	0.250
Natural vs. Constructed	0.002	1	0.002	0.126	0.733
Reference vs. OSPM	0.056	1	0.056	3.331	0.111
Within	0.118	7	0.017		
Total	0.176	9			

Table 3.6 Planned comparison table for BSOD.

Source of Variation	SS	d.f	MS	F	p
Treatments	0.002	2	0.001	1.475	0.301
Natural vs. Constructed	0.0019	1	0.0019	2.716	0.150
Reference vs. OSPM	0.0001	1	0.0001	0.138	0.723
Within	0.004	6	0.0007		
Total	0.006	8			

Table 3.7 Planned comparison table for CSOD.

Source of Variation	SS	d.f	MS	F	p
Treatments	0.0596	2	0.03	1.616	0.274
Natural vs. Constructed	0.0054	1	0.005	0.293	0.607
Reference vs. OSPM	0.0542	1	0.052	2.801	0.145
Within	0.111	6	0.018		
Total	0.170	8			

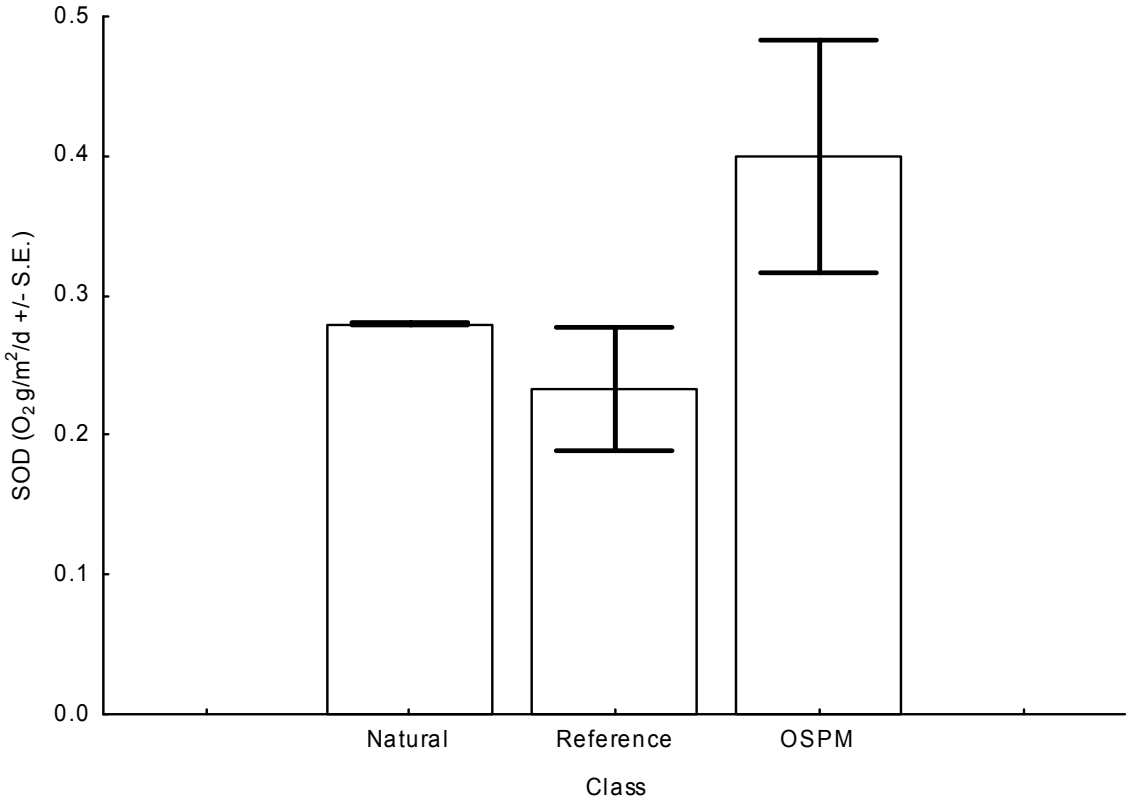

Figure 3.5. Mean (± S.E.) sediment oxygen demand (O_2 g/m^2/d ± S.E.) in natural (n=2), reference (n=3), and OSPM (n=4) wetlands for dark chambers in unvegetated zones of wetlands.

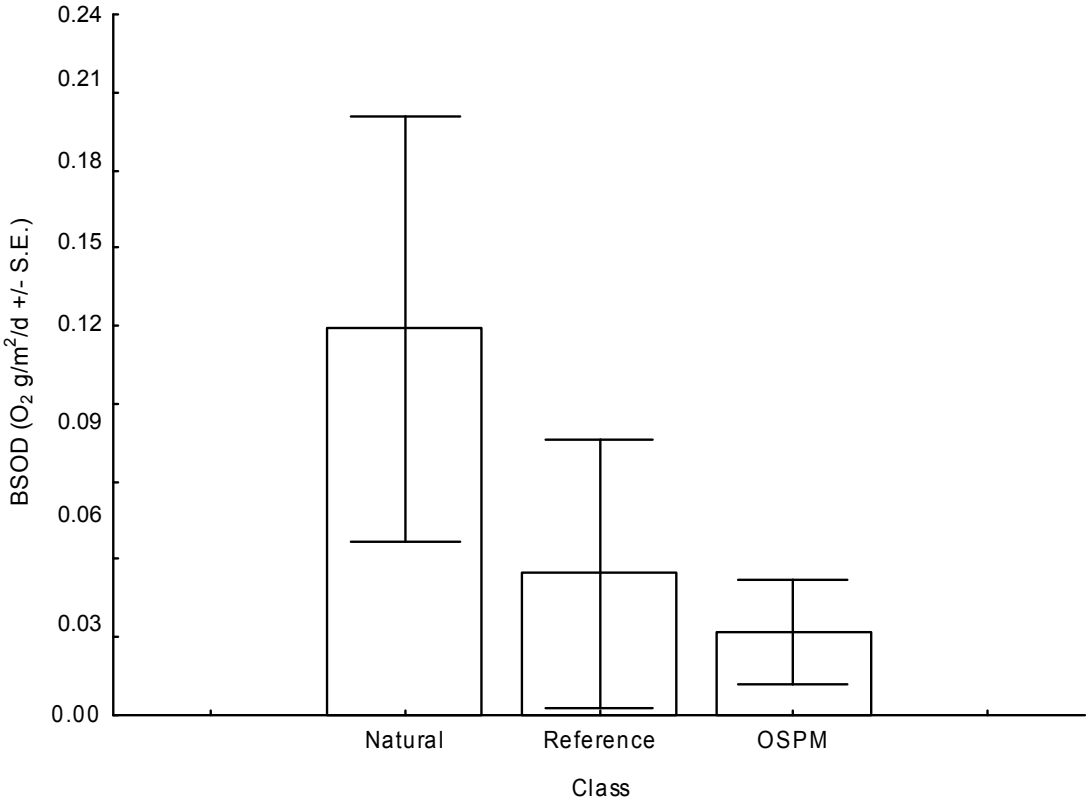

Figure 3.6. Mean (± S.E) biological sediment oxygen demand (O_2 g/m^2/d ± S.E.) in natural (n=2), reference (n=3), and OSPM (n=4) wetlands for dark chambers in unvegetated zones of wetlands.

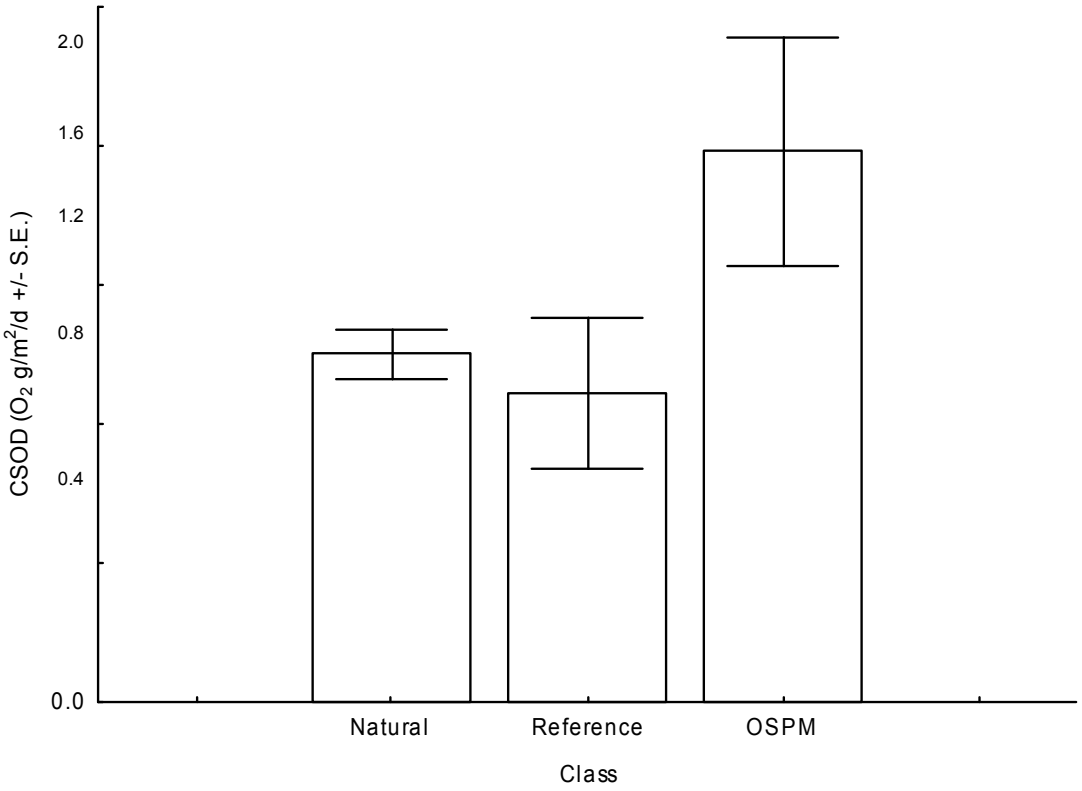

Figure 3.7. Mean ± S.E chemical sediment oxygen demand (O_2 g/m^2/d ± S.E.) in natural (n=2), reference (n=3), and OSPM (n=4) wetlands for dark chambers in unvegetated zones of wetlands.

Discussion

3.21 Sediment oxygen demand

Sediment oxygen demand was not significantly different among classes of wetlands. However, OSPM-affected wetlands had a SOD more than twice as high as reference wetlands 1.664 (O_2 g/m^2/d compared with 0.738 O_2 g/m^2/d). The lack of significance is most likely due to our small sample size. Our sediment oxygen demand values fall within range of literature values. 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 SOD rates observed in OSPM-affected wetlands suggest that OSPM had 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. Some of the wetlands tested contained OSPM-affected water over natural sediments, while 4-m CT was composed of OSPM-affected water and sediments but was still within the middle of the range of other OSPM SOD values. We had expected clay and organic matter to have different effects on biological SOD. However, no apparent trend was seen. 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 (chapter 4). Since CSOD values are ~ 10 times higher than BSOD in both reference and 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.

Reference and OSPM-affected wetlands averaged 30% and 44% (proportions of oxygen consumed from initial dissolved oxygen concentration) depletion in dissolved oxygen concentration, respectively. Differing percentages of oxygen depletion indicate that the increased SOD is the result of more rapid DO consumption in OSPM-affected

wetlands. An increased SOD can strain aerobic processes within a wetland food web by depleting DO concentrations at an accelerated rate (Connolly *et al.* 2004).

3.22 Biological sediment oxygen demand

Any differences observed in SOD are attributed 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 not significantly higher in reference than in OSPM-affected wetlands. This non significant trend will almost certainly be significant if we can increase our sample size if only by a few more wetlands.

The difference in BSOD between reference and OSPM-affected is almost 10-fold. 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 reference 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 (chapter 4) 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 (K. Frederick, University of Alberta, in prep.)), and submerged macrophytes (Slama 2010 in prep., pers. obs.) 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.

3.23 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, potentially underestimating 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. In chapter 4, I 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 mo., meaning I would predominantly find non-photosynthetic, anaerobic bacteria within the samples. I 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 uses stable isotopes to resolve bacterial carbon sources). Determining the isotope signatures of respiratory gas would more expensive and complicated (A. 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.

3.24 Chemical sediment oxygen demand

Our estimates of BSOD, suggest that oxygen consumption in OSPM-affected wetlands was dominated by the chemical oxidation. Roughly 90% of the oxygen was consumed chemically in reference wetlands, while close to 98% was chemically consumed in OSPM-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 95% responsible for oxygen uptake in a California 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 both 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 (in both wetland classes, more so in OSPM-affected) is driven by the oxidation of chemical constituents. These chemicals (NH_4^+, Fe (II), S^{2-}) are oxidized directly at the sediment-water interface where reduced chemicals from the sediments come into contact with oxygen.

The greater rate of CSOD (although not statistically significant) can 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. Water chemistry data show that ammonium levels are on average 0.35 ppm in

OSPM-affected wetlands and 0.14 ppm in reference wetlands. Plant root simulator (PRS) probes (although only showing bioavailable concentrations of compounds in the sediment) indicated twice as much ammonium in OSPM-affected wetlands (Slama in prep.).

Sulfide concentrations (measured using PRS probes) were variable among the wetlands. Higher concentrations of sulphides were expected (but not seen) in OSPM-affected wetlands. 4-m CT was the only OSPM-affected wetland with consolidated tailings sediment (we should see higher concentrations of sulphates and sulphides in these types of wetlands). 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. From our observations, we found that only ammonium was significantly higher in OSPM-affected than in reference wetlands (Slama in prep.). 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.

