Response of Peatland Microbial Community Function to Contamination by Naphthenic Acids and Sodium in the Athabasca Oil Sands Region, Alberta, Canada

by

Vinay Daté

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Author's Declaration:

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Reclamation of closed oil sands mining operations in former pristine boreal ecosystems of the Athabasca Oil Sands Region of Alberta, Canada (AOSR) requires construction of new fen land uses such as peatlands in order to meet the environmental regulatory requirements for restoration of 'equivalent landscape capacity' and because 'wetlands are required as an integral part of the reclaimed landscape' (Alberta Government 2000). Reconstruction rather than restoration is required due to the extensive disruption to the vegetation and hydrology of these sites inherent to the mining process. Such sites will be constructed with tailings sands forming part of the aquifer; consequently, they may be exposed through leaching to a variety of chemical contaminant species either not present (e.g. naphthenic acids) or present at significantly higher-than-baseline concentrations (Na^+) than in the pre-disturbance sites. The presence of these contaminants is likely to affect both the plant and microbial communities, which are the two major players in the carbon cycling function of peatland landscapes, and the effects of these contaminants on the microbial community is unknown in such landscapes. Oil sands process-affected water (OSPW) contains high concentrations of the contaminants to which these sites might be exposed. This study therefore tested the effects of OSPW on the aerobic and anaerobic carbon-cycling potential activity of the microbial communities of a variety of reference peatlands from the AOSR to determine the possible effects these contaminants might have on the communities of these constructed sites, through measurement of substrate-induced respiration (SIR) and methanogenic potential respectively. This study also measured the baseline aerobic and anaerobic carbon-cycling potential of these sites, to provide a reference baseline against which site managers might measure the development of such sites.

Aerobic carbon-cycling potential at the start of the growing season was not significantly different ($p=0.799$) between the hypersaline rich fen and the *Sphagnum*-dominated poor fen, which both had significantly greater aerobic carbon-cycling potential than the treed rich fen at the start of the growing season. The sites' aerobic carbon-cycling potential did not significantly differ between any pair of sites at midseason. The low potential of the treed rich fen was attributed to phosphorus limitation indicated by a substrate preference for low molecular-weight organic acids in that site. None of the sites displayed any significant change in overall SIR on exposure to OSPW, though the hypersaline site showed an SIR preference for saccharide compounds only under contamination, attributed to salt stress response from the high levels of Na^+ present in OSPW. The overall lack of effect of OSPW contamination was likely either due to short incubation times (6h) or the immobilization of OSPW contaminants through physical and chemical interactions with the peat substrate.

Control methanogenic potential was highest at the treed rich fen, significantly lower at the poor *Sphagnum*-dominated fen, and significantly lower than either of the other two sites at the hypersaline rich fen. The extremely low control methane of the hypersaline rich fen site was likely due to the presence of sulfate in the pore water of that site and inhibition of methanogenesis via the presence of a more thermodynamically favourable terminal electron acceptor. Exposure to OSPW significantly decreased methanogenic potential in both the treed rich fen and the hypersaline rich fen, but had no significant impact on methanogenic potential in the *Sphagnum*-dominated poor fen. As amendment with OSPW containing twice its usual concentration of Na^+ did not significantly further decrease methanogenic potential, it appears unlikely that high sodium concentrations are responsible for the inhibitory effect. The mechanism of resistance to OSPW inhibition in the *Sphagnum*-dominated poor fen is also

unclear, but may be the consequence of a more-resilient microbial community or the rapid consumption by the microbial community of any alternative electron acceptors that might be suppressing methanogenesis.

These results have implications for the construction of site-reclamation peatlands. Identifying the mechanism of resistance to OSPW contamination of methanogenesis in *Sphagnum* peat will inform choices about its use in the construction of such sites.

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Chapter 1 - General Introduction

1.1 - Background and Rationale for Research

Oil sands extraction activities in the Athabasca region of Northern Alberta require reclamation of the extraction site to a state of 'equivalent landscape capacity' (Alberta Government 2000) upon the cessation of oil extraction activities. Wetlands comprise much of the Athabasca region landscape (50% of total landscape cover), and 95% of these are fens (Vitt et al. 1996). Fen peatlands play an important role as global carbon-sinks, through accumulation of the partially decomposed plant matter known as peat (Christensen et al. 1997; Holden 2005; Turunen et al. 2002). Peat accumulation is contingent on the rate of accumulation of plant litter being greater than its rate of decomposition in the soil, owing to the persistent anoxic conditions caused by a high water table (Freeman et al. 2001; Moore and Basiliko 2006) throughout the majority of the soil column. The high water table and anoxic conditions are in turn a function of peatland structure.

The structure of a peatland comprises three layers, differentiated by oxygen availability, saturation status, and degree of peat decomposition (Clymo and Bryant 2008). The uppermost layer is the acrotelm, being that portion of the peat column which lies above the average of the annual minimum water table (Ingram 1978) and is therefore variably saturated and often aerated. As a consequence of this aeration, microbial activity in this layer is typically aerobic and activity levels much higher than in anaerobic conditions – most or all of the most readily utilized carbon pools are typically consumed in the acrotelm, leaving the deposited plant litter in a state of partial decomposition (Berg 2000). Conversely, the lowest layer of the peat column is the catotelm, being that portion of the peat column which is below the water-table year-round and is

consequently permanently anoxic (Ingram 1978). As a result, all respiration that occurs in the catotelm is anaerobic, and anaerobic carbon cycling is much slower than aerobic carbon cycling, leading to an accumulation of organic matter over time (Clymo et al. 1998). At depth the high-quality carbon sources have long since been consumed (Berg 2000), meaning that breakdown of carbon compounds may require assemblages of microbes working in concert(Thormann 2006; Thormann et al. 2003; Thormann et al. 2007). These factors combined lead to lower levels of microbial activity in the catotelm than in the acrotelm. Between these layers lies the mesotelm, generally defined as that part of the peat column that is occasionally but not permanently oxic due to being frequently but not permanently below the level of the water table. The nutrient and microbial activity conditions of the mesotelm can be distinct from conditions in either the acrotelm or catotelm, and need not always be intermediate between the two (Lin et al. 2014; Tfaily et al. 2014).

Once disrupted, restoration of this structure is not a trivial procedure. It is insufficient to simply replace the peat removed as part of the creation of open–pit mining sites. During the extraction period, this peat will have been drained and undergone dewatering and mineralisation, leading to significant changes in the physical properties of the peat such as increased density, increased water retention capacity, etc. (Price 1997). While some peat forming mosses are tolerant to brief exposure to saline conditions, extended exposure even at low concentrations has been shown to negatively impact several peat-forming moss species (Pouliot, Rochefort, and Graf 2013). In *Sphagnum* species high salinity renders the mosses unable to draw water from the highly-compacted peat (Mccarter and Price 2015). Alternative reclamation designs have been proposed to work around these issues by shaping the uplands of reclaimed sites to ensure adequate

groundwater supply and by adding a layer of live donor moss atop the decomposed peat layer (Price et al. 2010).

While the restoration of the plant community is necessary to resume carbon accumulation in a peatland, it is not sufficient, as it discounts potential variability in the carbon cycling by the peat microbial community. Microbial activity does not necessarily recover at the same time as the vegetation community (Francez et al. 2000), and if pre-disturbance peatland carbon-cycling activity is not resumed, carbon accumulation may not occur even though the vegetation community appears to have returned to its pre-disturbance state (Andersen et al. 2006).

1.2 - Monitoring Development of Reclaimed Peatlands

The development of newly vegetated or revegetated landscapes is understood to occur through a process known as succession - the iterated colonization of the landscape by several plant species or groups of plant species, with species succeeding each other based on facilitation (changes made a species or group of species creating favourable conditions for colonization by subsequent groups) or simply based on relative competitive advantage in the environment at the time of colonization, e.g. faster reproduction or greater survival ability (Li and Vitt 1995). However, reclamation strategies can sometimes bypass some of these successional stages through the active re-introduction of vegetation (e.g. planting or seeding) (Graf 2008). Vegetation communities in these landscapes might therefore comprise a mixture of target pool species (those species deliberately introduced into the landscape) and incoming species, desired or not (which establish themselves through stochastic processes) (Graf 2008; Priede et al. 2016). Over time, above-ground communities in fens will also be shaped through feedback from belowground

microbial communities, which can influence vegetation persistence directly (e.g. mycorrhizal association) or indirectly (e.g. through nutrient cycling) (Nwaishi et al. 2015).

In oil sands post-extraction landscapes, the usual pattern of succession may be complicated by the presence of contaminants (e.g. naphthenic acids, high sodium concentrations, high alkalinity residue from oil sands extraction processes, etc.) that were not present in the pre-extraction landscape. These can enter the site dissolved in the water supplied by the surrounding uplands and slopes, due to the use of tailings materials in the construction of these sites, and their presence can potentially cause the end-state reclaimed landscape to differ significantly from the pristine landscape in structure or landscape function (Purdy et al. 2005; Trites and Bayley 2009). Additionally, successful restoration of the plant community of a landscape may indicate a return to pre-disturbance carbon accumulation function, but the presence of those same contaminants may affect the peat microbial community differently than the plant community and it is the microbial community that is responsible for carbon remineralization. Our understanding of the effects of oil-sand derived contaminants on microbially-driven processes in peat is currently limited, but may prove to be critically important in predicting the outcome of fen reclamation projects.

1.3 - Objectives

The main objective of this project was to evaluate the impact of contamination on microbial communities from a range of fen types in Northern Alberta, and more precisely, we aimed to 1) characterize the aerobic microbial functional diversity of the Athabasca region and assess the effect of OSPW contamination on that activity, and 2) characterize natural diversity in the

methanogenic potential of those same sites and assess the effect of contamination with OSPW thereupon.

1.4 - Structure of Thesis

This thesis consists of six chapters. The first chapter is a general introduction to the topics to be covered in this thesis and outlines the goals and objectives of the thesis.

The second chapter is a literature review, intended to collect and synthesize the available information on microbial community carbon cycling functional diversity in peatlands and their responses to oil sands contaminants.

The third chapter contains an overview of the reference sites used in these studies and the methods common to both experiments conducted.

The fourth chapter addresses one of the two primary goals by investigating the effects of short-term exposure to oil-sands contaminants on peatland aerobic microbial carbon cycling potential (i.e. respiratory potential).

The fifth chapter addresses the second of two primary goals by investigating the effects of middle-term exposure to oil-sands contaminants on peatland methanogenic potential.

The sixth chapter comprises a summary of the conclusions drawn from the previous two chapters, places them in context of the larger body of research, and makes recommendations for future studies in the same field that might naturally follow from this work.

I conducted the lab work and data analysis for and wrote the first draft of this thesis.

Chapter 2 - Literature Review

2.1 - Introduction

Oil sands mining operations' collective footprint continues to expand in Alberta, estimated to cover an area of 1400 km^2 by 2023 (Alberta Government 1999). Many of the landscapes disrupted by these activities (approximately 45%) are fen peatlands (Vitt et al. 1996). Peatlands, while they may vary between being a carbon source or carbon sink on a small space or time scale, serve an important role as sites of carbon sequestration at the landscape scale via peat accumulation (Galand et al. 2003, Görres et al. 2013).