3.25 Conclusions

Rates of sediment oxygen demand and its components differ among natural, reference and OSPM-affected wetlands. Although not statistically significant (due to our small sample size), OSPM-affected wetlands had higher rates of CSOD and SOD but the lowest rate of BSOD. No difference was seen when natural wetlands were compared to constructed wetlands. CSOD was the largest factor in all cases but oxygen was consumed at a higher rate in OSPM-affected wetlands. We conclude that this is due to increased concentrations of reduced molecules, particularly ammonium. The fate of ammonium may be chemically or biologically driven but it appears to have an important role related to SOD. Greater rates of CSOD and overall SOD have the potential to alter ecosystem function. With dissolved oxygen being depleted at a higher rate, organisms such as zoobenthos 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. Slama (2010) 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.

APPENDIX

3.26 Light chamber deployment

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.

3.27 WOOTAnG in July

With apologies to Gordon Lightfoot; a song detailing our Fort McMurray exploits.

Em D
Em D G Em
C Em C Em C Em C Em
D Em

WOOTAnG in July
Gas trapping madness has touched the countryside
And through the veg and blackflies, we'd rather be inside
The beavers eat our rockets and our driver's permit de-nied

WOOTAnG in July
WOOTAnG in July
The weather starts to turn, they're calling for some snow
We run to get things finished, but our waders are filled with holes
And the hydrolabs aren't working, they won't record D.Ooooo

WOOTAnG in July
WOOTAnG in July
In the streets of Fort McMurray there's a friendly drunken sound
Jesse's throws a floor crawl,
And Kurt is passed out on ground
Upon the filthy carpet, his dropped room key can't be found

WOOTAnG in July
WOOTAnG in July
Jesse's done his thesis, now he'll set the world on fire
And the world cup plays at the hotel and Carsten begins to cry
And no one is breaking until Jan's will be SATISFI…ed

WOOTAnG in July

In the lab back at Windsor there's nothing that is known for sure
The cell-phone is ringing but Charlie lost it swimming
And they wonder how we sampled and they can't read our labels
And it wasn't just our writing and it's not that we weren't able

WOOTAnG in July
WOOTAnG in July
Chro-mata-graphic analysis drives the undergrads wild
They run a 1000 samples while Jesse and Carsten play at the lake
And the undergrads are sampling, and the grads are not awake

WOOTAnG in July
WOOTAnG in July
Safety hats and lifejackets are now the hipster trend
And the water's 6 feet deep, and we look like we're working, but we really just pretend
And the waders are in the ATCO and you scream "when will this end"

WOOTAnG in July
WOOTAnG in July
The samples are finally coming but our dreams are quickly dashed
And the blue truck isn't working and you've locked in all the keys,
And you curse the gods that made you, why does everything break on me??

WOOTAnG in July

In the Office the Synrude site, Terry laughs with great delight,

We run and cry to Lori C while the undergrads plan A mutiny
And then Chris B comes rolling in, to patch things up as best he can
There is no time to hesitate we really don't want to work that late
WOOTAnG in July
WOOTAnG in July
The wetlands in McMurrary are quiet and serene
But the beaver runs are hidden and strike terror to the heart
And wonder how you got here and you Wonder why'd I start?
Why didn't we just do lab work why do we love the field
But Saskatoon needs more samples our fates have all been sealed

WOOTAnG in July
WOOTAnG in July
Publication stardom has touched the countryside
And the researchers want to be us and the girls all want to see us Waiting for revisions, completing a CFRAW thesus

WOOTAnG in July

WOOTAnG in July

WOOTAnG in July

- *Jesse Gardner Costa, April 2010*

Appendix Table 3.1. 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 3.2 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
	AVERAGE	**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	0.721	NR	NR	NR
	AVERAGE	**0.738 ± 0.194**	**3.5 ± 3.5**	**96.5 ± 3.5**	**1.28 ± 0.896**
Test Pond 9	OSPM	1.440	0.21	99.79	1.902
Mike's Pond	OSPM	0.508	0	100	NR
Natural Wetland	OSPM	3.022	0.86	99.14	NR
4mCT	OSPM	1.687	4.27	95.74	0.134
	AVERAGE	**1.664 ± 0.519**	**1.34 ± 0.995**	**98.7 ± 0.993**	**1.02 ± 0.884**

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Chapter 4: Estimating relative abundance of bacteria within sediments of constructed wetlands in north-eastern Alberta using ATP luciferase assays

4.1 Expectations

In Chapter 4, I estimated microbial sediment biomass using an ATP assay as a proxy specific to bacteria. I sampled collected sediment cores to a depth of 14 cm from the study wetlands and measured ATP concentration, particle size distribution, loss on ignition carbon content, 1.0, 5.1 and 10.2 cm from the surface of each sediment core.

1) Since our wetlands fall under 'older' classification and Daly (2007) found higher abundances of heterotrophic bacterioplankton in 'older' OSPM-affected than in 'older' reference wetlands, likely due to water enriched carbon (from naphthenic acids > 20 mg/L) , I expected to find greater biomass in the sediments of OSPM-affected wetlands than in sediments of reference study wetlands as these sediments were also likely enriched with naphthenic acids and residual bitumen.

2) I expected to detect more bacterial biomass within the top layer (1-2 cm) of wetland sediments than deeper sediments. Microbial biomass tends to be higher in soils that contain greater organic content and higher concentrations of nutrients (carbon and nitrogen - Inubushi & Acquaye 2004). Microbial biomass decreases with increasing soil depth from aerobic surface layers to deeper anaerobic zones (Inubushi & Acquaye 2004). Furthermore, organic matter and nutrients accumulate at the sediment surface (Batzer & Sharitz 2006); fewer nutrients occur at greater depths, and consequently less microbial biomass is expected to occur there. Our study wetlands typically have shallow organic layers (1-5 cm), with clays or oil sands tailings below, reflecting the short periods of time since their construction. If microbial biomass is limited by the availability of nutrients and by soil type (loose organic layer vs. impermeable clay layer), then I would expect to find less microbial biomass in deeper sediments than in the surface sediment layer.

4.2 Introduction

Over the last 20-30 years microbes have become recognized as significant contributors to food-web function in a variety of ecosystems (Kuznetsov 1970, Christofferson *et al* 1990, Batzer & Sharitz 2006, Kraigher 2006 – oceans, lakes and

wetlands), especially aquatic systems (Simon 1987, del Giorgio & Williams 2005). 'Microbes' refer to a diverse variety of organisms including, algae, bacteria, protists and fungi (Batzer & Sharitz 2006). Their ubiquity in nature (Kladec & Knight 1995, Madigan *et al* 2003, Batzer & Sharitz 2006), versatile use of electron acceptors (Madigan *et al* 2003, Batzer & Sharitz 2006) and often abundant populations (Austin & Findlay 1989) greatly influence processes in the systems they inhabit. Understanding these effects requires basic characterization of the resident microbial communities. The objective of this study was to estimate the relative biomass and stratification of heterotrophic bacteria in sediments of wetlands located in the boreal region of north-eastern Alberta, Canada.

Microbiology's recognized importance can be partly attributed to the development of new measurement techniques. Older studies often estimated abundance (most probable number (MPN), Foyt *et al.* 1985) using staining techniques (Porter & Feig 1980), which could not distinguish between living and dead cells, and which required hours of counting under a microscope. Molecular techniques have been developed for determining microbial abundance and production. The adenosine triphosphate (ATP) assay uses a stable-form luciferase enzyme to measure relative numbers of active bacteria in water or sediment samples. Older studies that employed ATP were relatively inaccurate, largely due to the instability of the luciferase enzyme (Eydal & Pedersen 2007). Recent modifications of the procedure have produced assays that are more reliable, faster, and simpler than staining. The ATP assay is specific to live cells, accurate to within 10 cells/sample in some species (http://www.promega.com, Eydal & Pedersen 2007).

Micro-organisms play an important role in wetlands of north-eastern Alberta, where excavation of oil sands results in wetland loss and eventual replacement with 'constructed wetlands' (Oil Sands Wetland Working Group 2000). Newly constructed wetlands lack the habitat complexity (vegetative or otherwise) and community diversity that characterize mature wetlands, which can affect plant (J. Hornung, Suncor Energy, Inc., unpubl.) and invertebrate (Leonhardt 2003) communities. Microbes break down organic matter, influencing nutrient and carbon release within a system (Azam 1983, Batzer & Sharitz 2006, Ciborowski *et al.* 2006). They are the first colonizers of a new system, initiate food-web cycling, and facilitate colonization by higher trophic level

organisms (Austin & Findlay 1989). They play a crucial role in establishing food-web dynamics within these systems.

Wetlands built in the oil sands post-mining landscape typically incorporate Oil Sands Process Materials (OSPM; Fine Tailings Fundamentals Consortium FTFC 1995) - tailings clay, sand, and water containing elevated concentrations of salts, ammonia (NH_4+), naphthenic acids, and sulphate (SO_4^-). The constituents of OSPM-affected wetlands may impede macrophyte establishment (J. Hornung, Suncor Energy, Inc. unpubl., Bishay 1989 – in this thesis plant production and biomass are reported to not be significantly, negatively affected by OSPM even though values are slightly lower in OSPM wetlands), zoobenthic community composition (Leonhardt 2003) and production (Ganshorn 2003) and growth of invertebrates (Martin 2010). Although groups of microbes have been identified, the effects of OSPM on their abundance in wetland sediments have only begun to be characterized.

Groups of microbes that utilize these compounds have been identified in OSPM-affected wetlands. Sobolewski (1999) identified nitrate-reducing and sulphate-reducing bacteria within and at the interface of tailings sediment and water. Del Rio *et al.* (2006) and Videla (2007) have identified and studied microbes that metabolize naphthenic acids as a carbon source. Daly (2007) used 4'-6-diamidino-2-phenylindole (DAPI) staining to assess bacterioplankton in oil sands associated wetlands. She found that bacterial abundances were higher in 8-15 year-old OSPM-affected wetlands than in reference wetlands of equivalent age. These studies recognize the importance of microbial communities and potential for detoxification in OSPM-affected wetlands. These studies illustrate how the constituents of an aquatic system influence microbial community composition and ultimately carbon and mineral flow.

Methods

4.3 Study sites

The study wetlands are located in and near Syncrude Canada Ltd. and Suncor Energy lease areas in north-eastern Alberta (Chapter 2). We studied three categories of wetlands - OSPM-affected, reference, and natural.

OSPM-affected wetlands are constructed wetlands (formed in the mined landscape and containing mine tailings materials (OSPM: solids and water). The surficial and sediment pore waters exhibit elevated salinities and naphthenic acid concentrations, and their sediments may contain trace quantities of bitumen or petroleum coke. Sediments consist of mature fine tailings (MFT), consolidated tailings (CT) or clay, possibly covered ('amended') with a layer of organic materials (peat-mineral mix, stockpiled muskeg material, or stockpiled wetland sediments [FTFC 1995]).

Reference wetlands are either naturally formed or constructed wetlands located on mined sites. They do not contain mine-derived fine sediments or water (OSPM). However, wetlands that have formed in areas with sodic substrate may have salinities that approach those found in OSPM-affected wetlands. Substrate consists of sodic overburden (mostly clay), and may be covered ('amended') with organic materials (peat-mineral mix, stockpiled muskeg material, or stockpiled wetland sediments [FTFC 1995]).

Natural Wetlands are naturally formed, unaffected by OSPM, and are located in areas that have not been mined – relatively undisturbed areas. They may be of indeterminate age, or may be of very recent origin, being formed by flooding as a consequence of either beaver or human activity. Substrates represent the native soil that existed at the time the wetland formed plus organic material that has accrued since its formation. These wetlands are used to test whether reference wetlands, whose age and successional status are similar to those of OSPM-affected wetlands, differ in flux and production from their undisturbed, unconstructed, older counterparts.

Nine wetlands (4 OSPM, 3 reference, 2 natural) were studied. The chosen wetlands fit within the CFRAW matrix (Chapter 1), allowing one to test for differences among wetlands differing in age class, initial organic content and status (OSPM or reference or natural). Each wetland has been mapped (area, depths) and is subject to complementary ongoing studies of food web processes at higher trophic levels. (Ciborowski *et al.* 2006). Those data will be used to relate carbon flow to this work.

4.4 Water chemistry

Water quality measurements (dissolved oxygen, conductivity, salinity, and temperature) were made using a YSI model 85 meter. These measurements were taken

from each wetland at the time of gas collection and SOD sampling (see Table 4.1). Water was also collected once from each wetland in June 2009 for comprehensive water chemistry analysis (Syncrude 1995).

4.5 Microbial biomass sample collection

Field collection 2009: Wetland sediment samples were taken using 5-cm diameter x 21 cm long Lucite coring tubes to determine the distribution of microbial biomass in the upper portion (top 10 cm) of sediment. Analyses of cores previously taken from the field (appendix table 4.1) indicated that the organic layer in these wetlands did not typically exceed 5 cm in depth. The cores were collected contemporaneously with gas flux and sediment oxygen demand measurements as a complement to the studies undertaken in constructed wetlands (Chapter 2 & 3). A core of substrate (14 cm long) was taken 20 cm away from each gas-sampling microcosm site using 5-cm diameter coring tubes. Samples were taken prior to microcosm placement. The top and bottom of the tube was capped (retaining the wetland water overlying the sediment), sealed with Parafilm and electrical tape and stored in the dark, refrigerated at 4 ^{o}C until ATP analyses could be completed at the University of Windsor (November 2009). Cores were collected at 6 microcosm sites per wetland in each of 9 wetlands (54 samples) in early June 2009.

4.6 Organic carbon content (loss on ignition)

Sediment samples were processed in the laboratory to determine organic carbon content (through loss on ignition) and particle size distribution using methods based on ASTM designations D-2974-87, D-2977-71, and C-136-96a, with modifications for hand sieving. Excess water was poured off and wet mass of the sample (approximately 50 g) measured. The cores used for ATP analysis (sediment cores from OSPM-affected and reference wetlands) had been separated into three fractions; top, middle and bottom. Top samples consisted of the sediment layer extending from the water-sediment interface (0 cm) to 3 cm depth into the sediment. Middle-sample aliquots extended from the 5 cm to the 8 cm mark. Bottom samples were taken from the 10 –13 cm portion of the sediment core sample.

The sediment was spread on a sheet of aluminum foil, air-dried at room temperature for 48 h, oven dried at 105°C for 24 h, cooled in a desiccator, and weighed (to 0.01 g) to determine dry mass. Water content was calculated from oven dried sample using the equation:

$$\text{Water content (\%)} = \frac{[(A - B) * 100]}{A}$$

Where A is sample wet mass (g) and B is sample dry mass (g). The oven-dried samples were ground with a ceramic mortar and pestle to break up clumps of dried material following standard methods (Almedeij *et al.* 2010), transferred to a ceramic crucible and incinerated in a muffle furnace, at 550°C until a uniform colour was reached and weight remained constant (typically this took 2 h or more). Samples were weighed to the nearest mg. Loss on ignition was determined by first calculating % carbon content using the equation:

$$\text{Carbon Content \%} = 100 - \frac{(C \bullet 100)}{B}$$

Where C is sediment ashed mass (g) and B is oven dried mass (g).

4.7 Particle Size Distribution

Ashed sediment samples were used to determine the particle size distribution and median particle size. Ground samples were hand sieved through a standard brass sieve series. Mesh sizes were 1.0, 0.5, 0.25, 0.125, and 0.063 mm (there were no particles >2.00 mm). The dried sample was placed in the top sieve of the series. The stack of sieves was then agitated using a side-to-side and up-down motions for 2 min. Material retained in the top sieve was re-ground, returned to the top sieve, and the sieves were agitated again. The material remaining in the top sieve was emptied into a weighing boat, and the top sieve was discarded. The procedure was repeated with the materials in to 0.500 mm sieve. The process was repeated until the sediments retained in each sieve had been ground and re-sieved once. Subsequently, each size fraction was weighed to the nearest mg.

Median particle size distribution was determined by interpolation after plotting cumulative sample mass against a modified Wentworth Scale value – The Krumbein *phi* scale (log 2 (particle diameter)) (Krumbein & Sloss 1963).

4.8 Relative Microbial Biomass estimations

We measured the relative amount of living microbial material in cores collected from the study wetlands in June 2009 using the Promega BacTiter-Glo cell viability assay. We mixed a stable form of beetle luciferase (an enzyme that bioluminesces when in contact with adenosine tri-phosphate (ATP), Promega technical manual 2006). The PROMEGA proprietary solution containing luciferase extracts ATP from live bacterial cells, with which the enzyme reacts (ATP) and luminesces. The degree of luminescence is linearly correlated with the number of microbial cells present in a sample. Different species tend to have different concentrations of ATP within a cell (Eydal & Pedersen 2007), so the assay is typically calibrated with the species of interest to increase precision. However, field-collected samples contain a heterogeneous, wetland-specific mixture of species. This makes calibration of luminescence to a single-species calibration curve meaningless. As a result, exact cell densities could not be calculated from the luminescence values.

Microbial communities have been characterized in our study area (not the same study wetlands) (Sobolewski 1999, Hadwin *et al.* 2005, del Rio *et al.* 2005, Penner 2006, Videla 2007), However, the investigators studied or reported only on particular groups of species (naphthenic acid degraders (Hadwin *et al.* 2005, del Rio *et al.* 2005, Videla 2007)) or determined biomass in tailings only (Sobolewksi 1999, Penner 2006). This study evaluates differences in microbial biomass (ultimately carbon storage/utilization) among OSPM-affected, reference, and natural wetlands. Future investigations that more fully characterize microbial communities in these wetlands may permit lab calibration of the ATP-assay and empirically derive conversion factors that can then be retroactively applied the results of this study.

Cores were removed from cold storage and subsampled (5-mm diameter sediment core sample) on the day of measurement. A wide mouth (5-mm ID) pipette was inserted into the sediment sample. Twenty mm long plugs of sediment were then taken from this subsample core at distances of 1.0, 5.1, and 10.2 cm from the sediment-water interface (denoted top, middle and bottom, respectively). These depths, on average, corresponded to points at which sediment colour (and presumably characteristics) changed in each sample. The top 2 cm of any core typically consisted of loosely packed organic matter,

whereas it transitioned to denser materials, typically sand or clay at the bottom. Carbon content (estimated from loss on ignition) was determined from other sub-samples of each section of the core.

Cored sediments from chosen sediment depths were placed in each of three 15-mL vials and weighed before and after the addition of sediment to determine the amount of sediment used (usually 0.5-1.5 g). Four mL of water was added to a vial and mixed to create sediment slurry. Three additional slurry samples were taken from each core layer and autoclaved to serve as sterile reference samples (containing sediment but no living cells) from which to determine background luminescence for analyses. Reference sample luminescence readings were subtracted from live-microbe sample values to correct for residual luminescence.

The luciferase enzyme was mixed in a 1:1 ratio with BacTiter-Glo buffer solution, and kept on ice before use. Using a micropipette, 100 µL of enzyme was mixed with 100 µL of sediment slurry inside a 1.2-mL micro-centrifuge tube. Samples were incubated with the enzyme for 5 min. Each tube was vortexed for 10 s during incubation and shaken just prior to measurement. Luminescence was measured using a PROMEGA Glomax 20/20 luminometer (model # 2030-100) in relative light units (RLU) to generate a relative estimate of the number of live cells within each sample.

4.9 Statistical Analyses

Statistical analyses were performed using Statistica® version 7.0 (Statsoft, Inc., Tulsa, OK). Statistical significance of differences among OSPM-affected, reference and natural wetlands with respect to relative microbial abundance (measured in Relative Light Units) and stratification in the sediment were tested using a 3x3 (3 wetland classes, 3 sediment depths) factorial ANOVA. Significance levels were set at $p<0.05$. "Wetland" was the unit of replication (n= 4 for OSPM-affected n= 3 for reference, n= 2 for natural). All data were Log_{10} transformed prior to analyses. The relationship of both water content and Loss on Ignition (LOI) to ATP concentration (Relative Light Units) was evaluated using Spearman's rank correlation analysis.

Results

4.10 Wetland water chemistry

There were no consistent differences in salinity and conductivity between reference and OSPM-affected wetlands (Table 4.1). Reference wetlands ranges of salinity and conductivity were 0.2-1.5 parts per thousand (ppt) and 313-2,980 µS/cm, respectively. Consequently, mean values for reference wetlands did not differ significantly from those of OSPM-affected wetlands. The pH in OSPM-affected wetlands was typically higher than reference (7.97-8.87 vs. 7.18-8.52, respectively; Table 4.1). Naphthenic acid concentrations were 10-fold higher on average in OSPM-affected wetlands. All wetlands were well oxygenated (6.68-12.5 mg/L), with the exception of Beaver South (3.01 mg/L).

4.11 Qualitative Sediment Attributes

Generally, reference cores had dark organic layers that extended from the sediment surface only a few cm deep. There was a gradual transition from the dark colour of the organic surface layer to subsurface materials consisting of sand or hard grey clay. With the exception of Natural Wetland, OSPM-affected cores had little to no organic material and were made of soft, sandy grey clays. There was noticeable iron oxidation (reddish coloured precipitate) in some of the cores suggesting the presence of iron oxidizing bacteria in the sediments. Also, some of the cores showed a black film on the sides of the core tube, evidence of the presence of sulphides and sulphur reducing bacteria. Qualitative descriptions of each core sampled are summarized in Appendix Table 4.1.

Water content in the cores ranged from 68.5 % (Southwest Sands Beaver, top portion) – 24.5 % (Beaver South Wetland, bottom portion). Generally, water content decreased linearly from the top portion of a core to the bottom. Loose organic material is more porous than hard clays, so we would expect more water in the top portions of a core. Reference and natural wetlands tended to have higher water content than OSPM-affected wetlands. Percent water content was significantly positively correlated with ATP

Table 4.1. 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°	pH	Cond. (µS)	YSI DO (mg/L)	YSI Salinity (ppt)	NO_3 (ppm)	SO_4 (ppm)	Iron (ppm)	NH^{4+} (ppm)	Naphthenic acids (ppm)	ORP (mV)	Sediment Redox Potential (mV)
OSPM	Mike's Pond	NR	8.45	4550	NR	NR	BDL	565	BDL	0.137	26	106	-188.6
OSPM	Natural	17.7	8.85	1090	8.27	0.5	27	134	0.499	1.08	33	87	-206.0
OSPM	4 m CT	15	7.97	2300	6.68	1	1.4	335	0.04	BDL	30	124	-214.1
OSPM	Test Pond 9	23.9	8.87	2050	12.08	1	BDL	97.8	BDL	BDL	19	79	-220.5
Reference	Shallow Wetland	18	7.53	391	7.2	0.2	1.4	6.58	0.036	BDL	<1	113	-151.0
Natural Reference	South-west sands	13.8	7.92	1252	9.82	0.6	BDL	437	BDL	BDL	<1	119	-204.3
Reference	Beaver Golden	19.9	8.52	1737	12.5	0.9	2.3	825	BDL	BDL	2	99	-249.8
Natural Reference	High Sulfate	15.2	7.35	2980		1.5	2.2	1650	BDL	0.166	10	124	-350.8
Natural Reference	South Beaver Pond 5	15.9	7.18	313	3.01	0.1	0.47	2.84	0.894	BDL	<1	79	-128.6
		NR	7.90	1260	8.95	0.6	NR	NR	NR	NR	NR	66	-151.7

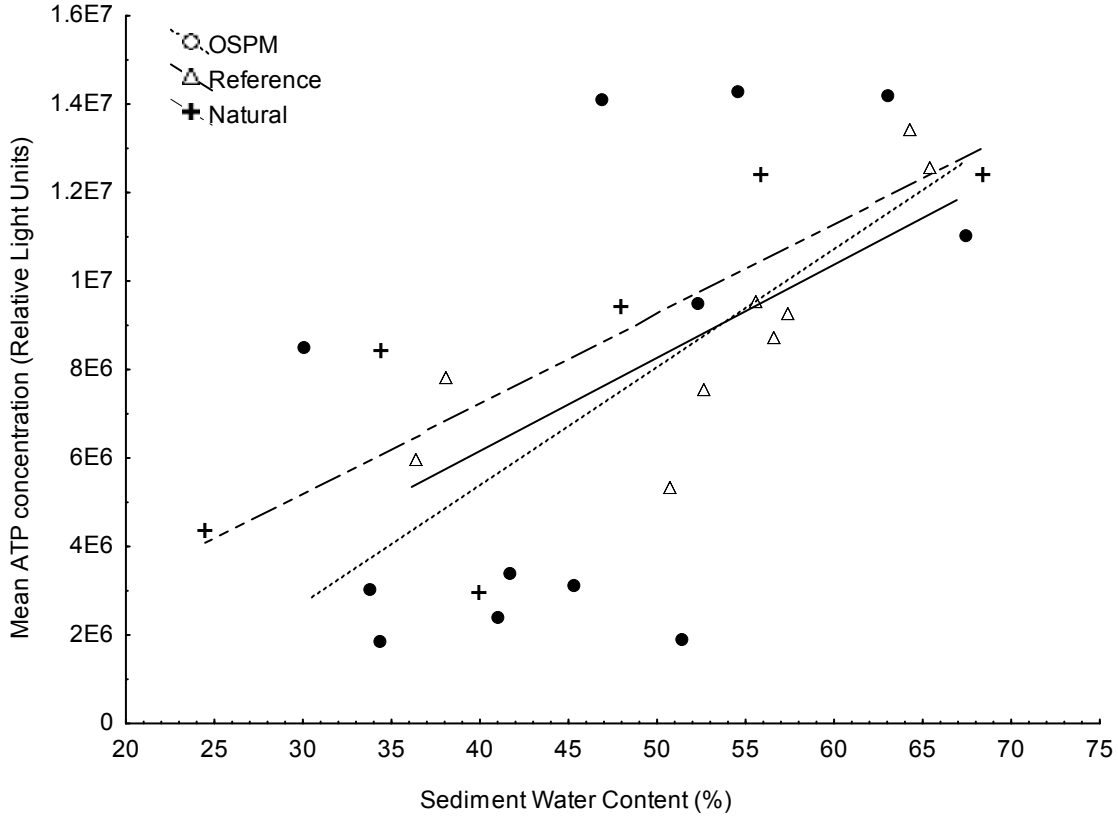


Figure 4.1. Scatter-plot of mean ATP concentration (Relative Light Units) vs. water content (%), comparing microbial biomass with % sediment water content among OSPM-affected, reference and natural wetlands. Lines are fitted by regression in Statistica.

Table 4.2. Particle size distribution Mean % weight (± S.E.) of core samples from each study wetland (9 wetlands) in 2009. Core samples were taken from the top (0 cm), middle (5.1 cm) and bottom layers (10.2), relative to the top of the sediment in. Median particle size was determined at the 50th percentile of each sample using cumulative percentages. Krumbein Log_2 values of 2 and 3 are identified as fine sand and very fine sand, respectively.