The Alberta government, as a condition of the leases granted to the oil companies, requires the restoration of impacted sites to a state of equivalent landscape functionality (Alberta Government 2000). In this case, this means that the reclaimed extraction sites will need to resume peat formation, among other requirements, to meet this criterion. This is complicated by the nature of the sites in question, as the majority of currently active extraction occurs by means of open-pit mining. Reclamation of such open-pit sites will be subject to stress conditions and contaminants not present or present in much higher concentration than in the undisturbed landscape, e.g. metal ions (vanadium, sodium) and organic compounds (naphthenic acids) (Pouliot et al. 2012; Purdy et al. 2005; Trites and Bayley 2009). Furthermore, the removal of the vegetation and peat overburden layers dramatically alters the hydrology of the site. As the formation of peat is dependent on hydrological conditions (specifically a consistently high water table), this proves a serious challenge to the construction of any functionally similar wetland in such a site.

Price et al. (2010) proposed a theoretical framework whereby a liner layer, donor peat, and

artificially constructed uplands could be used to create hydrological conditions suitable for establishment of peatland flora. Relying on hydrology and vegetation alone to judge the restoration state of a peatland ignores the microbial community, which are a crucial part of peatland carbon cycling function, and whose activity influences directly the rate of peat formation (by defining the speed at which deposited organic matter decomposes), and the influence of the assorted contaminants present in the site thereupon (Nwaishi et al. 2015). While it has been found that under natural conditions, microbial inocula from disparate peatland sources inoculated into the same environment will converge to a common structure and function, this does not hold true for inocula heavily impacted by site-atypical contaminants (Preston and Basiliko 2015). This suggests that the impact of the disturbances and contaminants present in any reclaimed oil-sands site may cause microbial community structure and function to diverge from what might be expected, given the hydrology and vegetation of the site. The effect that this would have on site carbon-cycling and peat-forming function is unclear.

It is therefore the aim of this chapter to provide a brief summary of the natural structure and carbon-cycling function of peatlands, the contaminants and disturbances with which the microbial community at such a reclaimed site may be presented, and the effects of such contaminants on microbial carbon-cycling activity in peatlands or other wetland types.

2.2 - Natural Functioning of Peatlands

2.2.1 - Peatland structure

Peatlands differ substantially from other environments in many respects, including soil structure and composition, organic matter content, nutrient availability, and carbon cycling function. The defining function of a peatland is peat accumulation, which occurs as a consequence of the rate

of decomposition of deposited plant matter being less than the rate of carbon accumulation through photosynthesis. This low rate of decomposition is typically a result of very low rates of oxygen exchange between the aerated and waterlogged portion of the soil – the mobility of oxygen in water is over 10 000 times less than its mobility in air (Weast 1989). For peat accumulation to occur, the water table at such a site needs to be consistently high to provide the necessary anoxic conditions throughout majority of the soil column, which will in turn slow overall decomposition enough to allow a net accumulation of plant matter (Clymo 1984; Moore and Basiliko 2006). These conditions result in the majority of labile or high-quality carbon being decomposed in the first few centimetres of soil depth, where oxygen is still available through diffusion from the surface, while the majority of the soil column comprises more recalcitrant, lower-quality carbon which has resisted decomposition in the oxic zone (Berg 2000). What decomposition does occur, is via anoxic pathways such as the production of methane, or methanogenesis, fermentation, or via coupling of CO_2 production to other anaerobic processes such as sulfate reduction or denitrification.

Non-carbon nutrients including Ca^{2+} are typically acquired as ions dissolved in water, and thus vary in availability with the peatland's water source. Mesotrophic fens, which are fed via mineral-rich groundwater flow in addition to rainwater input, acquire these nutrients in greater quantity than ombrotrophic fens, being those whose only source of water input is rainfall (Zoltai and Vitt 1990). The abundance of these nutrients also exerts a powerful influence on the vegetation and the microbial activity of these sites, which leads peatlands to be classified by their water input source – minerotrophic peatlands are referred to as fens, and ombrotrophic peatlands as bogs (Slack et al. 1980; Zoltai and Vitt 1990). Relative abundance of nutrients within fen sites is further subdivided; fens where these nutrients are comparatively abundant are referred to as

rich, and relatively nutrient-deprived fens are referred to as poor.

As is the case with most soils, carbon inputs are largely the function of the detritus of the plant community, while remineralization activity is a function of the microbial compartment (Van Der Heijden et al. 2008). Unlike most soils, however, aerobic decomposition is limited to the upper layer of the soil, known as the acrotelm, and the areas around plant roots, as these are the only zones with appreciable aeration and oxygen concentration. At greater depth lies the waterlogged, anoxic zone of the peat, called the catotelm, in which aerobic decomposition does not occur. Instead, remineralization of organic matter in the anoxic zones is more heavily dependent on the water chemistry. Should an ionic species more energetically favourable than carbon (e.g. sulfate, nitrate) be present, reduction of this species can be coupled to the remineralization of carbon compounds to CO_2. In the absence of any more favourable terminal electron acceptors in the anoxic zone, carbon is used, producing methane (CH_4) – this process is called methanogenesis. Methane is consumed in the peatland soil column mostly aerobically, and this process is called methanotrophy. Together, aerobic decomposition in the oxic zone and reduction-coupled remineralization to CO_2 in the anoxic zone or the opposing functions of methanogenesis and methanotrophy in the anoxic zone and the boundary between them constitute the microbial contribution to peatland carbon cycling.

2.2.2 - Decomposition

The decomposition pathways leading to CO_2 production occur in the aerobic zone near the surface of a peatland. These decomposition pathways are a function of aerobic bacteria and microfungi, and are generally the product of groups of microbial organisms called microbial consortia, where different organisms in the consortium catalyze different steps of carbon

remineralisation (Thormann et al. 2002; Thormann et al. 2007). These consortia are critical to peatland aerobic carbon breakdown, which differs substantially from decomposition in mineral soils. In mineral soil decomposition, a succession of distinct clades of microbes handle different stages of carbon breakdown (Heilman-Clausen 2001, Lumley et al. 2001). In peatlands, large groups of broad-activity microbes handle decomposition collectively; while an overall shift in composition is recognizable between the beginning and the end of breakdown, there is no clearly delineated cladistic succession in between (Thormann et al. 2003; Thormann 2006; Thormann et al. 2007). While decomposition has been shown to be fungally dominated in acid mineral soils (Blagodatskaya and Anderson 1998), acid peat soil decomposition has been shown to be bacterially dominated (Winsborough and Basiliko 2010).

Some specifics are known about the organisms involved in peatland decomposition - ascomycete fungi were found to degrade the cellulose and tannic acids present in *Sphagnum* moss tissue, while basidiomycetes degraded cellulose and insoluble phenolic compounds, including the polyphenolic matrix of *Sphagnum* cell walls (Rice et al. 2006). However, a relative absence of Basidiomycota from other peatland surveys (Thormann 2006), suggests that other organisms may substitute for Basidiomycota in aerobic decomposition. For example, Bacteroides were suggested to be responsible for bacterial breakdown of cellulose (Pankratov et al. 2011).

Controls on the fungal decomposer community composition were shown to be based primarily on litter quality, with total phosphorus, carbon, and nitrogen levels being good predictors of community composition, while rhizo-deposition from vascular plants did not significantly affect fungal composition (Thormann et al. 2003; Trinder et al. 2009). Fungal communities and fungal activity are largely limited to upper layers and aerobic conditions.

2.2.3 - Methanogenesis

Methanogenesis occurs when no more thermodynamically favourable compound or ion is available to serve as a terminal electron acceptor in respiration, and instead, carbon is used, which results in the production of methane, CH_4, as the final waste product instead of CO_2. This process is significantly less energetically efficient than aerobic respiration. In the presence of these alternative electron acceptors (e.g. sulfate, nitrate, Iron (III), etc.), competitive inhibition of methanogenesis can occur (Abram and Nedwell 1978; Balderston and Payne 1976; Van Bodegom et al. 2004; Bollag and Czlonkowski 1973; Ferry 2010, 2011; Karhadkar et al. 1987; Segers 1998). Methanogenesis can therefore only occur in peatlands under anaerobic conditions and in the absence of more favourable terminal electron acceptors, typically occurring either below the level of the water table or in anaerobic pockets within the mesotelm.

Methanogenesis is conducted purely by members of the archaeal phylum Euryarchaeota, which are obligate anaerobes, and are irreversibly inactivated on exposure to oxygen. Methanogens can be divided into groups based on the substrates they are capable of using, with the pathways using hydrogen gas and carbon dioxide (hydrogenotrophy) and acetate (acetoclasty) considered the most important (Galand et al. 2005; Segers 1998). Most methanogens are hydrogenotrophic, including members of the clades *Methanobacteriales*, *Methanomicrobiales,* and *Methanocellales* (Galand et al. 2002; Galand et al. 2005a; Galand et al. 2005b; Yavitt et al. 2006); there are considerably fewer known acetoclastic methanogens (Zinder 1993; Garcia et al. 2000), but members of clades *Methanosarcinales* and *Methanosaetaceae* are known to do so (Galand et al. 2002; Galand et al.et al. 2005b; Yavitt et al.et al. 2006). Other substrates are considered to provide no more than 5% of the methane produced in peatland sites, and thus will not be covered in this review.

A number of factors, both biotic and abiotic, influence the dominant methanogenic pathway of a peatland. These factors include peatland type, vegetation composition, soil pH, carbon substrate quality, temperature, and the depth at which methanogenesis occurs (Table 2-1). However, many of these factors are heavily interrelated – for example, peatland type directly influences micronutrient availability and pH, which in turn shape the vegetation community, etc. However, there are general trends towards one pathway or another for certain conditions (Table 2-1). In general, acetoclastic methanogens tend to predominate in conditions similar to those found in rich fens – high micronutrient availability, a neutral to slightly basic pH, higher peat carbon quality, methanogenesis occurring at shallower depths, and a vascular plant-dominated vegetation community. In contrast, hydrogenotrophic methanogenesis tends to predominate in conditions more similar to those found in ombrotrophic bogs – micronutrient scarcity, acidic pH, poor peat carbon quality, and a moss-dominated vegetation community, with few vascular plants. Oligotrophic fens, however, have been found to be dominated by different pathways in different studies (Galand et al. 2003; Juottonen et al. 2005), and which pathway dominates appears to be influenced decided largely by the balance of edaphic and vegetation conditions at the site in question.

It is also important to note that methanogenesis can vary heavily with position within a peatland – peatland microbial communities are, for example, perfectly capable of conducting predominantly acetoclastic methanogenesis in the mesotelm and predominantly hydrogenotrophic methanogenesis throughout the catotelm (Lin et al. 2014). Methane production potential is also fairly strongly linked to many of these factors – in general, those factors that promote hydrogenotrophy (low pH, low micronutrient availability, few vascular plants/abundant mosses, etc.) also tend to be correlated with lower methane production potential, while those

factors linked to predominantly acetoclastic peatlands tend to yield higher methane production potential.

Table 2-1: Description of key variables and their links to methanogenesis pathways in peatlands.