			Mean Particle size distribution % ± S.E. (sieve diameter size indicated in the row below – the number in brackets represents the Krumbein Log_2 value)						Median Particle size: Krumbein Log_2 value
Wetland Class	Wetland	Depth (cm)	Below 0.063 mm (5)	0.063 mm (4)	0.125 mm (3)	0.25 mm (2)	0.5 mm (1)	1 mm (0)	
OSPM	4m CT	1	7.0 ± 2.9	16.3 ± 3.8	39.0 ± 4.8	33.8 ± 3.5	3.9 ± 0.6	0.0 ± 0.0	3
		5.1	4.5 ± 2.2	21.4 ± 4.0	39.4 ± 4.9	31.5 ± 4.6	3.1 ± 0.6	0.0 ± 0.0	3
		10.2	6.8 ± 3.2	11.4 ± 2.4	43.7 ± 4.1	35.3 ± 5.7	2.8 ± 0.7	0.0 ± 0.0	3
	Mike's Pond	1	1.9 ± 0.4	11.0 ± 1.9	48.5 ± 2.2	28.9 ± 1.0	9.7 ± 3.5	0.0 ± 0.0	3
		5.1	2.8 ± 0.5	11.5 ± 1.3	35.1 ± 7.5	40.7 ± 11.0	9.9 ± 4.4	0.0 ± 0.0	2
		10.2	3.1 ± 1.1	9.8 ± 1.7	40.3 ± 4.5	34.7 ± 0.8	12.1 ± 6.4	0.0 ± 0.0	3
	Natural	1	3.1 ± 1.0	17.0 ± 3.6	51.9 ± 14.6	25.8 ± 11.5	2.1 ± 1.1	0.0 ± 0.0	3
		5.1	1.8 ± 0.7	11.6 ± 5.2	55.9 ± 16.6	26.4 ± 10.8	4.4 ± 3.2	0.0 ± 0.0	3
		10.2	2.3 ± 1.1	15.4 ± 6.6	62.0 ± 11.7	16.9 ± 8.2	3.4 ± 1.7	0.0 ± 0.0	3
	Test Pond 9	1	2.0 ± 1.1	13.6 ± 5.9	37.7 ± 11.4	37.2 ± 11.6	9.4 ± 4.9	0.0 ± 0.0	2
		5.1	1.9 ± 0.2	6.6 ± 0.2	21.7 ± 5.4	41.5 ± 4.8	28.3 ± 9.1	0.0 ± 0.0	2
		10.2	4.8 ± 2.2	11.0 ± 7.3	18.9 ± 5.7	50.7 ± 8.7	14.6 ± 6.5	0.0 ± 0.0	2
Reference	Golden Pond	1	18.5 ± 2.1	31.8 ± 4.5	30.8 ± 0.8	17.0 ± 7.7	1.8 ± 1.1	0.0 ± 0.0	3
		5.1	4.6 ± 1.7	18.5 ± 4.4	40.7 ± 3.4	29.9 ± 2.7	6.2 ± 3.5	0.0 ± 0.0	3
		10.2	20.1 ± 0.0	24.9 ± 0.0	22.0 ± 0.0	30.9 ± 0.0	2.2 ± 0.0	0.0 ± 0.0	3
	High Sulphate	1	11.5 ± 4.4	21.0 ± 1.4	38.0 ± 2.4	20.8 ± 1.4	6.4 ± 2.2	2.3 ± 4.0	3
		5.1	6.7 ± 1.0	19.2 ± 1.8	41.9 ± 1.9	26.2 ± 1.0	6.0 ± 1.6	0.0 ± 0.0	3
		10.2	4.6 ± 2.8	22.6 ± 4.3	43.0 ± 4.1	28.3 ± 1.6	1.5 ± 0.8	0.0 ± 0.0	3
	Shallow	1	6.2 ± 1.2	14.1 ± 5.6	33.4 ± 9.5	44.1 ± 14.2	2.2 ± 0.7	0.0 ± 0.0	2
		5.1	6.6 ± 3.1	13.2 ± 5.2	33.8 ± 7.2	44.8 ± 13.7	1.6 ± 1.4	0.0 ± 0.0	2
		10.2	5.7 ± 3.7	14.7 ± 6.3	38.2 ± 6.1	35.7 ± 12.8	5.7 ± 3.3	0.0 ± 0.0	2

Continued on next page

Natural	Beaver	1	9.4 ± 8.1	13.7 ± 5.9	29.5 ± 3.1	34.8 ± 5.7	12.6 ± 5.6	0.0 ± 0.0	2
	South	5.1	4.8 ± 2.7	15.4 ± 3.6	35.8 ± 5.0	36.0 ± 5.3	6.5 ± 2.1	1.5 ± 2.6	3
		10.2	2.4 ± 1.2	20.7 ± 8.2	43.1 ± 4.2	31.1 ± 2.7	2.8 ± 2.3	0.0 ± 0.0	3
	South	1	16.5 ± 1.1	35.4 ± 4.4	26.9 ± 2.2	20.6 ± 3.2	0.5 ± 0.5	0.0 ± 0.0	3
	west	5.1	3.7 ± 1.3	6.3 ± 1.4	38.5 ± 5.4	48.1 ± 4.7	3.4 ± 0.6	0.0 ± 0.0	2
	sands	10.2	8.2 ± 5.6	20.8 ± 8.5	34.3 ± 7.8	36.0 ± 8.6	0.6 ± 0.6	0.0 ± 0.0	3
	Beaver								

concentration for both OSPM-affected, reference and natural wetlands (Spearman's rank correlation, $p<0.001$ $n=9$, $r_s= 0.62$ and 0.76, respectively) (Figure 4.1).

4.12 Particle size distribution

Sieved sediment samples showed relatively uniform median particle size across wetland classes (Table 4.2). Most samples had a median particle size in the 0.125-mm range – identified as very fine sand on the Krumbein scale (modified from the Wentworth scale). Two wetlands, one reference (Shallow Wetland) and one OSPM-affected (Test Pond 9) both had median particle sizes of 0.25 mm (fine sand) sieve diameter for the bottom, middle and top portions of each core. The sediments of these wetlands were coarser than others. However, the differences are minimal, and median particle size still falls under the fine sand category. The median particle size fraction was almost always the modal fraction by weight of sieved samples.

4.13 Loss on ignition – organic content

Values of LOI within cores were variable among OSPM-affected, reference and natural wetlands, ranging from 23.8 % (Southwest Sands Beaver, middle portion) to 4.1 % (Beaver South Wetland, middle portion). We had expected to find more organic carbon in the top layer of each core. However, there was no consistent trend. Some wetlands had higher carbon content in the middle or bottom portions of the cores than in the top portions. Overall, the LOI in cores did not vary significantly among sediment depths ($p>0.05$; figure 4.2). Both OSPM-affected and natural wetlands showed no correlation, but reference wetlands had a non-significant positive trend between LOI and ATP concentration. Wetlands with noticeably more organic matter (Southwest Sands Beaver, Golden Pond – assessed from qualitative descriptions of each core used) showed higher % carbon content than wetlands with thin organic layers (Beaver South and Mike's Pond). One exception, 4-m CT had the 2nd highest organic percentage (22.4 %, middle portion), likely attributable to pockets of hydrocarbons that would have been burned off during incineration.

ATP concentration was not significantly correlated with LOI in either OSPM-affected, natural or reference wetlands (Spearman's rank correlation, $p>0.05$, $r_s = 0.17$ and 0.37, respectively) (figure 4.3).

4.14 2009 core samples

Mean values of the relative amount of bacteria (RLUs) for wetland class and each wetland for the top, middle and bottom portions of a core are summarized in Tables 4.3 and 4.4, respectively.

Relative bacterial biomass ranged from 14.1-1.8 million RLUs (Table 4.3). Surprisingly, the extremes of this range both occurred in one core from a single wetland (4- m CT Wetland). The top cm of this core had the greatest value, and the 10-cm assay gave the smallest value. Overall, relative biomass in OSPM-affected wetlands was only marginally higher in the top portion of cores compared with natural or reference wetlands, while lower when comparing middle and bottom portions (Table 4.3; Fig 4.4).

Bacterial abundances in the middle and bottom layers of sediments of most of the OSPM-affected wetlands (Mike's, Test Pond 9 and 4-m CT) were much less than the abundances in the top section of the core (Appendix figure 4.1). With the exception of Golden Pond, there was a linear decline in relative bacterial abundance from the top of the sediment to the bottom of the core (Appendix figure 4.1.). In Test Pond 9 (OSPM-affected) and Golden Pond (reference wetland), microbial biomass in the middle portion was lower on average than biomass in the bottom portion of the cores. The differences between the middle and bottom portions of Golden Pond and Test Pond 9 appear to be negligible. Both the middle and bottom portions of these two wetlands are clearly lower than biomass in the upper portion of the cores; this is the general trend for our study wetlands.

Using an analysis of covariance we compared wetland status (natural, reference and OSPM-affected) with sediment sample wet-mass as the covariate for each layer (top, middle, and bottom) of the wetland cores. Sediment mass was not a significant covariate for ATP luminescence for any layer of sediment (ANCOVA $p>0.05$, $F=2.56$)

Within a core, the relative number of bacterial cells (RLU) was significantly

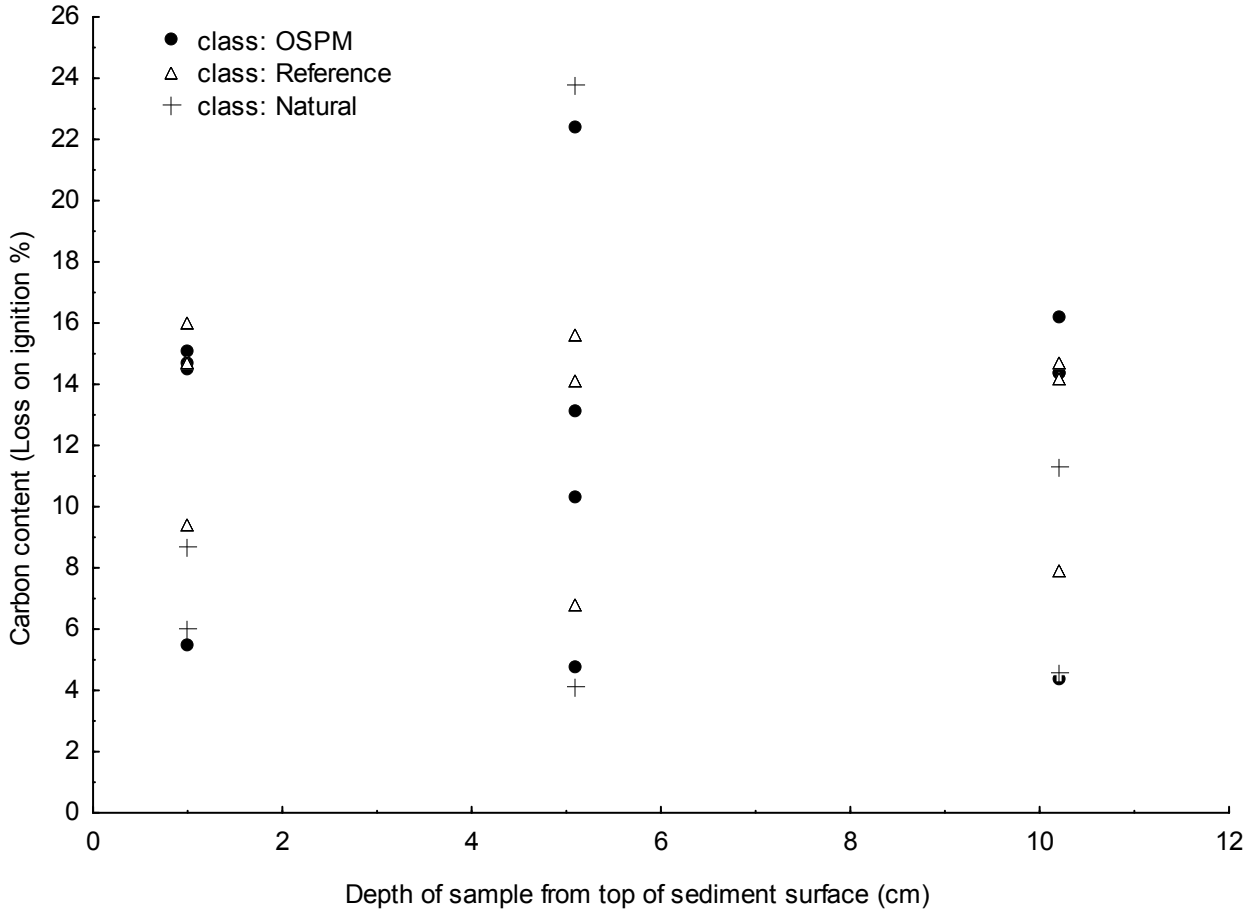


Figure 4.2. Scatter-plot of loss on ignition (LOI) carbon content I with depth of sediment sub-sample at 1, 5.1 and 10.2 cm in the sediment for OSPM-affected, reference and natural wetlands. There is no apparent trend for LOI with depth in the first 10 cm of the sediment.

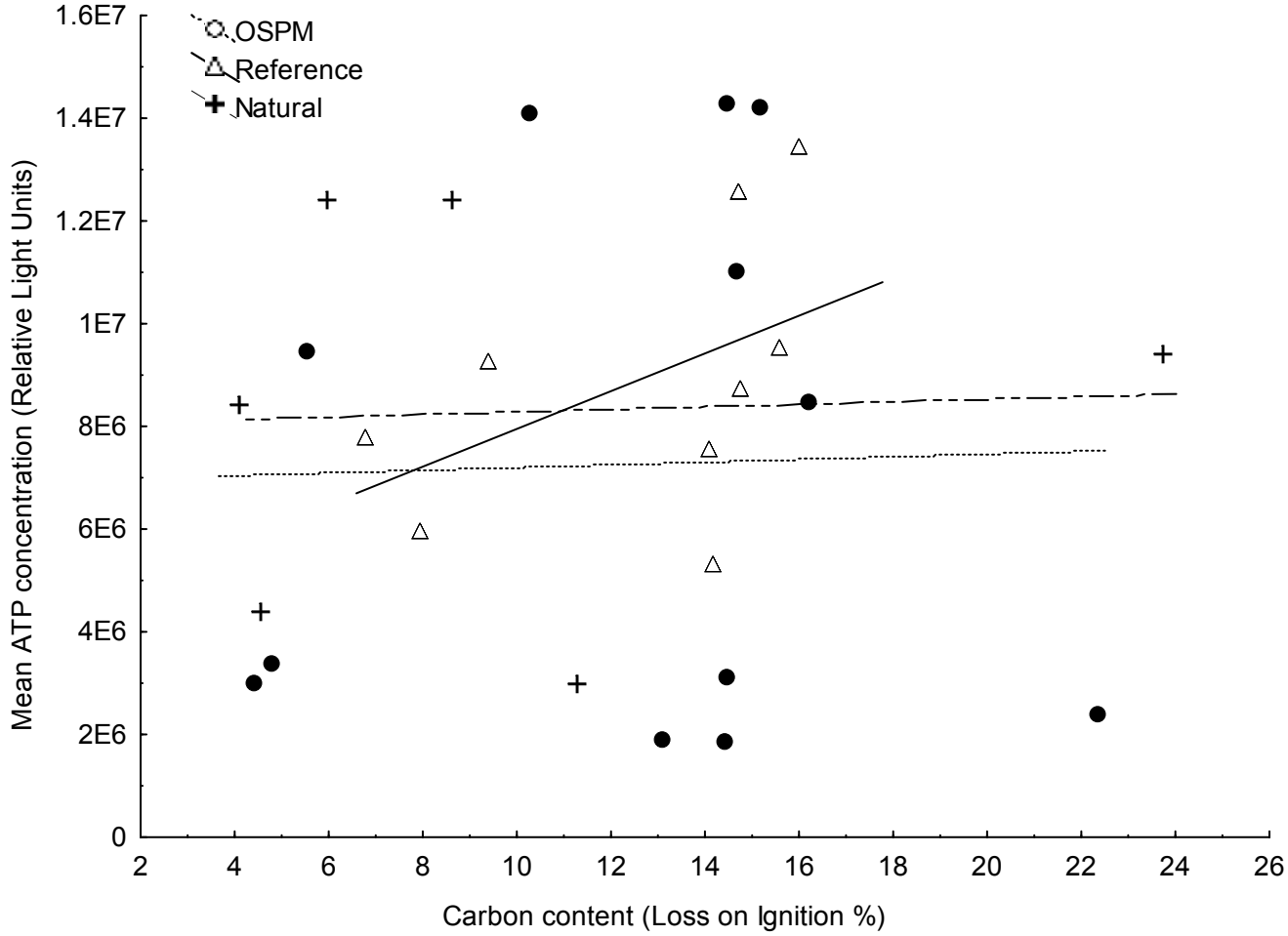


Figure 4.3. Scatter-plot of mean ATP concentration (Relative Light Units) vs. Carbon content (Loss on Ignition %), comparing microbial biomass with % sediment carbon content among OSPM-affected, reference and natural wetlands. Lines are fitted by regression.

Table 4.3. Mean (RLU ± S.E.) values of ATP concentrations in sediment cores samples from OSPM-affected, reference and natural wetlands, 2009. Top, middle and bottom refer to the subsample taken from each core relative to the top of the sediment in a wetland.

	OSPM-affected	**Reference**	**Natural**
Top portion (1 cm) of a core sample			
ATP Concentration (relative light units %)	12,171,201± 1,178,372	11,695,857 ± 1,287,395	12,372,440 ± 15,195
Middle portion (5.1 cm) of a core sample			
ATP Concentration (relative light units %)	5,383,440 ± 2,858,955	8,257,722 ± 608,178	8,866,671 ± 495,501
Bottom portion (10.2 cm)of a core sample			
ATP Concentration (relative light units %)	4,047,147 ± 1429784	6,636,141 ± 1,051,351	3,642,797 ± 696,465

Table 4.4. Mean (± SE) values of ATP concentrations in sediment cores, water content of sediments (%) and Loss on ignition (ash content %) of core samples from each study wetland (9 wetlands) in 2009. Core samples were taken from the top (1 cm), middle (5.1 cm) and bottom layers (10.2), relative to the top of the sediment.

Wetland Class	Wetland	Depth of Core (cm)	Mean ATP RLU ± S.E.	Mean Water content (%) ± S.E.	Mean Ash content (%) ± S.E.
OSPM	4m CT	1	$1.41 \times 10^7 \pm 2.32 \times 10^6$	63.1 ± 7.5	15.1 ± 2.3
		5.1	$2.39 \times 10^6 \pm 8.96 \times 10^5$	41.1 ± 1.0	22.4 ± 5.7
		10.2	$1.85 \times 10^6 \pm 5.88 \times 10^5$	34.4 ± 1.3	14.4 ± 2.6
	Mike's Pond	1	$9.45 \times 10^6 \pm 2.18 \times 10^6$	52.3 ± 8.4	5.5 ± 0.5
		5.1	$3.36 \times 10^6 \pm 1.01 \times 10^6$	41.7 ± 0.9	4.8 ± 0.4
		10.2	$3.00 \times 10^6 \pm 7.74 \times 10^5$	33.7 ± 3.1	4.4 ± 0.5
	Natural	1	$1.41 \times 10^7 \pm 1.24 \times 10^6$	54.6 ± 13.5	14.5 ± 1.8
		5.1	$1.39 \times 10^7 \pm 5.35 \times 10^6$	46.9 ± 12.1	10.3 ± 2.3
		10.2	$8.25 \times 10^6 \pm 8.00 \times 10^5$	30.1 ± 3.7	16.2 ± 3.6
	Test Pond 9	5.1	$1.87 \times 10^6 \pm 4.24 \times 10^5$	51.4 ± 1.3	13.1 ± 0.0
		1	$1.10 \times 10^7 \pm 1.52 \times 10^6$	67.5 ± 5.0	14.7 ± 7.3
		10.2	$3.08 \times 10^6 \pm 1.22 \times 10^6$	45.3 ± 0.9	14.4 ± 0.2
Reference	Golden Pond	1	$1.34 \times 10^7 \pm 2.61 \times 10^6$	64.2 ± 8.0	16.0 ± 6.5
		5.1	$7.54 \times 10^6 \pm 1.42 \times 10^6$	52.6 ± 12.9	14.1 ± 7.1
		10.2	$8.71 \times 10^6 \pm 1.25 \times 10^6$	56.6	14.7
	High Sulphate	1	$1.25 \times 10^7 \pm 2.03 \times 10^6$	65.5 ± 6.0	14.7 ± 2.8
		5.1	$9.47 \times 10^6 \pm 2.04 \times 10^6$	55.6 ± 10.9	15.6 ± 3.9
		10.2	$5.29 \times 10^6 \pm 1.22 \times 10^6$	50.7 ± 8.0	14.2 ± 2.9
	Shallow	1	$9.18 \times 10^6 \pm 7.48 \times 10^5$	57.4 ± 1.7	9.4 ± 2.0
		5.1	$7.77 \times 10^6 \pm 7.84 \times 10^5$	38.1 ± 5.5	6.8 ± 1.9
		10.2	$5.91 \times 10^6 \pm 1.53 \times 10^6$	36.4 ± 9.0	7.9 ± 3.8
Natural	Beaver South	1	$1.24 \times 10^7 \pm 2.10 \times 10^6$	55.9 ± 4.0	6.0 ± 0.8
		5.1	$8.37 \times 10^6 \pm 1.99 \times 10^6$	34.5 ± 5.1	4.1 ± 0.5
		10.2	$4.34 \times 10^6 \pm 1.22 \times 10^6$	24.5 ± 2.3	4.6 ± 1.8
	South west sands Beaver	1	$1.24 \times 10^7 \pm 1.84 \times 10^6$	68.5 ± 15.9	8.7 ± 1.7
		5.1	$9.36 \times 10^6 \pm 2.10 \times 10^6$	48.0 ± 9.0	23.8 ± 11.4
		10.2	$2.95 \times 10^6 \pm 4.28 \times 10^5$	40.0 ± 8.3	11.3 ± 1.5

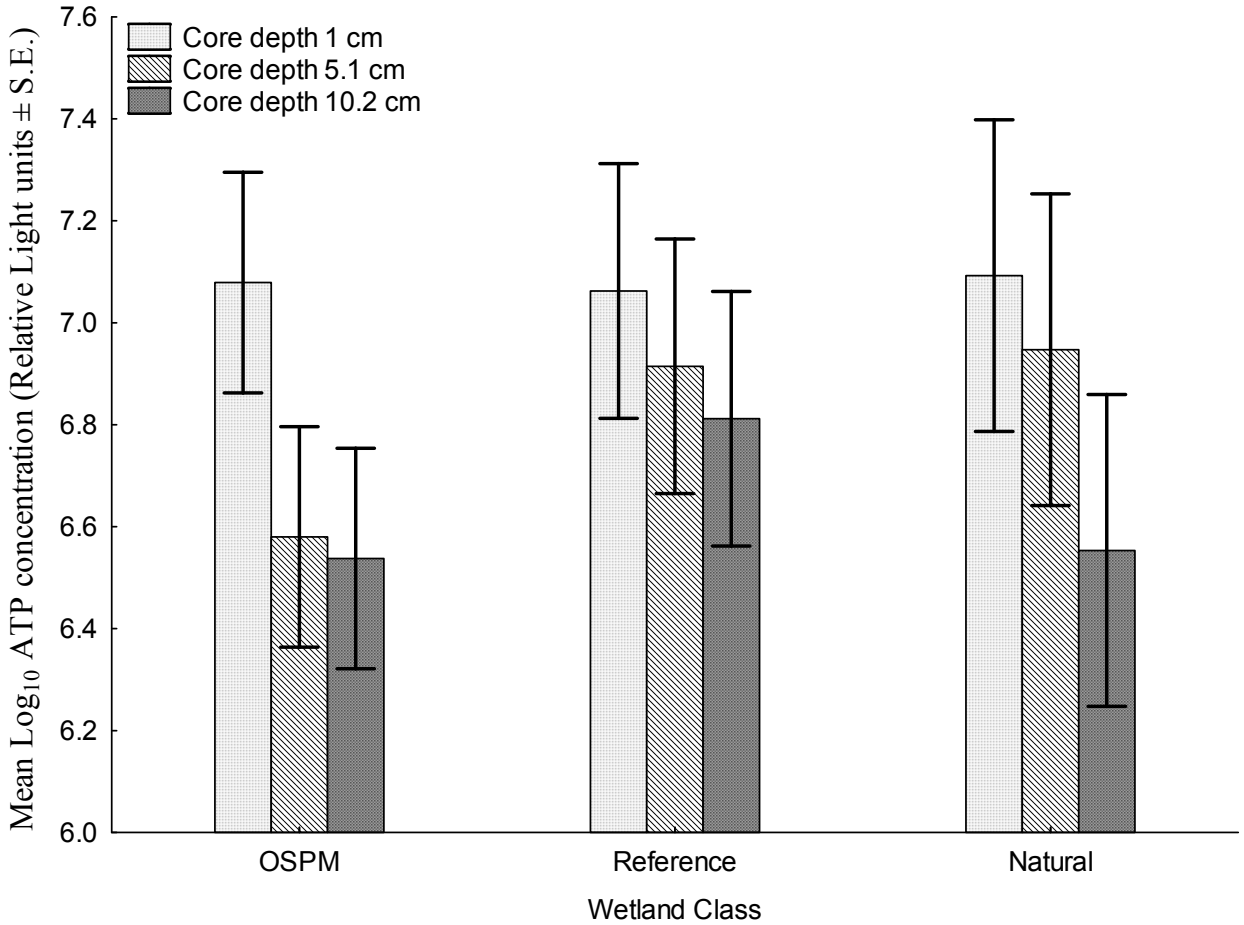


Figure 4.4. Mean (± S.E.) relative ATP concentrations in sediment cores samples from OSPM-affected, reference and natural wetlands, 2009. Core depth refers to the depth each ATP sample was taken from the top of the sediment within a core

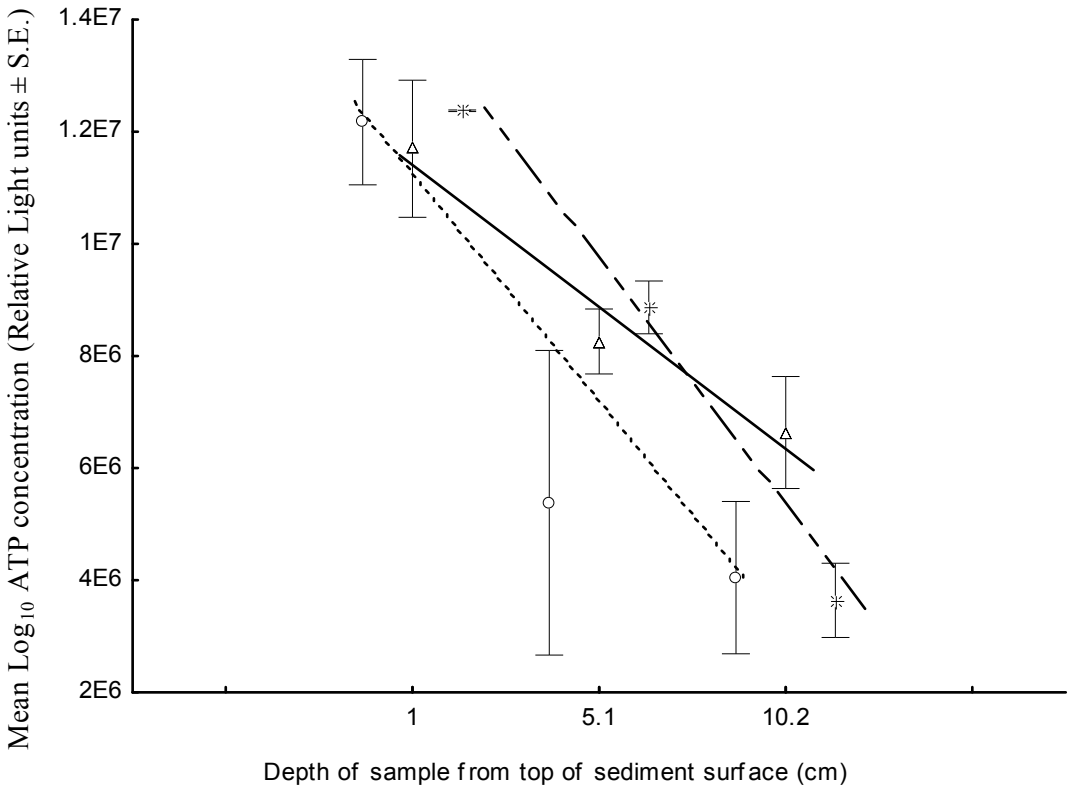


Figure 4.5. Mean (± S.E.) values of ATP concentrations in sediment cores samples from OSPM-affected, reference and natural wetlands, 2009. The slope of the line in each graph shows the decline of relative bacterial cell numbers with increasing depth. Regression lines fit by Statistica.

higher in top portion of a core sub-sample (1 cm) than in the lower sub-samples of a core (5.1 cm, and 10.2 cm), with relative cell numbers dropping the deeper the sub-sample (ANOVA $p<0.05$, $F= 8.08$, Figure 4.4). The decline in cell numbers with depth was linear but heterogeneous between OSPM-affected and reference wetlands (homogeneity of slopes (test of parallelism $p<0.001$ figure 4.5). Cell numbers were lower in deeper sediments in OSPM-affected wetlands than in reference wetlands. Within a wetland core, the middle and bottom samples were not significantly different from each other; there were consistently more bacteria in the middle portion of reference and natural cores than the bottom layer of either reference or natural cores (Figure 4.4).