Edaphic Factor	Acetoclasty	Hydrogenotrophy	References
Peatland type/nutrient abundance	Mesotrophic, oligotrophic (Nutrient rich to moderately nutrient deficient)	Oligotrophic, ombrotrophic (moderately nutrient-deficient to highly nutrient-deficient)	(Galand et al. 2005; Yavitt et al. 2006)
pH	Slightly basic to slightly acidic conditions (pH 5 – 8)	Acidic conditions (pH 3-4.5)	(Görres et al.et al. 2013; Juottonen et al. 2005)
Substrate quality	Moderate labile carbon	Little labile carbon	(Duddleston et al. 2002; Hornibrook et al. 2000; Kotsyurbenko et al. 2004)
Vegetation community	Vascular plant-dominated community (esp. e.g. Carex)	Moss-dominated community (esp. Sphagnum), few vascular plants	(Galand et al.et al. 2003; Lin et al. 2014; Robroek et al. 2015)
Depth	Methanogenesis occurring in mesotelm	Methanogenesis occurring in catotelm	(Galand et al. 2002, 2003; Lin et al. 2014)
Temperature	Higher temperatures (20 °C)	Lower temperatures (4 °C)	(Metje and Frenzel 2007)
Average Water Table Depth	Deeper water table	Higher water table	(Galand et al. 2005b)

2.2.4 - Methanotrophy

Methanotrophy is the microbial carbon-cycling process that uses methane as a carbon source, and can occur under either aerobic or anaerobic conditions. In peatlands, the better-studied form by far is aerobic methanotrophy, carried out by obligately aerobic members of the bacterial phylum Proteobacteria.

There are two major subgroups of aerobic methanotrophs (Type I and II respectively), differentiated by the form of the methane monooxygenase (MMO) enzyme (which catalyzes the first step of methane assimilation) they produce, their method of assimilating methane, and the class to which they belong. Briefly, Type I methanotrophs only produce the membrane-bound form of this enzyme complex (also called the particulate form or pMMO), assimilate carbon

through a pathway called the ribulose monophosphate (RuMP) pathway, and belong to the Gammaproteobacteria. Type II methanotrophs, however, produce both the membrane-bound and a soluble secreted form of MMO (sMMO), assimilate carbon through the serine pathway, and belong to the Alphaproteobacteria. Type II methanotrophs are generally more tolerant of lower pH but tend to prefer warmer temperatures, while Type I methanogens tend to be neutrophilic but also includes the more psychrophilic methanotrophs. In general, Type II methanotrophs prefer bogs, while Type I tend to dominate in fens, with pH – and incidentally peatland type - being the primary control over this separation (Belova et al. 2006; Chen et al. 2008; Dedysh 2002; Dedysh, Panikov, and Tiedje 1998; Hanson and Hanson 1996; Jaatinen et al. 2005; Kolb and Horn 2012; Segers 1998). Irrespective of type, aerobic methanotrophy requires the oxidation of methane to methanol as its first step, and this process requires a steady supply of both O_2 and CH_4.

Table 2-2: Summary of main characteristics of aerobic methanotrophic pathways in peatlands.

Type	Class	MMO type	C fixation pathway	Peatland type	Edaphic conditions
Type I	γ-Proteobacteria	pMMO only	RuMP pathway	Mesotrophic, oligotrophic	Neutral pH, colder temperatures
Type II	α-Proteobacteria	sMMO and pMMO	Serine pathway	Ombrotrophic	Acidic pH, warmer temperatures

Anaerobic oxidation of methane is also possible, and occurs in marine (Martens and Berner 1974; Reeburgh 1976), terrestrial (Blazewicz et al. 2012; Raghoebarsing et al. 2005), and freshwater systems (Ettwig et al. 2010). These reactions are not generally conducted by a single organism, but instead are carried out by multi-organism consortia, typically containing members of archaeal clades ANME-1, -2, or -3, in conjunction with sulfate-reducing bacteria (Barnes and

Goldberg 1976; Martens and Berner 1977). Sulfate reduction is not the only process coupled to anaerobic methanotrophy in this way, simply the most common; coupling of nitrate reduction to anaerobic methane oxidation has been observed in freshwater environments, possibly owing to greater nitrate availability in non-marine waters than sulfate (Raghoebarsing et al. 2006). Furthermore, organisms related to the members of the consortia carrying this reaction out have been detected in freshwater ecosystems worldwide, indicating that it may be a fairly common process (Bakermans and Madsen 2002; Koizumi et al. 2003; Raghoebarsing et al. 2006; Stein et al. 2001). Most of the research regarding anaerobic oxidation of methane thus far, however, has been in anoxic marine sediments. One of the few peatland studies, by Gupta et al. (2013), found evidence of anaerobic oxidation of methane occurring in peatlands, restrained by terminal electron acceptor availability. However, anaerobic methane oxidation activity was not controlled by methanogenic activity, and while the process appeared to be coupled to an alternative electron acceptor, they were not able to determine its identity. As such, the principal mechanism, controls, and overall importance of anaerobic oxidation of methane in peatland carbon balance is not yet well understood (Caldwell et al. 2008; Raghoebarsing et al. 2005; Smemo and Yavitt 2011).

2.3 - Disturbance and Contamination

Much of the bitumen present in the Athabasca deposit is too far below the surface to be accessed without steam-injection well methods (Foote 2012). The most readily accessible deposits - those nearest the surface - are the ones that have been most heavily exploited to date, generally via open-pit mining. This process entirely removes the layer of vegetation that overlies the peat and the peat itself, to permit access to the bitumen deposits below (Alberta Government 2014). The

term ‘oil sands’ refers to deposits of a mixture of sand grains and bitumen, in which sand grains are enclosed by a thin bitumen film. Bitumen is a thick, tarlike form of crude oil, highly viscous and resistant to flow (Masliyah et al. 2004). Due to bitumen’s high viscosity, oil sands deposits require considerable pre-processing prior to transportation to a refinery. The most common pre-processing method involves washing this sand with hot water and a strong base, such as sodium hydroxide (Sui et al. 2016). This process produces large quantities of wastewater and tailings enriched in sodium and contaminated with those few chemicals present in the bitumen that are water-soluble, including naphthenic acids, a class of polycyclic aromatic hydrocarbons (Clark and Pasternack 1932; Sui et al. 2016) known to have toxic effects on animals and plants (Dokholyan and Magomedov 1984; Franklin et al. 2002; Kamaluddin and Zwiazek 2002; Rogers et al. 2002). These tailings are redirected to a constructed pond to allow the particulate matter to settle in preparation for re-use of the water. However, once operations have concluded, a large quantity of this water will remain in tailings ponds, and some contaminants may be incorporated into reclaimed landscapes through the use of tailings sand in those landscapes.

2.4 - Contaminant Effects

In the constructed fen-upland system, both the peat microbial community and the vegetation will be exposed to many of the contaminants described above in the form of oil sands process-affected water (OSPW). OSPW may contain elevated concentrations of NaCl on the order of 1000 mg/L (Franklin et al. 2002) and naphthenic acids (NAs) at unknown concentrations, both of which have the potential to alter peat microbial community's structure or function. The effects of these contaminants on microbial community activity in a peatland environment are as yet poorly understood.

The three major functions of microbial carbon cycling respond quite differently to input of high concentrations of salt or oil-derived contaminants in peatland or wetland systems. In one example, crude oil released to a swamp increased microbial aerobic respiration, although it reduced community diversity – it is presumed a small number of oil contaminant-tolerant species took over the majority of respiration activities (Nyman 1999). In a different study, addition of OSPW into a rich fen peatland microcosm was found to significantly reduce catabolic diversity of the microbial community, although there was a considerable delay between the application of OSPW and the manifestation of deleterious effects on respiration (Rezhanezhad et al. 2012a). This was attributed to the structure of the peat itself immobilizing some of the contaminants, thereby retarding their toxic effect (Rezanezhad et al. 2012a; Rezanezhad and Price 2011; Rezanezhad et al. 2012b).

The effect of naphthenic acids on microbial communities of oil sands tailings sediments of the Athabasca region has been well studied, and the concentration of naphthenic acids in their source environment proved to be a significant control on microbial community structure(Hadwin et al. 2006). These communities have also proved capable of decomposing both commercial mixtures of naphthenic acids and samples extracted from oil sands tailings ponds, concurrently reducing their toxic effects (Herman et al. 1994). Both functions were more effective on the commercial preparation than the site-extracted one, however.

The effect of naphthenic acids on methanogenesis in aquatic environments is also well understood, as tailings ponds provide an ideal site for investigating this interaction. In general, naphthenic acids can suppress methanogenesis near-completely but temporarily, with the duration of the suppression being proportional to the quantity of naphthenic acids in the environment (Holowenko et al. 2000, 2001). The persistence of the methanogens during this

period is indicated by the successful isolation of *Methanosaeta* from such a tailings pond, indicating that methanogenic microbes can indeed adapt to high naphthenic acid conditions (Harner et al. 2011).

The effect of high sodium concentrations on methanogenesis in aquatic systems has also been studied, where the addition of sodium, through the input of saltwater, decreased but did not completely suppress methanogenic activity. This change in activity was not accompanied by a change in community composition, indicating methanogens appear capable of adapting to sodium contamination also (Edmonds et al. 2009). Similarly, methanogenesis was likewise suppressed by 25% by the addition of 40-80 mM salt to a non-peat-forming wetland (Denier van der Gon and Neue 1995).

Methanotrophy appears more sensitive to salinity than methanogenesis: in the case of Denier van der Gon and Neue (1995) above, where addition of 40-80 mM salt reduced methanogenesis, it almost completely suppressed methanotrophic activity. Similarly, while a study of a hypersaline wetland microbial mat found detectable methane efflux with salinity between 8.5 and 13.2%, but suppression of methanotrophic activity did not change this efflux, indicating complete suppression of methanotrophy (Conrad et al. 1995). However, Saidi-Mehrabad et al. 2013 found that there was detectable methanotrophic activity and organisms in tailings ponds in the Athabasca Oil Sands region, and thus it appears that methanotroph adaptation to the presence of both high salinity and naphthenic acids is possible. There presently appears to be no literature about the effect of naphthenic acids on methanotrophy in peatlands.

2.5 - Summary

The literature suggests significant resilience of methanogenic activity to modification by

contaminants – naphthenic acids seem to only delay the beginning of methanogenesis, while high salinity seems to inhibit, but not to inactivate, methanogenic activity. In contrast, methanotrophy is much more sensitive than methanogenesis to salinity, being more heavily inhibited than methanogenesis at similar concentrations. This suggests that any constructed peatland whose water input included OSPW might resume normal methanogenic activity but reduced methanotrophy, leading to a larger efflux of methane than in a similar undisturbed fen, and may influence the reconstructed fen's carbon-accumulation rate or capacity. However, if alternative terminal electron acceptors are abundant in the tailings sands used in these constructed sites, then these sites will have very little methanogenic or methanotrophic activity, instead remineralizing carbon through the release of CO_2 coupled to the reduction of those more favourable terminal electron acceptors.

This is hardly a certain outcome, however, owing to a number of complicating factors. First, this review focused only on two of the main contaminants in OSPW – naphthenic acids and salt – while the full OSPW solution, which may be applied to constructed wetlands, contains considerably more contaminant species (e.g. vanadium, nickel, etc.), which may also alter the fate of carbon input to these sites. Second, all the studies found on NA and Na^+ contamination on methanotrophy and methanogenesis were conducted in either aquatic environments or non-peat-forming wetlands, and thus do not account for the potential attenuating effect of the peat substrate found by Rezanezhad et al. (2012). There is therefore very little information on the effect of whole OSPW on microbial aerobic respiration potential, and none on its effects on anaerobic respiration potential. By studying the impact of oil-sand derived contaminants on carbon cycling in three different types of peat found within the Athabasca region and formed by contrasting vegetation types, this research will aim to fill this gap in knowledge.

Chapter 3 - Materials and Methods

3.1 – Site Descriptions

Field sampling for this study was conducted in three undisturbed peatlands located within the Athabasca Oil Sands Region. The region's climate is characterized as a boreal continental climate, which entails long, cold winters and short summers, resulting in a mean annual temperature of 1°C and a mean annual precipitation of 418.6 mm, based on data collected from 1981-2010 (Environment Canada, 2015). These peatlands were chosen as sampling sites, as they encompassed a gradient of vegetation types and physicochemical regimes that represent the range of fen peatlands in Northern Alberta. As such, they could be used as reference baselines for the state of microbial activity in pristine peatlands and to gauge possible responses of reclaimed peatlands to contaminant addition. These sites were:

1) Poplar fen, a moderate-rich treed fen located 20 km northwest of Ft. McMurray, characterized by vegetation survey as containing treed poor fen and treed rich fen ecosite phases (Beckingham, Archibald, and Corns 1996). Sampling was conducted in the treed rich fen ecosite phases, whose vegetation is dominated by *Larix laricina, Betula glandulosa, Equisetum fluvatile, Smilacina trifoliata, Carex prairea, Carex diandra,* and *Stellaria longipe*. The moss layer included *Tomenthypnum nitens, Campyllium stellatum and Hylocomnium splendens.*
2) Saline fen, a rich fen located 10 km south of Ft. McMurray. Saline fen was characterized as containing shrubby rich fen and graminoid rich fen ecosite phases, and sampling was conducted in the marsh grass fen community phase, where the peat and groundwater contain very high concentrations of NaCl, and whose vegetation is dominated by

Calamagrostis inexpansa, *Carex tenax*, and *Hordeum jubatum*. The sparse moss layer included *Campyllium stellatum*.

3) Pauciflora fen, a poor fen located 40 km south of Ft. McMurray. Pauciflora fen is characterized as containing treed poor fen and shrubby poor fen ecosite phases, and sampling was conducted in the latter, whose vegetation is dominated by *Picea mariana*, *Carex aquatilis*, and *Chamaedaphne calyculata*. The moss layer was dominated by *Sphagnum angustifolium* and *Sphagnum magellanicum*.

Table 3-1: Means (standard errors) of physico-chemical properties of the three sampling sites. EC = Electrical conductivity (measured in 2015), WL = water level.

Site	**pH**	**EC (µS/cm)**	**Soil Moisture(%)**	**WL(cm)**
Pauciflora	4.01 (0.1)	33.5(2.3)	73 (1)	-0.3 (0.21)
Saline	6.5 (0.3)	>4000	92 (4)	-5.3 (2.44)
Poplar	7.0 (0.01)	230.8 (6.0)	69 (3)	-5.4(0.81)

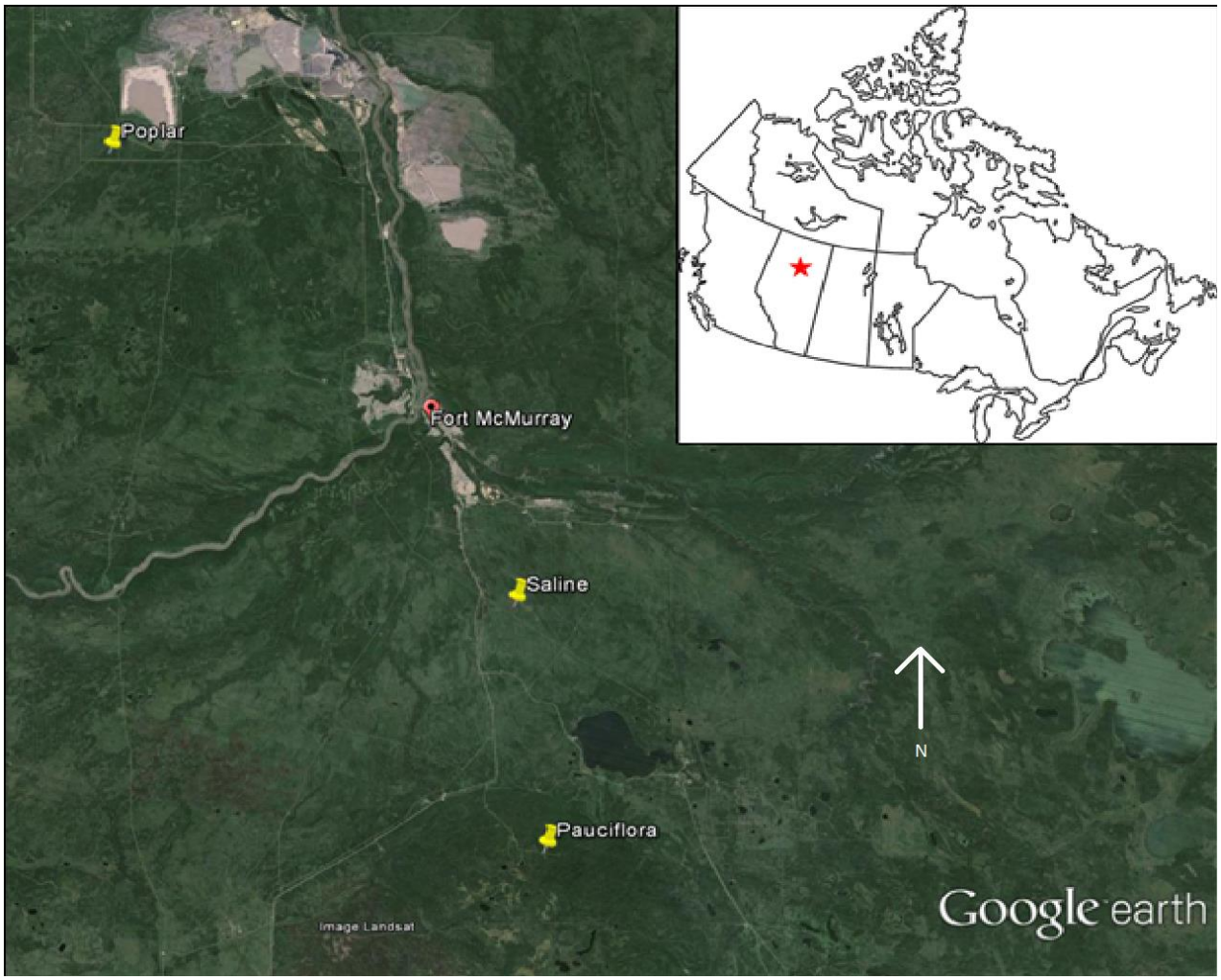

Figure 3-1: Map of field sites

Figure 3-2: The Pauciflora Sphagnum dominated poor fen. Photo credit: Roxane Andersen

Figure 3-3: The Poplar rich treed fen. Photo credit: Roxane Andersen

Figure 3-4: Saline fen. Photo credit: Roxane Andersen

All sampling sites were characterized as regionally typical, all were subject to influence by surface water and groundwater, all had a stable water table at or near the surface, and had 40 cm or more of accumulated peat (A. Borkenhagen, personal communication) at the time of sampling. Site physicochemical properties for each of the reference sites are summarized in Table 3-1; a map of the area with the locations of the field sites is shown in Figure 3-1 and figures 3-2, 3-3, and 3-4 depict the sites in question. Exact EC data are not available for Saline, as the instruments used had an upper limit of 4000 µS/cm.

3.2 - Sampling Methods

At each site (Pauciflora, Saline, Poplar), five replicated composite peat samples were extracted by hand via the inverted Ziploc bag technique. For each composite sample, five cores 5 cm in radius by 15 cm in length were taken around pre-existing plots used for greenhouse gases measurements (not part of this study). The five cores thus sampled were homogenized in the field to make the composite sample, then sealed in a Ziploc bag and stored at 4°C until analyzed. Samples were taken at the start of the growing season (early May 2014) and in the middle of the growing season (late June 2014).

3.3 - Analyses

3.3.1 - Aerobic microbial activity

Microbial community aerobic respiration potential was measured via the MicroResp© method (Campbell et al. 2003). In this method, 96 - well deepwell microplates (holding 1.2mL per well instead of the few hundred microliters of ordinary microplates) are filled with 0.3 g of fresh peat per well, with each plate being filled exclusively with peat from one sampling point. These plates are refrigerated if not to be used immediately, but are allowed to incubate at room temperature in the dark for 72 hours prior to beginning the assay regardless of whether or not they were refrigerated. The assay entails the addition of 25 μL of substrate solution to each well and the attachment of a detection microplate to the deepwell plate via the use of a specially made gasket and clamp. These substrates are typically chosen from common root exudate carbon sources – in this experiment l-alanine, arginine, l-cysteine-HCl, and l-lysine, α-ketoglutaric acid, l-arabinose, citric acid, γ-aminobutyric acid, L-malic acid, and oxalic acid, d-fructose, d-glucose, N-acetylglucosamine, and trehalose were used, alongside Milli-Q water and OSPW from the Suncor lease as controls. All substrate solutions were made with both OSPW and Milli-Q water

as solvents. All solutions were made to the lower of 300 mg/mL or their limit of solubility and aliquots of these solutions were diluted (if necessary) to deliver 30 mg substrate g^{-1} soil water via the application of 25 μL substrate solution. Alanine and N-acetylglucosamine, were the exceptions, being diluted (if necessary) to deliver 7.5 mg substrate g^{-1} soil water instead. In this experiment, applications were in triplicate, allowing both the control and OSPW-amended versions of each substrate to be run on a single plate. The detection gel was made with a solution of 150 mM potassium chloride and 2.5 mM sodium bicarbonate, along with the indicator dye cresol red at a concentration of 12.5 ppm (w/w), mixed into molten 1% Noble agar at a temperature not exceeding 65 degrees Celsius. 150 μL of this mixture was then dispensed into each well of a 96-well microplate and allowed to cool. The absorbance of the detection plates was measured at 570 nm prior to use, and only plates with a coefficient of variance of absorbance less than or equal to 5% were used. Upon addition of substrates the detection plate was clamped to the deepwell microplate and incubated in the dark for 6 h, whereupon the detection plate absorbance at 570 nm was read once again. The change in colour over the 6-hour incubation period reflected the degree of substrate-induced respiration.

3.3.2 -Anaerobic methane production

Anaerobic methane production was measured via flask incubation, where 10 g of wet peat from each field site was inundated with water sufficient to submerge the peat – either Milli-Q water, OSPW from the Suncor lease, or OSPW with sufficient (1331 mg/mL) NaCl added to double the Na^+ concentration of the solution. Each flask was sealed with a butyl rubber stopper under an anaerobic (N_2) environment using a glovebox. The flasks were incubated in the dark for 6 weeks and the headspace sampled at 0, 1, 2, 5, 7, 14, 21, 28, 38, and 42 days with a gas syringe. Prior to sampling the syringe was flushed three times with 99.9% N_2 gas; then a 20 mL sample was taken

and placed into a LabCo Exetainer and the sampled gas volume replaced with 99.9% N_2 gas. Samples were analyzed on a Shimadzu GC-2014 gas chromatograph with flame ionization detector for methane content.

3.3.3 -Data analysis and statistical methods

All data was analyzed with the R statistical software (R Core Team 2016). Specific details regarding the analyses conducted and functions and packages used for analysis of aerobic and anaerobic microbial activity are found in chapters 4 and 5 respectively.

Chapter 4 - Building a reference baseline for the evaluation of aerobic microbial activity in the Athabasca Oil Sand region, near Fort McMurray, Alberta, Canada

4.1 – Introduction

Microbes are responsible for a large majority of nutrient cycling in soil, and are therefore indispensable to the function of any ecosystem (Van Der Heijden et al. 2008). Furthermore, studies have shown that the soil microbial community has the potential to shape the plant community of their ecosystem, by mediating the cycling and availability of nutrients (Bragazza et al. 2015; Lamers et al. 2012; Lin et al. 2012; Myers et al. 2012), or by enhancing the ability of the flora to resist an environmental stress (Qu et al. 2015). Microbial activity (nutrient utilization profiles, catabolic evenness, etc.) varies with ecosystem, land use and other environmental factors. The effect of this variability in microbial activity on the overall soil function (especially nutrient cycling function) is still poorly understood (Degens et al. 2001; Fisk et al. 2003).