Discussion

4.15 Carbon Content – Loss on ignition

Ignition: We had expected that both bacterial biomass and carbon content would decrease the deeper into the sediment we measured (as seen in soils, Nelson *et al.* 1994). This was true for bacterial biomass but not for carbon content, one possible energy source for bacteria. Carbon content was nearly uniform from the top of sediments down to clay layers (10 cm). The upper layers of cores typically had loose organic layers, expectedly rich in carbon, whereas the bottom layers of cores were usually sand or clays. The presence of hydrocarbons in the sediments, particularly in OSPM-affected sediments (noticeable in 4 m CT and Test Pond 9, even though Test Pond 9 was constructed of clay, not OSPM) would have altered the amount of carbon in those samples.

It is unknown why reference and natural sediments showed no differences among layers in the cores. Hydrocarbons are a natural constituent of soils in the areas (OSWWG 2000). However, hydrocarbons (bitumen) were not noticeable in the sediments, and are usually not present in high concentrations, certainly not to the extent of OSPM sediments. Thus, the differences in microbial biomass can not be explained by organic carbon content; water content in the sediments may be a better predictor of biomass.

Water content in the cores was significantly correlated with ATP concentration in reference and OSPM-affected wetlands; both variables decreased deeper into the sediments. We expected that water content would be lower in clays than in the looser top

organic layers. The fairly strong correlation of water content and ATP concentration (r_s = 0.63 and 0.76 for OSPM and reference, respectively) coupled with the lack of correlation with organic content was surprising. Nelson *et al.* (1994), observed higher microbial biomass in soils that contained more water soluble carbon; water soluble carbon was shown to decrease with depth. This fraction of carbon was readily available to microbes and it was postulated that lower microbial biomass was due to inaccessibility of carbon. This may be the case in our wetlands, where more water-saturated sediments have more water soluble carbon, which is more easily used by microbes than insoluble forms of carbon such as hydrocarbons (Nelson *et al.* 1994). As a result we see ATP concentrations (a surrogate of microbial biomass) decreasing with water content into the deeper sediments.

4.16 Particle size distribution

There were no differences in mean particle size between wetland classes or among layers of core samples. Although there is evidence that smaller particle sizes allow for higher microbial diversity and biomass in soils (Sessitsch *et al*. 2010), particle size appeared to have no effect on ATP concentrations in our study wetlands' sediments. Underlying substrate in wetlands has been observed to influence microbial communities (Gutknecht *et al.* 2006). Many of the reference wetlands' substrates are made of sandy clays with an overlying rich organic layer, whereas the OSPM-affected wetlands tended to be lined with soft clays with little to no top organic layer. We ran a test of parallelism among core samples taken from the top, middle and bottom of a core with sample depth as our covariate. The slope (change in biomass vs. depth) was statistically significantly different (depth's effect depended on wetland status) among wetlands (Figure 4.5). The reference and natural wetlands show a clear linear drop the deeper the core sample, while the OSPM-affected wetlands show a large drop from top layer samples to the middle layer, little to no change between the middle and bottom samples. This trend is most apparent in wetlands built with clay substrates and with little to no organic layer on top, suggesting influence from the substrate type. Particle size, however, was not different at different depths or between OSPM-affected and reference wetlands; it is possible that the

physical and chemical properties of the underlying substrate may influence microbial communities rather than particle size.

With the possibility of recalcitrant carbon (hydrocarbons or otherwise) confounding our loss on ignition observations and no apparent trend with particle size in our wetland sediments, water content seems to be the best predictor of ATP concentration within our sediments. Future studies should resolve labile and refractory sources of carbon in the sediments; this may yield better predictors of determining microbial biomass and ultimately, carbon flow through a wetland.

4.17 Relative bacterial biomass

We did not find an overall significant difference in relative bacterial numbers (RLU) among natural, reference and OSPM-affected wetlands; on average there were more bacteria in reference than in OSPM-affected wetlands (Table 4.3). We had expected to find higher bacterial biomass in OSPM-affected than in reference wetlands because of previous findings of higher bacterioplankton abundances in overlying waters of OSPM-affected wetlands than reference wetlands (Daly 2007). There are 3 likely possibilities that may explain our counterintuitive results. Each of these should be evaluated in future studies.

1) Microbial communities and biomass may differ as postulated. However, each community is suited to its environment and grows to roughly the same densities that each wetland will support.
2) Within a wetland, there is considerable site to site variation, and we had insufficient replication to provide the power necessary to distinguish differences among wetlands. There is evidence of this from examining the cores (see core descriptions in Appendix table 4.1), as well as variation associated with gas flux measurements taken at the same time of core collection.
3) There is high variation in the amount of ATP per cell produced by different species (species diversity/community structure can be addressed by quantitative Polymerase Chain Reactions (PCR)) of bacteria that potentially large differences in bacterial abundances are masked.

4.18 Differences in microbial communities

Wetland water chemistry, sediment substrates and related research conducted during this study (Chapter 2 & 3) all suggest that there should be marked differences in the microbial communities among the 3 wetland classes.

OSPM-affected wetlands tend to have elevated salinities, naphthenic acid and sulphate concentrations relative to reference and natural wetlands (Table 4.1), which promote development of salt tolerant/halophilic, naphthenic acid metabolizing (del Rio *et al.* 2006) and sulphur reducing (SRB) (Batzer & Sharitz 2006, Gutknecht *et al.* 2006) bacterial species, respectively. When microbial communities are identified in reference wetlands we would expect to find fewer or none of these types of organisms compared to the OSPM-affected wetlands. There is indirect evidence of the presence of SRBs in OSPM-affected wetlands, manifested in the form of methane gas flux (Chapter 2) measurements made at the time the sediment cores were collected. More methane was released in reference than in OSPM-affected wetlands for both 2008 and 2009 studies. Note - a reference wetland, High Sulphate, aptly named for its high sulphate concentrations (table 4.1) released no measurable amounts of methane during our study periods. Sulphur-reducing bacteria inhibit methanogenic bacteria (Batzer & Sharitz 2006), and little or no methane was released (chapter 2) from wetlands whose waters had higher concentrations of sulphates (Table 4.1). This suggests that reference wetlands support methanogenic bacteria and lack (or have few) sulphur-reducing bacteria relative to the OSPM-affected wetlands.

Sobolewski (1999) and other studies (del Rio *et al.* 2006, Penner 2006, Videla 2007) have characterized microbial functional groups within tailings and newly constructed OSPM-affected wetlands. Nitrate-reducers, iron-reducers, sulphate-reducers, and methanogens were found within tailings ponds. Knowledge of functional group characterization over community composition will be important to improve the understanding of the biogeochemical cycling of minerals within these wetlands.

Comte & del Giorgio (2010) observed no correlation between community composition and community function across a number of gradients (including boreal wetlands in southern Quebec). Therefore, determining the community composition may not shed light on the movement of carbon or minerals. An approach to trace respiratory

end-products such as carbon dioxide (as done in this thesis, chapter 2 & 3) is likely more useful than community characterization. These comparisons are important in providing guidance for wetland reclamation strategies as microbial communities will play a role in nutrient and biogeochemical cycling in these wetlands (Batzer & Sharitz 2006).

The use and amount of oxygen present at the water-sediment interface also influences microbial community composition. Aerobic or anaerobic processes dominate in near-surface sediment layers, depending on the amount of oxygen present and how quickly it is depleted or replaced (Batzer & Sharitz 2006). Higher (2x) oxygen consumption at the water-sediment interface was observed in OSPM-affected than in reference wetlands (chapter 3). Since microbial biomass in the top portion of sediments is apparently no different among wetland classes, higher (~2x) oxygen consumption in OSPM-affected wetlands than natural or reference suggests that differential oxygen use may be chemically driven rather than biologically mediated in OSPM-affected wetlands.

4.19 Within-Wetland variation

There was considerable variation with respect to bacterial biomass within the sediments of each wetland (Table 4.4). We had anticipated high variation among core samples, consistent with observations of high spatial variability of gas flux found among microcosms from gas collected in the study wetlands (Chapters 2 and 3) in 2008 and 2009. Other studies also document high variability both in gas flux and spatial heterogeneity (del Giorgio & Williams 2005, Batzer & Sharitz 2006, Chanton *et al.* 1989) in aquatic systems. Gas flux serves as a surrogate for microbial activity (del Giorgio & Williams 2005, Gutknecht *et al.* 2006, Bubier 1995, Chanton *et al.* 1989), which is a function of microbial biomass. We assumed that differences in gas flux should be due to differences in microbial activity, due to either (or both) microbial production and biomass. In terms of biomass, this does not seem sufficient to explain differences in methane and carbon dioxide flux from these wetlands.

The depth and type of substrate differed from core to core within a single wetland (see core descriptions Appendix table 4.1). For example, some Golden Pond cores had a thick top organic layer (≥7 cm) whereas other Golden Pond cores were mainly clay. Some of the cores taken from Golden Pond had evidence of high iron oxidation (presence

of orange precipitates), whereas others exhibited no oxidation whatsoever. Wetland sediments and the microbial assemblages living within them (especially in reference and natural wetlands), are not uniformly distributed. In addition, we worked within the 30-70 cm range of water depth within these wetlands; some areas of these ponds are 3 - 4 m deep. Sampling in these deeper areas would likely add to the within-wetland variation. Future studies should focus on detailed characterization of different areas of a wetland and use planned comparisons to compare similar areas in reference wetlands with OSPM-affected wetlands.

4.20 ATP assay

With no prior knowledge of the of cellular ATP concentration within micro-organisms living within wetlands sediments I assumed that concentrations of ATP would be relatively similar among bacterial species, however, environmental factors may invalidate this assumption. Temperature, nutrient availability (starved or not) and growth phase (dividing or metabolizing) of the bacteria are important factors influencing the concentration of ATP in microbes (Stoek *et al*. 2000, Eydal & Pedersen 2007). Differences in temperature, nutrient availability and growth phase would add more variation within wetlands rather than among them and thus would minimize the chances of mistaking a non-significant result with a significant one. In order to resolve these issues and estimate accurate measures of biomass within these wetland sediments, future studies should focus on the following approach:

1) Identification – At the very least, groups of organisms (methanogens, sulphur reducers etc.) should be identified to determine the functional feeding groups that are dominant within each wetland. Based on the observations and expectations in this thesis, and technical reports from the oil sands industry (Sobolewski 1999) one could tailor culture media to the expected organisms and resolve the community by functional groups. If species identification is desired, Polymerase Chain Reactions (PCR) and phospho-lipid fatty acid (PLFA) analysis are two very specific (and expensive) techniques for species identification (Gutknecht *et al.* 2006).

2) Biomass turnover - Bacterial production can be assessed to determine cell turnover, a proxy for carbon cycling. Radiolabelled leucine and thymidine incorporation

are often used to determine cell turnover. This technique has proven useful. It is specific to live heterotrophic bacterial cells (Kirchman 2001). Heterotrophic bacteria uptake radiolabelled leucine (and/or thymidine) added during experiments (Kirchman *et al* 1985, Buesing & Gessner 2003). Leucine can be incubated with fresh sediment samples where bacteria uptake free amino acids and use them to make proteins. Rates of incorporation of leucine into proteins are monitored by extracting proteins and measuring their radioactivity, giving protein synthesis rates. Synthesis rates are used to give an estimate of cells produced per sample over an experimental period (typically 1 h).

Daly (2007) used leucine incorporation to determine bacterioplankton turnover rates within our study wetlands. She found that reference wetlands had higher turnover rates than OSPM-affected wetlands, suggesting quicker cycling of carbon within these wetlands. Combined with biomass estimates one could estimate the quantity and movement of carbon within a wetland. Bacteria associated with sediment can have higher densities than are measured in the water column (Kirchman 2001). Determining the quantity and flow of carbon from sediment microbial communities may prove to be a large component of carbon flow within the sediments.