Northern peatlands are an example of an ecosystem where the understanding of variability in microbial activity would be valuable. Indeed, northern peatlands store about one-third of the world's terrestrial carbon, a disproportionately large fraction compared to their land area (Blodau 2002; Limpens et al. 2008; Tarnocai 1999). Their capacity to store carbon is a result of the imbalance between uptake and net losses, which include respiration by micro-organisms (Clymo 1984). Any disturbance which changes this imbalance (e.g. by inducing greater microbial respiration) could cause the disturbed peatland to change from a sink to a source of carbon to the atmosphere (Kim et al. 2012; Yavitt et al. 1987). In light of ongoing concerns regarding carbon emissions and their effect on global climate change, a more in-depth understanding of the primary control on carbon release in the world's largest terrestrial carbon sinks is more important

than ever. Yet, little is known about how microbial activity in these systems varies with peat botanical composition and properties, surface vegetation, physicochemistry, etc. (Bardgett et al. 2008).

The Athabasca Oil Sands region (AOSR) of Alberta, Canada, is pertinent to discussions both in terms of peatland microbial responses to changes and of peatland carbon cycling in general - the area is dominated by wetland environments (>50% of the terrestrial surface area), 95% of which are minerotrophic peatlands, or fens (Vitt et al. 1996). The region is also the location of extensive bitumen mining operations, expected to cover an area of 1400 km^2 by 2023 (Alberta Government 1999). Bitumen is extracted from these sites through open-pit mining, which requires the complete removal of the surface vegetation and the underlying peat, and thus severely disrupts the ecosystem functions of these sites, including carbon accumulation functions (Johnson and Miyanishi 2008; Rooney and Bayley 2012; Turetsky et al. 2002). Previous studies have indicated that severely disrupted peatlands have limited ability to regenerate either vegetation structure or microbial community function without intervention (Andersen et al. 2010; Elliott et al. 2015). However, the Alberta government's land use regulations require restoration of leased sites to a state of 'equivalent land capability' (Alberta Government 2000). Given that 'equivalent land capability' should include the resumption of healthy soil activity – which in peatlands includes carbon accumulation functions - one of the goals of ongoing experimental reclamation efforts is to understand the role of microbial community in the carbon cycling processes across the range of natural peatlands in the oil sands region.

It has been shown that microbial community structure and functions lagged behind recovery of vegetation composition in peatlands harvested for horticultural peat fibre, when compared to natural regional reference systems (Andersen et al. 2006). At the moment, only two constructed

peatlands exist within the Athabasca Oil Sands region (Ketcheson et al. 2016). Nevertheless, to be accountable for their land reclamation strategies, the oil industry will require a means of determining whether constructed peatland sites have achieved functions, such as microbial activity, that are equivalent to their natural counterparts. It is therefore necessary to acquire information that encompasses the range of peatland types that a constructed site might resemble in the future, for use as a reference baseline.

The construction of such a baseline is further complicated by the location of reclaimed sites: they must be constructed at the heart of the open-pit mining sites and thus cannot avoid exposure to effluent-enriched oil sands process water (OSPW) from tailing materials used in constructing upland landscapes that supply water to constructed fen watersheds. OSPW is known to contain high levels of both salt (especially sodium, Na) and naphthenic acids (NAs), both of which have been shown in mesocosm experiments to affect plant communities (Pouliot et al. 2012) as well as the activity of exposed microbial communities (Degens et al. 2001). One study observed a decrease in microbial catabolic diversity in peat samples exposed to OSPW, although there was a delay between exposure and the onset of deleterious effects (Rezanezhad et al. 2012a). It is therefore reasonable to hypothesize that the microbial activities of these constructed fens, even given full recovery of the peat microbial community, would exhibit some differences relative to their undisturbed state. Understanding how microbial communities in reference peatlands respond to OSPW addition will be useful to contextualize observations in constructed sites.

The objectives of this study were therefore twofold – 1) to characterize the aerobic microbial functional diversity of a variety of reference fen types found in the Athabasca oil sands region, and 2) to assess the impact of the addition of OSPW on these functions. We hypothesized that 1) microbial activity would vary between sites as a function of their unique vegetation and

biogeochemistry, and 2) that the addition of OSPW would generally lead to a reduction in microbial activity in all samples.

4.2 - Materials and methods

4.2.1 - Measurement of substrate-induced respiration with MicroResp©

To evaluate catabolic activity, the MicroResp™ method adapted for peat (Artz et al., 2006) and as described in Rezanezhad et al. (2012a) was used, with changes noted below. The carbon sources used fell into three functional groups: amino acids (comprising l-alanine, arginine, l-cysteine-HCl, and l-lysine), carboxylic acids (comprising α-ketoglutaric acid, citric acid, γ-aminobutyric acid, L-malic acid, and oxalic acid,) and saccharides (comprising l-arabinose, d-fructose, d-glucose, N-acetylglucosamine, and trehalose). All these carbon sources were made in solution in two variants – one using Milli-Q water as a solvent, one using OSPW as a solvent. The OSPW used was taken from a tailings pond on the Suncor lease, near Fort McMurray, Alberta, and had Na^+ concentration of 1331 mg L^{-1}. Due to technical difficulties, the concentration of naphthenic acids in the OSPW solution was not measured (Rubi Simhayov, personal communication). All solutions were made to 300 mg/mL of the respective carbon source, or to saturation, for those carbon sources whose maximum solubility was below 300 mg/mL. Then, 25 µL of each carbon source (both variants) was applied to triplicate wells, and included Milli-Q water and OSPW without any carbon source as controls. The absorbance of the detection plate was measured at 570 nm both before inoculation and after 6 hours of incubation in the dark at room temperature, and the change in absorbance used to calculate the CO_2 produced in each well.

4.2.2 - Statistical methods

All data were subjected to a normality test before use in statistical analysis; where data were found to be non-normal they were log-transformed before use. All statistical analyses were performed in R (R Development Core Team, 2013) with non-core packages and functions used as noted below.

The overall microbial activity (as measured by average well colour development, or AWCD) and the catabolic evenness (as quantified by Simpson's Diversity Index) were compared between treatments on each reference fen at each sampling date using a nested analysis of variance (ANOVA) using the function 'aov' in the R core package. Significant differences in the overall carbon utilization profiles of these sites within a given sampling date and contaminant treatment were tested for using non-parametric permutational analysis of variance (PERMANOVA) using the function 'adonis' in the package 'vegan' (Oksanen et al. 2016). Differences in patterns of carbon utilization between sites were analyzed by nested MANOVA (using the function 'manova' in the package 'stats') and post-hoc difference tests. Significant differences between sites across sampling dates were determined using ANOVA and post-hoc difference tests (using the functions 'TukeyHSD' in the 'stats' package and 'multcompLetters4' in the package 'multcompView' (Graves et al. 2015).

To determine the relationships between environmental variables and microbial function, the midseason data was analyzed using redundancy analysis (function 'rda' in the package 'vegan') with a forward selection of explanatory variables (function 'ordiR2step' in package 'vegan'). The significance of each constrained axis was tested using Monte-Carlo permutations (using the function 'anova' from the 'stats' package (R Core Team 2016). Only the midseason data were used in the ordination because no physicochemical data were available for the start of season.

We tested the influence of different groups of variables (edaphic and vegetation) to assess their respective influence on the overall utilization of carbon sources by the microbial community through variation partitioning (using the function 'varpart', package 'vegan').

4.3 - Results

4.3.1 - Variability in site overall respiration response

Contrary to initial expectations, the addition of OSPW had no significant short-term impact on either the community respiration potential (AWCD) ($F=0.67$, $p=0.68$) or community catabolic evenness ($F=0.27$, $p=0.95$). On the other hand, sampling date had a significant effect on both community respiration potential ($F=281.16$, $p<2x10^{-16}$, d.f.=1) and catabolic evenness ($F=23.23$, $p=3.12x10^{-6}$), as did sampling site within a given sampling date ($F_{AWCD}=41.33$, $p<2x10^{-16}$, d.f.=4, $F_{even}=10.37$, $p=1.49x10^{-7}$, d.f.=4). The influences on overall carbon utilization patterns (as indicated by permutational ANOVA) were very similar to the responses of AWCD- sampling date and site within sampling date had a significant effect on pattern of carbon utilization, while treatment within sampling site within sampling date did not significantly affect carbon utilization patterns overall (Table 4-1).

The respiration potential of the Pauciflora and Saline microbial communities did not differ significantly from one another, and the respiration potential of both communities was found to be significantly greater than that of the Poplar site. Additionally, the microbial respiration potential of the Pauciflora and Saline sites decreased significantly between the start of season and midseason sampling dates, while no such difference was observed at the Poplar site.

In contrast, the catabolic evenness profiles of the Poplar and Saline field sites were not significantly different from one another, but both differed significantly from the Pauciflora site. Differences in catabolic evenness were not consistent between dates: the catabolic evenness decreased significantly over time in Pauciflora, increased significantly in Saline, and did not vary significantly between May to June in Poplar (Figure 4-1).

SIR only differed significantly between Pauciflora and Saline samples in start-of-season samples for d-fructose, d-glucose, and trehalose, and only differed in midseason samples for arginine and l-malic acid. In both Pauciflora and Saline samples, SIR was significantly higher at start-of-season than midseason for all carbon sources and controls. In contrast, Poplar sample SIR did not differ significantly between sampling dates in response to any carbon sources except l-malic acid and oxalic acid, which were both higher at the start of the growing season. In Poplar samples, SIR was significantly lower than in Pauciflora or Saline samples at the first sampling date for all carbon sources except arginine and oxalic acid, which did not vary between sites. In contrast, SIR was similar across sites for most C sources in midseason samples except for α-ketoglutaric acid, citric acid and oxalic acid, for which SIR was significantly higher in Poplar than in Pauciflora or Saline samples (Figure 4-2).

Table 4-1:Analysis of variance (ANOVA) results for average well colour development (AWCD) and catabolic evenness and non-parametric permutational ANOVA (Adonis) results for overall site carbon utilization, measured using the outputs from the MicroResp™ experiment. Parent factors are separated from subsequent nested factors by colons. Significant effects are highlighted in bold. Significance threshold: p-value < 0.05.

Variable and model	D.f.	F-value	p-value
AWCD			
Date	1	228.66	**$<2x10^{-16}$**
Date:Site	4	38.74	**$<2x10^{-16}$**
Date:Site:Treat	6	0.67	0.68
Residuals	168		
Catabolic evenness			
Date	1	22.64	**$4.18x10^{-6}$**
Date:Site	4	10.11	**$2.36x10^{-7}$**
Date:Site:Treat	6	0.27	0.95
Residuals	168		
Multivariate ANOVA			
Date	1	157.24	**0.001**
Date:Site	4	32.01	**0.001**
Date:Site:Treat	6	0.988	0.469
Residuals	168		

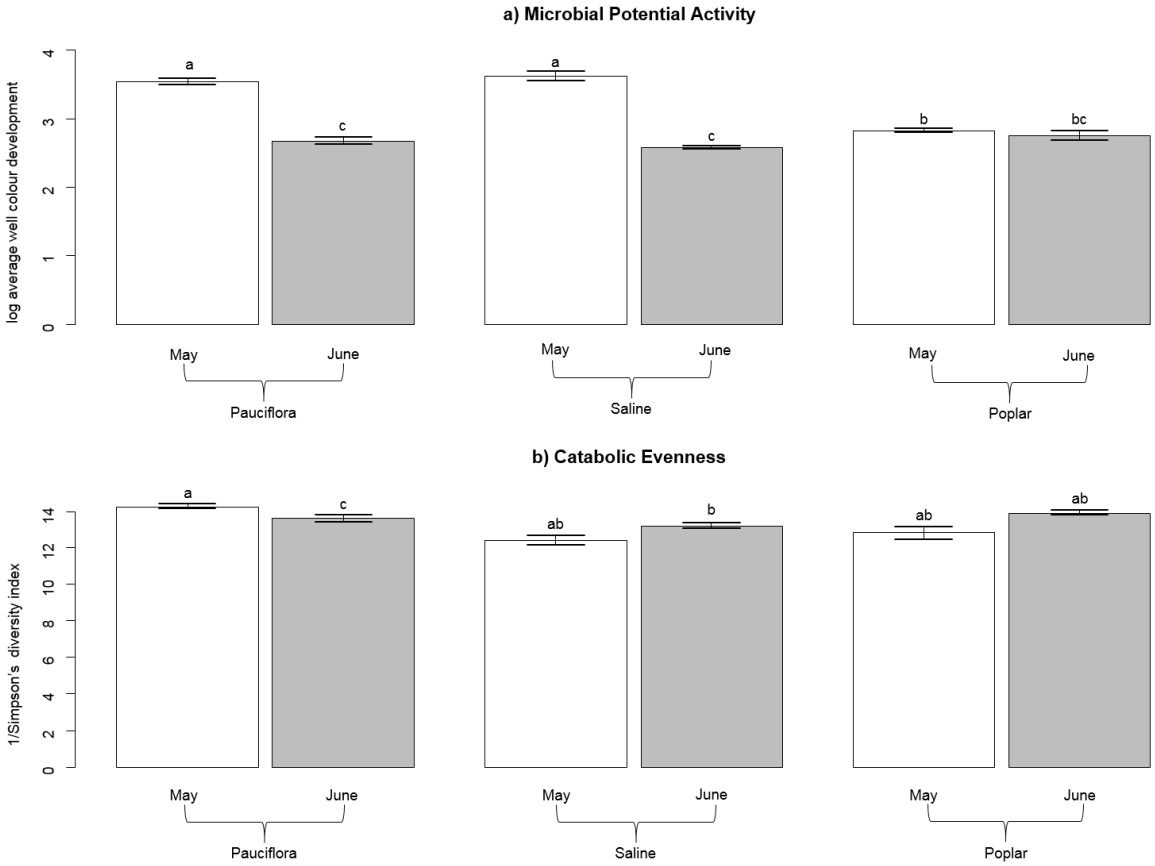

Figure 4-1: a) Microbial potential activity and b) catabolic evenness divided by field site and sampling date. Samples collected at the start of season are indicated by white bars while samples collected at midseason are indicated by grey bars. Groups that share a letter are not significantly different.

Forward selection of variables based on an initial redundancy analysis indicated that water table depth and soil moisture content were the two least powerful explanatory environmental variables. Upon their removal, 27.7% (p=0.001) and 10.0% (p=0.001) of the total variance of potential microbial community response in potential CO_2 production was explained by the first two axes of the redundancy analysis (Figure 4-3), respectively. The measured edaphic variables (pH, electrical conductivity, mean water table height, soil moisture content) explained only 3.8% of the total midseason variation in microbial respiration potential across all sites and treatments, while vegetation-related variables (vascular plant species richness, moss species richness, and percentage canopy cover) explained 23% of the variation, with 2.8% of the variation explained

by the intersection of vegetation and edaphic variables. The percent cover of the canopy and soil water electrical conductivity (EC) were the most important environmental factors in shaping substrate preference, with EC positively correlated with utilization of D-fructose and D-glucose, and greater canopy cover percentage and greater moss species richness related to greater utilization of citric acid, oxalic acid, and α-ketoglutaric acid.

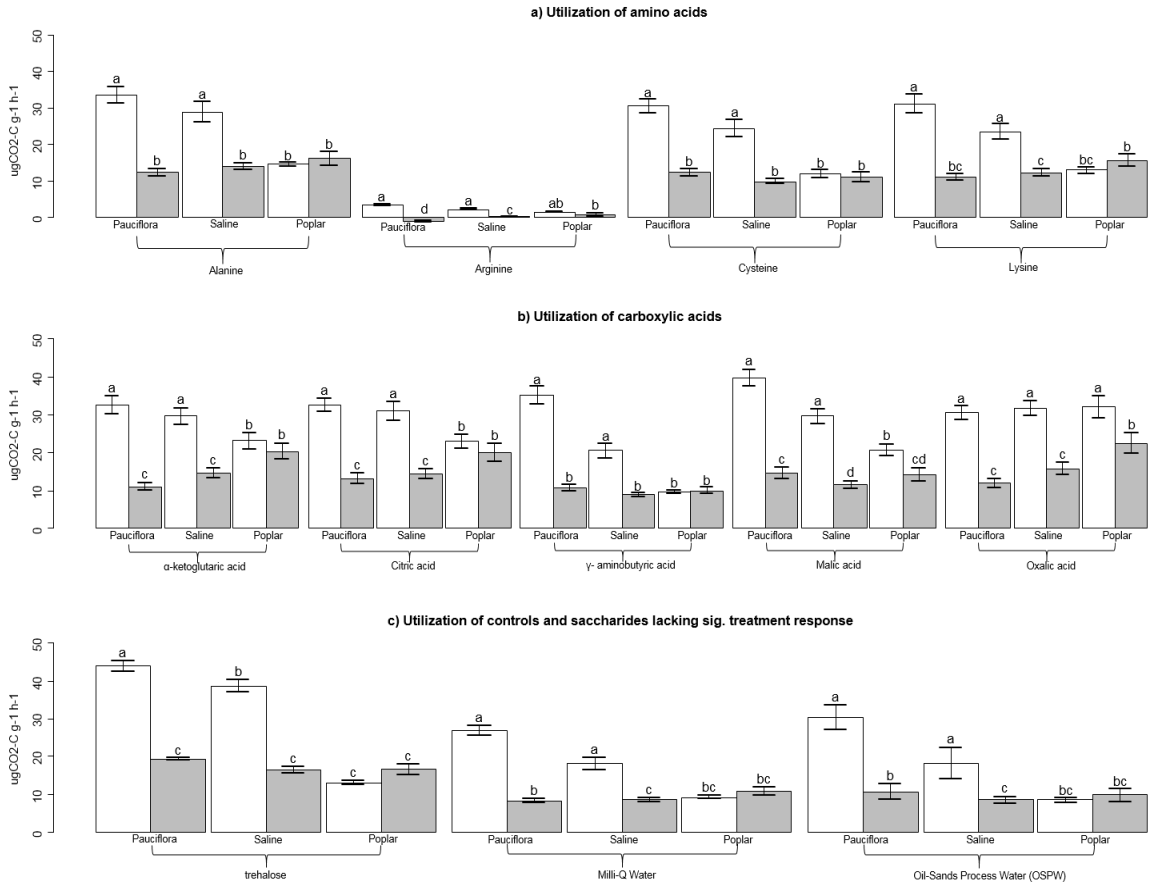

Figure 4-2: Carbon utilization profiles organized by carbon source functional group. White bars indicate start of growing season samples, grey bars indicate midseason. Samples within a carbon source that share a letter are not significantly different, as determined by Tukey post-hoc test.

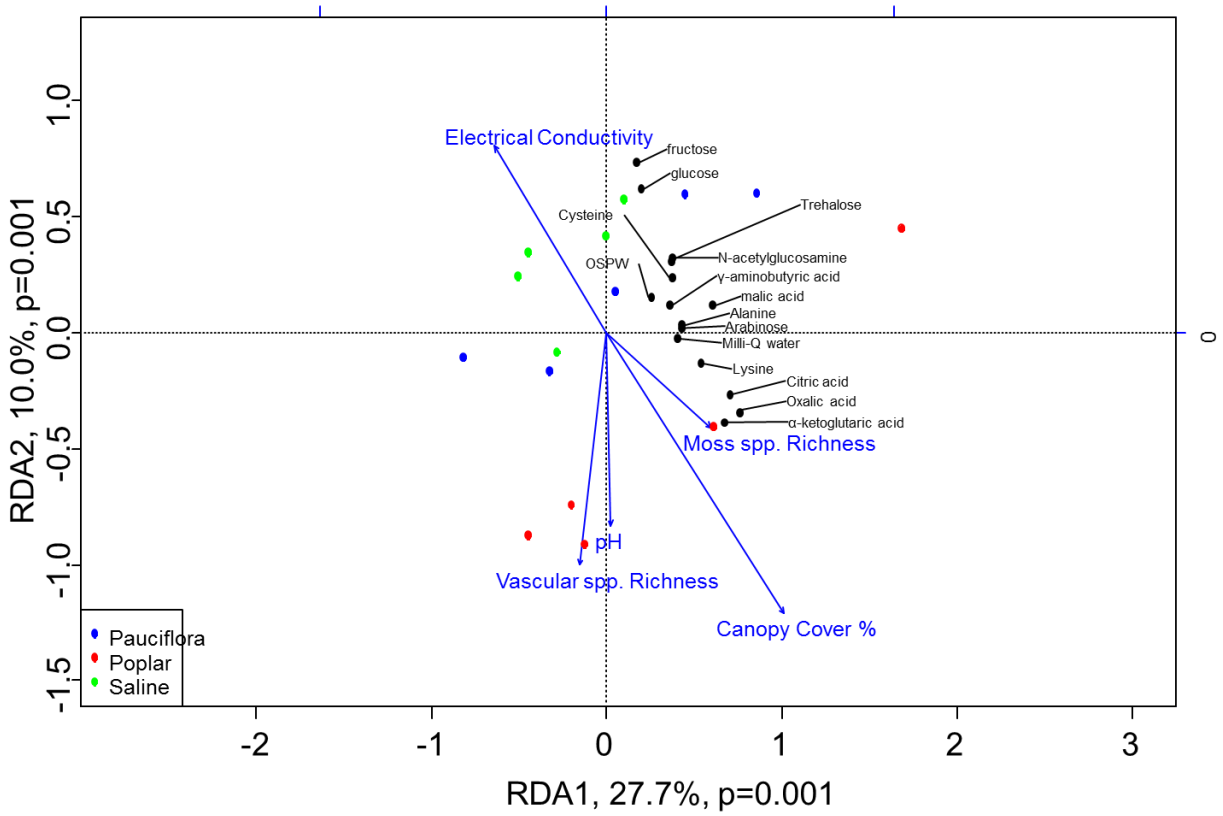

Figure 4-3: Redundancy analysis output for midseason data. Averaged sampling points are indicated by coloured dots, while carbon sources are indicated as red text and explanatory variables as blue arrows and text.

4.3.2 - Effect of OSPW contamination on microbial community function

While overall site respiration did not differ significantly in response to treatment with OSPW, treatment with OSPW did significantly affect SIR D-glucose (F=3.46, p=0.003) and marginally significantly for arabinose (F=2.25, p=0.040), D-fructose (F=2.30, p=0.037), and N-acetylglucosamine (F=2.3226, p=0.03) (Table 4-2).