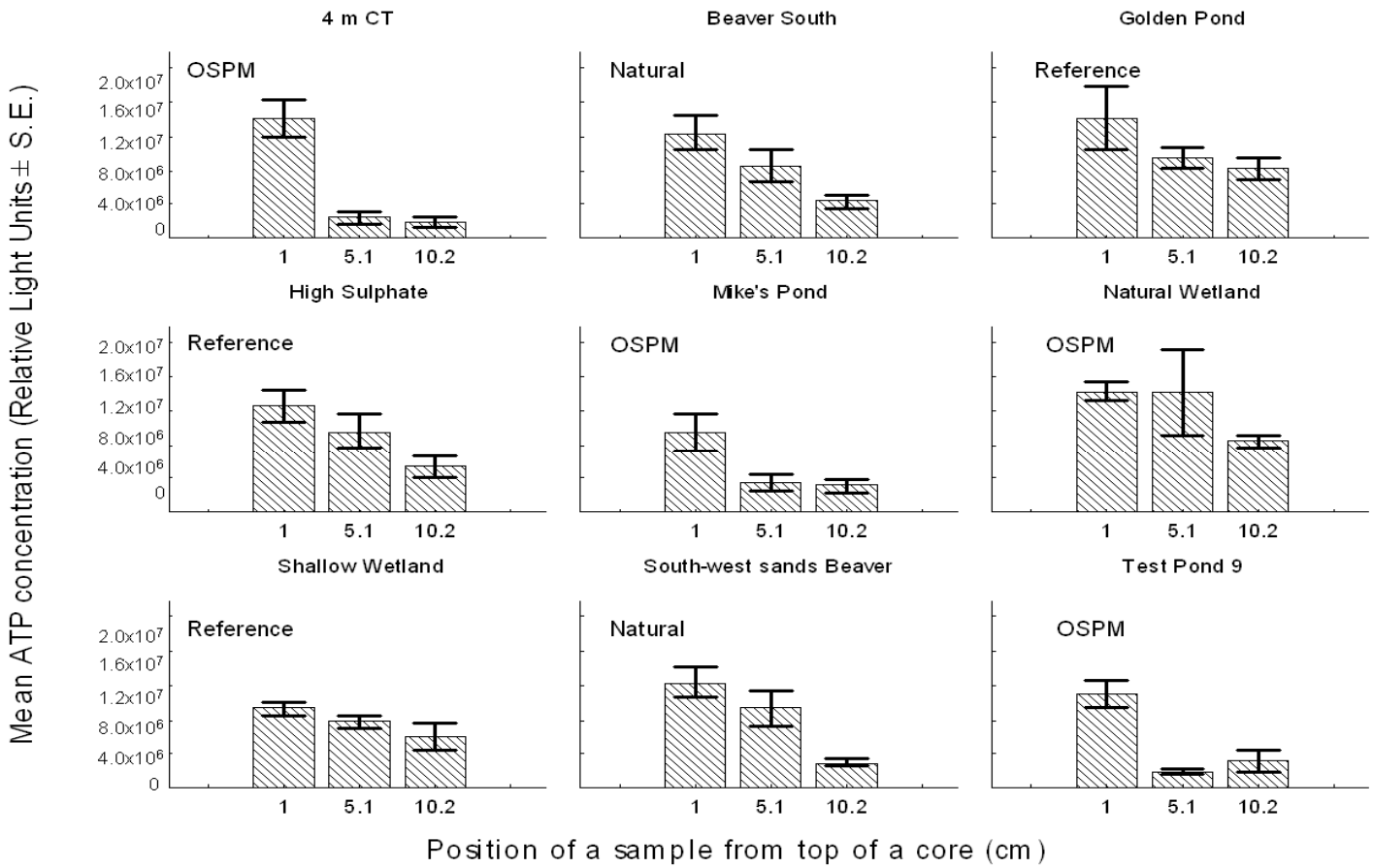
3) Calibration- ATP-assays can be run with known numbers of cells from wetland samples to build a standard curve of ATP concentration vs. cell numbers. Once calibrated, these values can retroactively convert this chapter's data into cell numbers. If the proportion of species within samples is known, multiple-species assays can be performed to account for any interactions among species on the signal output. For the purpose of this thesis and the CFRAW project, it would be most efficient to focus on functional groups of microbes rather than individual species and determine their roles within wetland food-webs.

4.21 Conclusion

We compared the relative amount heterotrophic bacteria in the top 10 cm of sediments in constructed and natural wetlands of north-eastern Alberta. We found no statistically significant differences among natural, reference and OSPM-affected wetlands. We did observe a decrease in ATP concentration with increasing depth. The rate of decrease was lower in reference wetlands than in OSPM-affected wetlands. In

terms of respiration (chapters 2 & 3), we would expect more respiration in sediments with higher microbial biomass, the fact that biomass is highest in the top portion of the sediment would suggest the wetland gas we observed in chapter 2 & 3 may be produced predominantly by this top layer. Biomass was correlated with water content but not with loss on ignition. Particle size of the sediments also had no effect on ATP concentrations observed. The composition of the sediments likely influenced the amount and type of microbes living within them. Overall, our observations demonstrate a need for identification of microbial functional groups to investigate acclimation and/or differentiation of microbial assemblages in their wetlands to better understand these organisms' role in wetland food-webs.

Although there are other logical possibilities that might account for the patterns observed: e.g., initial inoculation during wetland construction, successional stage of the wetland, influence of higher trophic levels on microbe assemblages, we currently do not have the evidence to consider them more than speculative. The three possibilities explained to account for the lack of differences in ATP concentration among wetland classes are likely the most important next questions to pose about microbial communities - their importance in wetland food-webs and how to measure microbes in constructed wetlands. Addressing any one of these possibilities will shed light on the others.



Appendix Figure 4.1. Mean (± S.E.) values of ATP concentrations in sediment core samples from each study wetland in 2009. (4 OSPM-affected, 3 reference wetlands and 2 natural wetlands).

Appendix Table 4.1. Qualitative descriptions of each core that was sampled for ATP analysis from 2009. This table gives a basic description of the composition and thickness of each layer (top, middle and bottom, 1, 5.1 and 10.2 cm from the top of the sediment surface respectively. Core # refers to which sampling site the core was taken within a wetland

Wetland Class	Wetland	Core #	Position of a sample from top of a core (cm)	Description
OSPM	4m CT	3	1	<0.5 cm loose organics material,
			5.1	Consolidated tailings throughout core (sand
			10.2	and clay), more bitumen deeper into a core
		4	1	<0.5 cm loose organics material,
			5.1	Consolidated tailings throughout core (sand
			10.2	and clay), more bitumen deeper into a core
		2	1	<0.5 cm loose organics material,
			5.1	Consolidated tailings throughout core (sand
			10.2	and clay), more bitumen deeper into a core
	Mike's Pond	1	1	
			5.1	Light grey coloured clay throughout core, no
			10.2	organic material
		5	1	
			5.1	clay topped with black organic material
			10.2	throughout the core
		4	1	
			5.1	clay topped with black organic material
			10.2	throughout the core
	Natural	4	1	Thing organic layer ~1 cm)
			5.1	
			10.2	fine sand
		6	1	
			5.1	Loose organic material ~ 7 cm
			10.2	sand
		5	1	
			5.1	Loose organic material ~ 7 cm
			10.2	sand
	Test Pond 9	3	1	Soft light grey clay throughout, black near
			5.1	the top of the core, possible sulphur
			10.2	oxidation
		6	1	
			5.1	
			10.2	Soft light grey clay throughout
		1	1	Soft light grey clay throughout, black near
			5.1	the top of the core, possible sulphur
			10.2	oxidation
Reference	Golden Pond	4	1	
			5.1	
			10.2	Loose black organic material, very fine
		3	1	~6 cm of rich black organic material

			5.1	
			10.2	Hard grey clay
		2	1	3 cm of rich black organic material
			5.1	Hard grey clay lots of iron oxidation around
			10.2	the sides of the core
	High Sulphate	2	1	3 cm of rich black organic material
			5.1	
			10.2	Dark grey clay
		3	1	3 cm of rich black organic material
			5.1	
			10.2	Dark grey clay
		5	1	
			5.1	
			10.2	Black organic material throughout
	Shallow	5	1	1 cm loose organic layer
			5.1	organic material mixed with clay and sand
			10.2	clay and sand
		4	1	1 cm loose organic layer
			5.1	organic material mixed with clay and sand
			10.2	clay and sand, iron oxidation present around the bottom of the core
		2	1	3 cm of loose organic material
			5.1	
			10.2	Clay
Natural	Beaver South	3	1	1 cm organic layer
			5.1	Hard grey clay
			10.2	
		4	1	2 cm organic layer
			5.1	Hard grey clay
			10.2	
		5	1	Hard grey clay
			5.1	
			10.2	
	South west sands Beaver	6	1	2 cm rich, black organic material
			5.1	organic material mixed with blackish clay
			10.2	blackish clay
		4	1	2 cm rich, black organic material
			5.1	organic material mixed with blackish clay
			10.2	blackish clay
		3	1	2 cm rich, black organic material
			5.1	organic material mixed with blackish clay
			10.2	blackish clay

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Chapter 5: General discussion, recommendations and conclusions

5.1 General Conclusions

The goal of this project was to characterize and quantify carbon flow via sediment-associated microbial respiration in permanently flooded areas of constructed wetlands in northeastern Alberta. Additionally, we determined the effects of oil sands process material (OSPM) on microbial biomass and respiration through gas flux, sediment oxygen consumption and by measuring ATP concentrations in wetland sediments. The research presented in this thesis provides a baseline understanding of the movement of microbial respiratory carbon end-products within constructed wetlands in the northern boreal area. We investigated the release of carbon dioxide and methane from sediments and related it to use of oxygen at the water sediment interface and the microbial biomass of bacteria within wetland sediments.

Overall we found that:

1) The sediments of most study wetlands were exporters of carbon dioxide and methane, OSPM-affected wetlands released less gas than reference wetlands, which released less gas than natural wetlands, and gas release overall was variable;

2) Oxygen use was primarily a result of chemical oxygen demand (COD) rather than biological oxygen demand, and COD was proportionately greater in OSPM-affected wetlands than in reference wetlands;

3) Microbial biomass (estimated from concentrations of ATP extracted from sediments) did not differ between OSPM-affected and reference wetlands. Microbial biomass declined with increasing sediment depth. Biomass was correlated with sediment water content, but not with organic carbon (Loss on ignition carbon content) or particle size in OSPM-affected and reference sediments.

All measures associated with microbial activity were highly variable, indicating strong spatial heterogeneity in these biological processes. These systems are complex and heterogeneous, and the movement of carbon depends on a number of factors. This work will help to illuminate the role of microbial communities within a food-web.

5.2 Implications

The study wetlands released relatively little carbon dioxide and methane compared to other boreal wetlands (Del Giorgio & Williams 2005, Table 2.8). Despite low emissions, most wetlands released CH_4 and CO_2 and were supersaturated with CO_2. Although most of these wetlands would be considered a 'carbon source', the true measure of a carbon sink would consider the net effect of the primary production/ respiration ratio and see whether more carbon accumulates than is respired (del Giorgio *et al.* 1997, Kayranli *et al.* 2010). If these wetlands are carbon sources then we would conclude that the wetland systems are heterotrophic and would need continuous or recurring allochthonous carbon inputs to sustain a food-web. Del Giorgio *et al.* (1997) reviewed production and respiration in a number of aquatic systems and found that unproductive systems (<100 µg C/L d) are most often heterotrophic. These young study wetlands would be classified as 'unproductive' and are most likely heterotrophic systems. It is important to note that flux values taken from our study include only permanently inundated areas of a wetland. Future work should compare production and respiration values in both the wetland and 'wet meadow' periphery (including carbon respiration and input from emergent plant zones) to determine whether constructed wetlands are net autotrophic or heterotrophic. These wet meadows likely have more respiration than permanently inundated areas of wetlands (this study) and will likely bridge the gap between our reported values and literature values.

If these wetlands are heterotrophic, they will not accumulate organic matter and thus won't develop into a condition that performs the equivalent function of pre-mining peatlands. Furthermore, adding organic amendments may only provide a carbon pool that is largely respired back into the atmosphere. Perhaps the study wetlands are still relatively young (< 20 y), but with extensive macrophyte development within and around the wetland, macrophyte communities may shift from net heterotrophy to net autotrophy, resulting in the long-term accumulation of carbon.

In terms of reclamation, there are 3 factors that the CFRAW research group studies because they are thought to be important influencing factors for wetland communities – wetland age (Craft *et al.* 2002), vegetation (ultimately carbon accrual)

(Craft *et al.* 2002) and wetland status (OSPM-affected vs. reference) (Ciborowski *et al.* 2006).

Our wetlands are young relative to natural wetlands, which have developed over the course of decades or centuries (Bridgham *et al.* 2006). The study wetlands may not have the carbon stores to utilize and release as much carbon as other boreal wetlands. We investigated whether wetland age influenced a variety of dependent variables; carbon flux, sediment oxygen consumption, relative microbial biomass but found no consistent, significant relationships. This may be a result of our operational criterion for designating 'older' and 'young'; we classified wetland age (≤7 years = young, 8+ years = older) on the time by which the number of invertebrate families stabilizes following wetland creation (Leonhardt 2003). Although this operational classification may be better than arbitrary assignment, it is evidently inappropriate for the non-invertebrate components of the food-web that I studied. Scatterplots (not shown) of methane and carbon dioxide flux show no relationship with wetland age. We do not have enough data points to properly test this relationship; however, it does suggest that wetland age may not be a useful metric to predict gas flux. It also suggests that flux does not increase linearly with time over the range of wetland ages that were available for examination in this study; increased gas flux may not be an appropriate endpoint for successful reclamation, but can give insight into carbon movement from a system.