Table 4-1: Multivariate analysis of variance (MANOVA) results for the carbon sources whose individual SIR differed significantly with treatment. Parent factors are separated from subsequent nested factors by colons. Significance threshold: p-value <0.05.

C source and model	D.f.	F-value	p-value
Arabinose			
Date	1	158.16	<0.0001
Date:Site	4	32.35	<0.0001
Date:Site:Treat	6	2.25	0.041
D-fructose			
Date	1	184.18	<0.0001
Date:Site	4	77.74	<0.0001
Date:Site:Treat	6	2.30	0.037
D-glucose			
Date	1	133.10	<0.0001
Date:Site	4	55.77	<0.0001
Date:Site:Treat	6	3.46	0.0030
N-acetylglucosamine			
Date	1	100.00	<0.0001
Date:Site	4	24.44	<0.0001
Date:Site:Treat	6	2.32	0.036

The Saline site presented the most consistent response to amendment with OSPW; addition of contaminant increased SIR for every sampling date – carbon source combination, except for arabinose at the start of the growing season, where SIR was nonetheless visibly increased, but not sufficiently to change significant difference groups as determined by a TukeyHSD post-hoc test.

The Pauciflora response was overall similar to the Saline response at start of season, with OSPW exposure increasing start-of-season SIR for all four carbon sources, though not significantly in any case. At midseason the response was much more variable; OSPW provoked a significant decrease in response to D-fructose, while having no significant effect on arabinose, D-glucose and N-acetylglucosamine response.

The Poplar community response was more variable still, showing no significant change at either timepoint for OSPW-amended D-glucose, a trend towards increase in D-fructose SIR at both timepoints, a significant increase at start of season but a significant decrease at midseason for arabinose, and a significant decrease at start of season but no significant change at midseason for N-acetylglucosamine. (Figure 4-4)

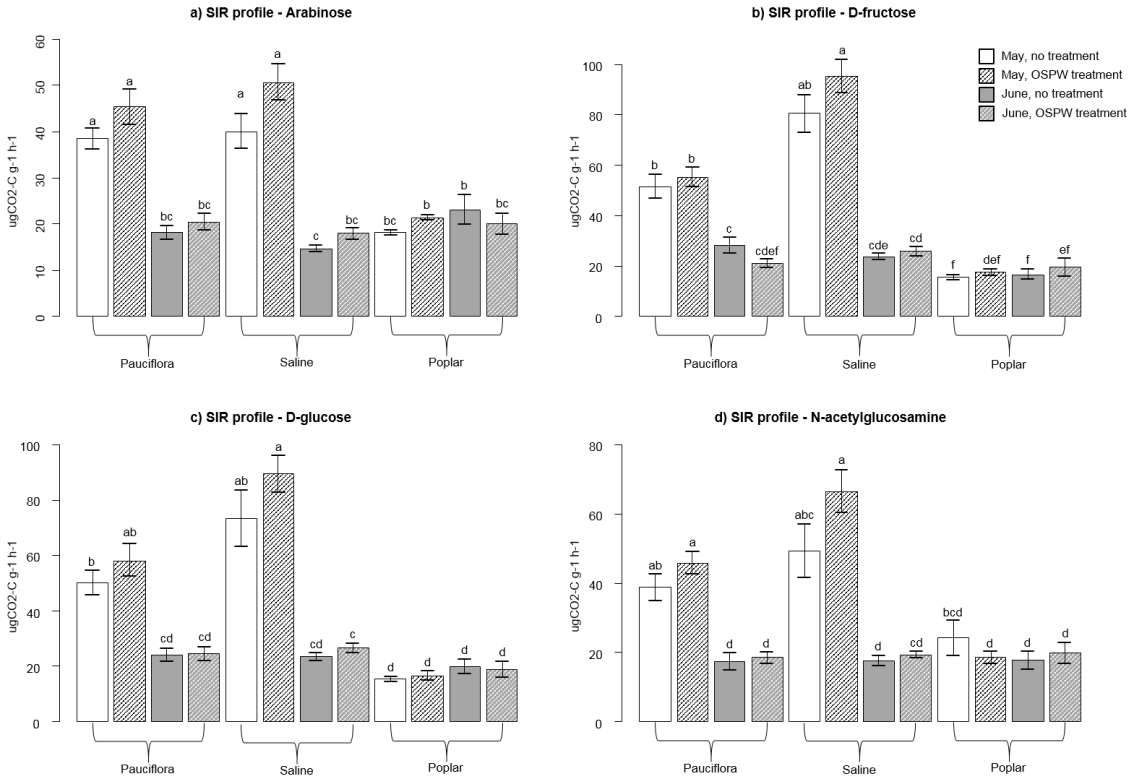

Figure 4-4: SIR profiles of those carbon sources varying significantly with treatment. White bars indicate the sample was taken at start of season, grey bars indicate midseason. Crosshatched bars indicate treatment with OSPW, while bars without indicate the control treatment. Samples within a carbon source that share a letter are not significantly different, as determined by Tukey post-hoc test.

4.4 - Discussion

4.4.1 - Microbial community diversity of reference fens

While in general the individual substrate-induced response of the field sites followed the pattern set by the overall respiratory response (AWCD), three carbon sources (citric acid, α-ketoglutaric acid, and oxalic acid) provoked significantly different responses for at least one sampling date. Citric acid and α-ketoglutaric acid both caused significantly higher midseason Poplar SIR than at the other two sites. Oxalic acid raised start-of-season SIR in Poplar to a level that was not significantly different from that of the other two sites, and a midseason response significantly

higher than in the other two sites. If this spike in SIR were simply a result of the uptake and consumption of these carbon sources, it would likely be observed in response to any of the carbon sources used in this experiment regardless of functional group (which was not the case), unless the microbial community exhibited an unusually strong preference for carboxylic acids over saccharides or amino acids as carbon sources.

If this effect were actually the consequence of such a strong microbial community substrate preference, it would have likely been observed for all five carboxylic acids used in this experiment, rather than only these three. As neither other carboxylic acids nor other common labile carbon sources provoked such a response in the Poplar site, it seems unlikely that carbon substrate availability is the factor limiting microbial community response at the Poplar site. Both oxalic acid and citric acid, however, are thought to play a role in mobilization of soil micronutrients, nitrogen, and phosphorus (Clarholm et al. 2015; Dotaniya et al. 2014; Taghipour and Jalali 2013; Wei et al. 2010). Such studies have found that different low molecular mass organic acids (LMMOA) display peak efficiency in different environments, with citric acid found to be more effective for nutrient mobilization in low-pH forest soils (Clarholm et al. 2015; Wei et al. 2010), while oxalic acid is more effective in calcareous soils or soils with a higher pH (Clarholm et al. 2015; Dotaniya et al. 2014; Seshadri et al. 2014; Taghipour and Jalali 2013). This suggests that phosphorus limitation or the limitation of some other organic acid-mobilizable nutrient may be responsible for the lower start-of-season Poplar microbial community activity. These nutrients may not be limiting in the other sites, given that the addition of citric acid at the Pauciflora site or oxalic acid at the Saline site did not significantly increase respiration. This is consistent with the findings of Lin et al. (2014), who found that acrotelm peat at a boreal forest peatland site displayed signs of phosphorus limitation, and attributed a hotspot of activity in the

mesotelm to the microbial secretion of organic acids and C-P lyases. It would be relatively simple to test for this theory by repeating this experiment, but adding a source of labile phosphorus to every carbon source solution; if phosphorus is actually limiting at Poplar and not at the other sites, this should bring SIR levels for Poplar up until they are more in line with the other two sites, while increasing the SIR response from the other two sites somewhat less.

One unusual response shared across all sampling dates and sites was the low – and occasionally-negative – SIR response to arginine. As the incubations were done in the dark, it is unlikely that the addition of arginine provoked an anabolic response (i.e. consumption of CO_2) where every other amino acid did not. It is possible, however, that the catabolic response provoked by arginine might have interfered with the MicroResp™ assay method. The means of detection of CO_2 production in MicroResp™ is through dissolution of CO_2 into a gel containing the pH indicator cresol red; when dissolved, some portion of the CO_2 forms carbonic acid and dissociates, lowering the pH and causing a colour change in the pH indicator (Campbell et al. 2003). If catabolism of arginine released some gas (e.g. ammonia) that would increase the pH should it dissolve in the detection gel, it might mask any respiration actually occurring.

Overall, variation in midseason microbial aerobic carbon cycling potential was found to be more strongly controlled by plant species richness and canopy cover than the measured edaphic variables. It is possible that the very low explanatory power of edaphic variables compared to vegetation variables is due to the selection of the variables in question, and the inclusion of different edaphic variables (e.g. other groundwater ion concentrations) might explain more of the observed variation. Alternatively, this disparity might reflect a greater diversity of plant community composition than of inorganic soil conditions between the sites, as the site plant community compositions are fairly distinct from one another, while the large disparity in

electrical conductivity (and by extension salinity) between the sites may have been compensated for by the adaptation of the microbial community at the Saline site to high salinity, thus minimizing its ability to affect carbon cycling potential.

4.4.2 - Impact of OSPW contamination on aerobic microbial community function

Contrary to the hypothesis, contamination with OSPW did not significantly change the overall microbial potential activity or catabolic evenness of any of the three reference sites. One possible explanation for this arises from the physical structure and chemical properties of the peat soil. Peat soil structure has been known to immobilize nutrients and contaminants alike, both as a function of its function of its physical structure. The complex physical structure of peat, which contains flow paths of variable tortuosity based on pore size, and dead-end pores which can serve to sequester contaminants, has been found to contribute significantly to the ability of peat to immobilize or slow the passage of metal ions and large organic contaminants (Allen et al. 1994; Brown et al. 2000; McLellan and Rock 1988; Rezanezhad et al. 2012b). However, due to the homogenization of the peat samples and the very small quantity of peat used for this assay, it appears unlikely that the secondary structure of the peat itself is responsible for the attenuation of toxic effect.

However, the chemical composition of peat contains abundant carboxyl, phenol, and alcohol functional groups, as a consequence of the very slow decomposition of organic matter in the anaerobic part of the peat column. These functional groups have been shown to form chelation complexes that immobilize and limit the bioavailability of heavy metal ions (Clemente and Bernal 2006; Kumpiene et al. 2007; Lee et al. 2013). Previous studies of the movement of OSPW and NaCl through peat have shown that the amount of NaCl and NAs adsorbed onto the

peat in a contaminant uptake experiment was an order of magnitude higher than the amount of the same contaminants in the liquid phase once the OSPW had traveled through a 40 cm peat column (Rezanezhad et al. 2012a). It is therefore possible that some attenuation of potential toxic effects of the contaminants occurred through sorption of the contaminants to the peat substrate itself. Additionally, this experiment only measured substrate-induced respiration, which is necessarily a short-term response. Thus, any long-term toxic effects of OSPW would not have been detectable in the time frame of the experiment. A longer-term aerobic incubation would be the easiest way of detecting any such long-term effects, and the addition of a second contaminant treatment consisting solely of salt at the same concentration as in the OSPW used would aid in determining the degree of influence of NAs and NaCl on respiration responses in the short- and middle-term.