Microbial communities change as conditions change; the concept of succession and categories of old and young are inappropriate if microbial habitats are ephemeral (Jaatinen *et al.* 2008). Microbial biomass and respiration depend largely on bioavailable organic carbon (Nelson *et al.* 1994). Available organic carbon depends on carbon stores and water chemistry & regimes (Craft *et al.* 2002, Kayranli *et al.* 2010). We would expect then that changes in microbial biomass and gas flux would correlate with carbon accrual and availability (soluble carbon vs. recalcitrant). Carbon accrual may be a function of age (Craft *et al.* 2002, Hossler & Bouchard 2010) because macrophyte community establishment is a major source of carbon within natural systems (Bridgham *et al.* 2006). If macrophyte establishment is time-dependent, then carbon accrual within a wetland is also dependent on time. Constructed wetlands may take 3-5 years to establish macrophyte communities comparable to natural wetlands (Craft *et al.* 2002) and

anywhere from 70-300 years to accumulate carbon comparable to natural wetlands (assuming the wetlands are on trajectory to accumulate carbon, Hossler & Bouchard (2010).

In our study systems, macrophytes will provide carbon to these wetlands but organic amendments (usually peat) are seen as a way to jumpstart carbon accumulation (fast-track a wetland's 'trajectory' to natural equivalent) within these wetlands or at least provide a carbon source for organisms to establish (Bruland *et al.* 2009). Although no correlation between microbial biomass and organic content was found (chapter 3 – although biomass was related with water content which likely reflects the availability of soluble carbon (Nelson *et al.* 1994), wetlands with noticeably more organic matter (from qualitative assessments) appeared to release more gas than wetlands with clay linings. High Sulphate Wetland was an exception to this, where water chemistry was likely the controlling factor in gas flux.

As well, plants can 'vent' greenhouse gases out of a wetland, depleting carbon stores within wetland sediments (Kayranli *et al.* 2010). A thick organic layer that releases less gas than vegetated areas may increase the rate of carbon accumulation, but without vegetation there is no self-renewing source of detrital material (not as big as decaying plant matter at least). Studies are underway to address carbon venting from plants in our study wetlands (F. Mollard, University of Alberta, in prep.).

Carbon accrual depends not only on deposition of organic matter, but also on retention of carbon stores. Craft *et al.* (2002) found that over the course of 15 years, permanently (or more frequently) flooded created saline-wetlands established macrophyte communities and increased soluble carbon and nitrogen faster than drier constructed saline-wetlands. Anoxic sediments are less likely to release gas and more likely to accumulate carbon (Kayranli *et al.* 2010), therefore if organic amendment has any chance to be an effective reclamation strategy wetlands need to be permanently flooded throughout the year. Our study wetlands are usually flooded. However, wet meadows surrounding some our wetlands dry later into the summer and may not benefit from amendment.

It seems reasonable that the prevailing conditions of a wetland – organic carbon pool (soluble carbon vs. recalcitrant, carbon accrual vs. loss), temperature and water

regimes, and water chemistry are more likely to influence gas flux (among other biological components) within a wetland (Kayranli *et al.* 2010). Age may only correlate with some of these factors; for example, salty constructed wetlands can be flushed of their salinity over time, or macrophyte communities establish and wetlands begin to accrue organic matter. These processes would certainly affect microbial communities (Abril & Iverson 2002, Batzer & Sharitz 2006). Processes that affect microbial energy sources and their chemical environment will most likely be the most important determinants of whether a wetland produces a lot of gas or not.

A third alternative is that "legacy effects" may persist long after local environmental conditions have changed. Mummey *et al.* (2002a) studied microbial communities (fungi and bacteria) in reclaimed and pre-mining soils and found lower biomass and species richness in the reclaimed sites compared to pre-mining sites, even 10-20 y after reclamation. If this observation holds in wetlands, almost all of our study wetlands will require many years before their behaviour converges with natural sites. There is little evidence that the carbon sources, upon which the microbial community depends, have changed greatly as a function of time since wetland creation in our wetlands (Chapter 4).

Unlike the study of Mummey *et al.* (2002a), the CFRAW compares reclaimed using tailings materials to other reclaimed sites that do not use tailings materials to reconstruct the landscape. This has the benefit of studying wetlands in similar conditions at the same time. It is somewhat unreasonable to compare undisturbed wetlands that are decades or centuries old to newly formed wetlands (although this was the rationale for studying two 'natural' wetlands - an out-group for our analyses). Although an observer would likely find differences, that information would yield no predictive power as to how new wetlands may look or function when they mature. The lack of differences in gas flux and ATP sediment concentrations between OSPM-affected and reference wetlands may show that although these constructed wetlands differ from undisturbed sites, they function and progress as any newly forming wetland might. Alternatively, small-scale spatial heterogeneity may have been so great that our sampling design lacked the power to detect significant differences. The latter is more likely as trends in my data suggest that reference wetlands produce more greenhouse gas than OSPM-affected and natural

wetland produce more gas than reference. As well, microbial biomass in the lower layers of wetlands sediments appears to be higher in reference than in OSPM-affected wetlands. There is likely some negative impact of OSPM on these wetlands, even if we could not detect them in this study. Long term monitoring would be required to assess whether reference and OSPM-affected wetlands diverge in terms of gas flux and whether constructed wetlands converge to function similar to natural wetlands in the area. Larger sample sizes and microcosms with larger sampling areas may improve our power to detect differences.

In contrast to wetland age, amendment and construction materials, water levels and temperature are two covariates that can immediately and directly influence the composition and function of wetland microbes (Batzer & Sharitz 2006, Kayranli *et al.* 2010). Hydrologic regime (either groundwater or precipitation input/discharge) changes from year to year and among seasons, altering wetland water levels (Zedler 2005). Lowering water levels or periods of drying usually result in an increase of respiration due to an influx of oxygen that promotes organic matter decomposition (Batzer & Sharitz 2006, Kayranli *et al.* 2010). Increased decomposition alters nutrient levels, which influence the growth of organisms within the wetland. As well, decreasing water levels may concentrate salts or other ions (Zedler 2005) changing osmotic conditions and potentially stressing microbes or promoting the growth of salt tolerant species. This effect is frequently observed in the oil sands constructed wetlands and may be especially important in OSPM-affected wetlands where salinities are already high. Great fluctuations in water regime may lead to a situation where microbial communities never reach an 'undisturbed' state. Rather, gas flux and community structure continually change in response to the current and recent hydrological state.

Temperature will also affect microbial communities. Warmer or drier conditions cause ephemeral wetlands (especially those sustained by surface water run-off) to dry out and increase respiration, making sediments less likely to accumulate organic matter (Bridgham *et al.* 2006). The area within the wetlands we studied did not typically dry, but water levels decreased throughout the summer, and temperatures rose (pers. obs.). We did not find any significant differences among sampling times throughout the summer of 2008. However, we worked in at similar depths for all wetlands, plus

differences may have been masked by high variability as seen throughout the data set. Clearly, the wetlands exhibit phenological change through the summer, reflecting the seasonal change of plant, biofilm and invertebrate communities, although we could not detect the microbial consequences of these differences. The microbial changes that accompany seasonal wetland variation are likely most pronounced at the wetland margins (wet meadow areas), which are most subject to wetting and drying.

Perhaps the best criteria would involve aging all study wetlands and performing regression analyses instead of forcing categories unto the data. This approach is more expensive and labour intensive (one would need many more replicates, i.e. wetlands), which may increase wetland-wetland variability - as we've already seen, no two wetlands are created equal.

5.3 Recommendations for oil sands research

Research in the Athabasca oil sands' lease sites within reclamation areas provides the opportunity to apply our research to make recommendations to the oil sands companies that may directly influence reclamation strategies or direct future research in the area. This section will briefly outline observations (to provide general guidelines) from this thesis as well as suggesting future research that may complement my data.

1 a) Most of the study wetlands were exporters of carbon dioxide and methane. The sediments of OSPM-affected wetlands released less gas than reference wetlands, which released less gas than natural wetlands.

b) Compared with other boreal wetlands (Table 2.8), our study wetlands are not significant sources of greenhouse gases. However, our estimates did not take into account the substantial respiration that may occur from the emergent vegetation and wet meadow zones around the periphery of the study wetlands.

c) Methane is not released from permanently inundated wetland sediments when sulphate concentrations in the overlying water are approximately 400 ppm or higher.

2 a) Oxygen consumed at the water-sediment interface of all wetland classes was predominantly (~90%) chemically consumed, suggesting the sediment-associated

microbes in these wetlands are not very productive, especially those of OSPM-affected wetlands.

b) Since OSPM wetlands have low gas emissions and are likely unproductive, then these wetlands can potentially accumulate organic matter. OSPM-affected wetlands are lower exporters of carbon than are natural or reference wetlands and can benefit organic amendment. Further studies are needed to determine a suitable amount of organic amendment for these wetlands.

3 a) Unamended OSPM-affected wetlands have less microbial biomass than reference or natural wetlands. This is likely due to a thinner accumulated organic layer in OSPM-affected wetlands; these wetlands would benefit from organic amendment to `jump start` microbial communities.

4 a) Spatial variation was an important source of variation for all variables studied within this thesis. Gas flux, SOD and relative microbial biomass varied among samples taken only a few cm apart. This made it difficult to generate representative estimates for entire wetlands, which cover hundreds of m^2. Gas flux and SOD measurements would benefit from a larger sampling apparatus that covered greater surface area, minimizing small scale variation in order to facilitate comparisons among wetlands.

b) Temporal variation is expected to be more important than spatial variation in terms of estimating yearly carbon mass balance. Estimates of gas flux and SOD throughout each season must be measured to determine whether reclaimed wetlands will accumulate organic matter on an annual basis.

c) Wetland to wetland variation was also an important source of variation within my studies; variation was greater among reference wetlands than among OSPM-affected wetlands. Wetland classification in the future should account for variation in water chemistry and hydrology to help reduce wetland to wetland variation within a wetland class.

5 a) Gas flux and relative microbial biomass (Chapters 2 & 4, respectively) were determined but not combined. We need quantitative estimates of biomass as well as

biomass turnover rates (microbial production) for sediment microbial communities within our wetlands.

b) Future studies should combine gas flux estimates with microbial biomass to determine gas to mass ratios; this will estimate carbon assimilation efficiencies for the microbial community. Knowing how much carbon is respired with respect to how much carbon accumulated as biomass will show whether wetland sediments are likely to accumulate organic matter over time or not.

5.4 Future studies

Each data chapter concluded with recommendations of topics that require further investigation. The likely next step, however, should consider the sum of the observations already presented. This research should be combined with all other estimations of carbon (organic or inorganic) flow among compartments of a food-web to determine a mass balance of carbon movement within constructed boreal wetlands (Kovalenko *et al.* 2010). Studies in estuaries by Craft *et al.* (2002) and Więski *et al.* (2010) parallel our CFRAW work; they study a suite of constructed and natural wetlands varying from fresh to saltwater. They study carbon accrual and changes in macrophyte communities as wetlands age (studies in the area go back 20 years or more). Within these studies they note the negative impact of salinity on plant production and carbon accrual, which can affect the time until convergence with local natural wetlands.

CFRAW differs from these studies in that we study the movement of carbon in and out of a wetland as well as the effect of mining disturbance and tailing materials on these processes. Convergence with natural wetlands for any particular process is one of many endpoints of this research, but in terms of this thesis, this work will help to resolve carbon inputs/outputs from constructed boreal wetlands. A determination of whether the CFRAW suite of constructed study wetlands are net sinks or sources of carbon requires at the very least a comparison of respiration rates (this thesis, Daly 2007 for microbial estimates; F. Mollard, University of Alberta, in prep for emergent vegetation estimates) to rates of production (primary production: H. Chen, University of Waterloo in prep., C. Wytrykush, University of Windsor, in prep. M.C. Roy, University of Alberta, in prep., K. Frederick, University of Alberta in prep.), and even secondary production: Ganshorn

(2002) Leonhardt (2003), Martin (2010), among others). External inputs of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) should be included for more precise estimates of carbon flow. The inclusion of organic matter decomposition estimates across wetland classes (C. Wytrykush, University of Windsor, in prep.) would give an estimate of carbon turnover. When combined with production and respiration measurements, we can estimate the amount of carbon stored in biomass (plant, microbes, invertebrates), the amount of carbon available for organisms (decomposition), how much carbon is lost from the system (gas flux) and how much carbon is externally loaded within the system (DIC and DOC inputs) – a balance sheet of carbon movement within constructed and natural wetlands.

The research in this thesis provides a key component of understanding carbon movement through a wetland food-web. Specifically, I have quantified sediment-associated biological respiration rates of permanently inundated sedments, a component of decomposition processes. Carbon is the common currency connecting all organisms within a wetland. This allows us to study carbon dynamics without the need to know where exactly the carbon comes from. The 'coarse' scale of this study then is not just a survey of carbon flow from sediment microbial communities but a quantification of the net effect of carbon movement from numerous organisms living with a wetland. Since microbes are a dominant component of inland freshwater systems (Batzer & Sharitz 2006), future research will no doubt lead to the characterization of microbial functional groups, time and funds permitting. Further research may resolve sources of the carbon measured in this study via stable isotopes (Videla 2007, Daly 2007, K. Jurkowski, University of Windsor, unpubl.), but all of these components will have to tie their work back into the wetland as a whole, and in the case of the oil sands, the consequences of reclamation. This thesis lays a foundation for future microbial ecology studies as well as providing carbon movement estimates for one-half of the production/respiration (decomposition) ratio; a simplified metric that will tell us whether or not reclaimed wetlands accumulate carbon in a manner similar to their pre-mining counterparts.

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