While overall respiration potential and catabolic evenness was not significantly influenced by treatment with OSPW, the individual SIR responses of arabinose, D-glucose, D-fructose, and N-acetylglucosamine did vary significantly with treatment. All of these substrates are monosaccharides or monosaccharide derivatives, and SIR response to the only disaccharide substrate, trehalose, did not vary with treatment. It is possible that this represents a stress response to the increased NaCl concentrations in the OSPW, as increased salt concentrations necessarily place some degree of stress on microbes (Galinski and Trüper 1994). Naphthenic acids (NAs) are large molecules foreign to the peat environment, whereas Na^+ is part of membrane concentration gradients in both single-celled and multicellular organisms. It seems more probable, given the short duration of the assay, that an increase in Na^+ concentration was more likely responsible for any stress response than toxicity of NAs.

Furthermore, of the three sampling sites, only the Saline site responded in a largely consistent manner, with OSPW significantly increasing SIR for all substrate-sampling date combinations except for start of season arabinose response. When compared to the responses from the Saline site, the Pauciflora and Poplar responses are both small and inconsistent, responding differently to treatment across the four carbon sources and/or across dates. The Saline site is so named because the groundwater and soil contain unusually high concentrations of NaCl. While the microbial community of this site are halophilic or halotolerant by necessity, these adaptations are not without energetic cost. Sudden changes in the osmotic pressure of the extracellular environment, if not speedily adapted to, will shrivel or burst the cell through osmotic water loss or uptake (Galinski and Trüper 1994). It is therefore likely that the microbiota of the Saline site would display the largest immediate metabolic response and therefore substrate demand of the three sites.

In contrast, the increase in salt concentration may not exceed the threshold necessary for stress response mechanisms to be activated in the microbiota of the other two sites. It is possible that when these osmotic stress responses are triggered, the increased metabolic load leads to a preference for those substrates for which uptake mechanisms are already in place and which require few intermediate transformations in order to be catabolized for energy, e.g. simple sugars. The lack of such a preference response for trehalose might be explained by its role in resisting salt stress, both in plants and microorganisms; thus, addition of trehalose alongside salt concentration might attenuate the stress the additional salt causes. In contrast, the relatively small and variable responses of Pauciflora and Poplar samples to OSPW might suggest that the threshold to salt stress may be different across the sites due to variability of soil physicochemical conditions or that the microbial communities of the Pauciflora and Poplar fens may be better

adapted to withstand these stresses than the Saline community. As the Saline fen is a hypersaline site, it seems probable that its community is highly specialized to survive in that environment, while the less extreme Poplar and Pauciflora communities are comparative generalists. This might account for their lack of response to OSPW, while Saline's specialization does not allow it the functional diversity to resist OSPW contamination. As this experiment in general gave very little insight into the link between microbial community structure and functional potential, integration of 16s rRNA sequencing experiments before and after exposure to OSPW would allow greater insight into the role of microbial community structure in resistance to OSPW toxicity.

4.5 - Conclusions

Both microbial functional diversity and microbial community carbon cycling potential were found to vary significantly by reference site. Carbon cycling potential was significantly greater at the start-of-season than the midseason time points at the Pauciflora and Saline sites, but these two sites did not differ significantly from each other in respiration potential at either time point. Conversely, respiration potential did not vary significantly with time of season at the Poplar site, but Poplar start-of-season potential was significantly less than at the other two sites, while midseason respiration potential was not significantly different from that of the other two sites. With respect to microbial functional diversity, the Poplar site microbial community evidenced a strong respiratory response to certain low molecular weight organic acids, while the Saline community response to saccharides when under additional salt stress via the addition of OSPW as a contaminant. Both site-specific responses are reasonably explained by site biogeochemical conditions, as the Saline microbial community could be expected to react more quickly to further

salt stress than those of the other two sites due to existing hypersaline conditions, while a strong preference for specific LMMOA indicates a potential phosphorus limitation at the Poplar site.

Contrary to expectation, addition of OSPW did not significantly reduce overall site carbon cycling potential activity in any samples, and the only significant response provoked by OSPW treatment was the aforementioned increase in saccharide utilization at the Saline compared to the control treatment at the same timepoint.

The above can tentatively be considered good news for the reclamation projects currently ongoing and yet to come in the Athabasca region, as the lack of significant effect of OSPW on aerobic microbial carbon cycling suggests that reclaimed sites constructed under the current model may be able to resume normal aerobic microbial carbon-cycling function provided that the site vegetation complement can be restored, given that it is the major controlling factor on microbial activity. Both findings regarding patterns of microbial community activity response can be of immediate value in already-constructed reclamation sites as a 'baseline' against which the microbial community function of the developing constructed site can be compared. In conjunction with monitoring of the edaphic and vegetation-related variables of such a site, this may allow managers of such sites to make informed predictions about the site's eventual successional trajectory.

However, it is by no means certain that OSPW will have no effect on aerobic microbial activity overall, as the duration of the assay period in this study was only six hours, which was likely insufficient time for deleterious effects to make themselves known, given the ability of peat to both physically and chemically retard the transport of metal ions and organic contaminants. Future studies on the matter should include longer-term incubations to determine the detrimental

effects of OSPW on microbial community function over timescales that better approximate the duration of contaminant exposure reclaimed peatlands may face.

Chapter 5 - Contamination by oil sand process-affected water impacts potential methanogenic activity in fens of Northern Alberta

5.1 - Introduction

Wetlands are a very frequent landscape type in Northern Alberta, with up to 50% of the landscape being wetlands; of these wetlands, approximately 90% are fens (Vitt et al. 1996). These fens are subject to a range of hydrological and physicochemical conditions, with water input sources ranging from mineral-rich streams or groundwater flow in the case of rich fens, to water input principally via precipitation, in the case of poor fens (Vitt and Chee 1990). These conditions, in turn, influence the fen's vegetation composition (Chee and Vitt 1989; Slack et al. 1980), which dictates both the physical structure and chemical conditions of the fen through the physical and chemical properties of the deposited plant litter, respectively. The high water table typical in peatlands (Ingram 1982) causes decomposition of this deposited plant litter to be slower than its accumulation (Berg 2000), and creates the anoxic conditions where methanogenesis can occur. While the rate of microbial activity under such conditions is much lower than in aerobic conditions, the sheer volume of anoxic peat in any peatland means that anaerobic processes are still an important component of the carbon cycling function of any peatland (Clymo 1984; Wright et al. 2011). Methanogenesis is the most common anaerobic carbon catabolic pathway in fens (reference). Fens are significant sources of CH_4 release to the atmosphere (Mitsch et al. 2013), and methane is a significantly more potent greenhouse gas than CO_2 (Forster et al. 2007).

Vegetation and edaphic conditions both impact belowground processes in fens including heterotrophic respiration by microbial communities, which leads to the production of CO_2 in aerobic conditions and to methanogenesis - the production of CH_4 - and in some cases the

production of CO_2 in anaerobic conditions. For instance, the roots of vascular plants both introduce small oxic zones into otherwise anoxic peat and provide a source of labile carbon for peat microbes in the form of root exudates, the latter of which can influence the function of the microbial community (Yan et al. 2008). Non-vascular vegetation (e.g. *Sphagnum* mosses in poor fens) also affect their environment through means other than litter deposition – in *Sphagnum*'s case, through acidification of their environment by release of H^+ ions in exchange for Ca^{2+}. This in turn influences CH_4 production, as decreasing pH has been shown to significantly decrease methanogenesis (Ye et al. 2012).

However, water chemistry – specifically, redox potential - is the most significant environmental control on a fen's methanogenic function. Methanogenesis occurs due to the unavailability of alternative terminal electron acceptors under anoxic conditions, which dominate the majority of the peat column. Under anoxic conditions, carbon can be used instead of oxygen as a terminal electron acceptor, although as the redox potential of carbon is significantly lower than that of oxygen, the reactions are significantly less energetically favourable. Moreover, carbon is also a less favourable electron acceptor (i.e., has a lower redox potential) than several other compounds that can be found in fen environments (e.g. NO_2^-, NO_3^-, Fe^{3+}, SO_4^{2-}). The presence of these ions in solution can lead to the competitive inhibition of methanogenesis (Balderston and Payne 1976; Van Bodegom et al. 2004; Bollag and Czlonkowski 1973; Karhadkar et al. 1987), as resources used by both kinds of microbes (e.g. CO_2 + H_2, acetate) are more efficiently consumed by organisms that utilize more energetically favourable substrates (Klüber and Conrad 1998a, 1998b; Watson and Nedwell 1998). Hydrological conditions in the fen may also increase abundance of alternative electron acceptors – drought conditions and the lowered water tables they bring will oxygenate previously anoxic peat, which has been shown to allow for the

regeneration of alternative electron acceptors, leading to suppression of methanogenesis, which can last for several weeks beyond rewetting, as any more favourable electron acceptors must be reduced before methanogenesis can be thermodynamically favourable (Eimers et al. 2003; Freeman et al. 2002; Knorr et al. 2009).

As oil companies conclude extraction activities at oil sands extraction sites, there will be an associated rise in the number of reclamation projects including wetlands and upland systems. Greater knowledge of how varying edaphic conditions influence anaerobic microbial activity in fens will be useful to interpret and predict anaerobic responses in their constructed counterparts.

Post-mining landscapes are characterized by the presence of complex chemical compounds and ion species at higher concentrations than normally appear in the region (e.g. Na^+), and contaminants that are entirely foreign to the landscape under normal circumstances, such as naphthenic acids (NAs) (Purdy et al. 2005; Trites and Bayley 2009). The impacts of these contaminants, especially NAs, on fen anaerobic functions, such as methane production, are poorly understood. Predicting the interaction between contamination, existing environmental conditions and methanogenesis at such sites will be complicated, and a greater understanding of the impact of contaminants on methane production in fen peat is thus urgently needed.

To quantify these effects and provide a baseline for the monitoring of developing constructed fens in the area, this study aimed to 1) Measure the natural range of CH_4 potential production in three fen types of the Athabasca region of Alberta, 2) Characterise which environmental variables influence potential CH_4 production, and 3) Determine the effect of oil-sands process-affected water (OSPW) and NaCl-amended OSPW on potential CH_4 production in these reference systems. We hypothesized that the methane production potential of the peatlands varies significantly with the biogeochemical properties unique to each type site, that addition of OSPW

significantly inhibits methanogenesis at all sites, and that the addition of further salt causes greater inhibition than the addition of OSPW alone.

5.2 - Materials and Methods

5.2.1 - Sample Incubation and Analysis of Methane Production

Mid-season peat samples from each field site were bulked, and 10 g of peat from each site was placed within a 125 mL Erlenmeyer flask and suspended in sufficient water to inundate the peat. The water used to inundate the peat was either Milli-Q water, serving as a control, OSPW, or OSPW amended with sufficient salt (an additional 1331 mg L^{-1}) to double the total salt concentration of the water. Post-inundation, the flasks were evacuated of air and filled with a nitrogen atmosphere in a glovebox, then sealed with a butyl rubber stopper. Each site-treatment combination was made in triplicate. After the initial replacement of the headspace, the flasks were incubated in the dark at a temperature of 24.5 °C. The flasks' headspace was sampled via gas syringe at 0, 1, 2, 5, 7, 14, 21, 28, 38, and 42 days. Each sampling removed 20 mL of the headspace gas and replaced it with 20 mL of 99% N_2 gas. Prior to each sampling, the gas syringe was flushed three times with the same 99% N_2 gas and the headspace was mixed by repeated filling and emptying of the syringe without removing it from the septum. Samples, once withdrawn from their flasks, were stored in Labco Exetainers ® at 4°C until processed. Samples were processed on a Shimadzu GC-2014 gas chromatograph using the manufacturer-provided LabSolutions software (version 5.71 SP2), and analyzed for CO_2, N_2O, O_2, N_2, and methane (CH_4) content. Two replicate time series – one each from the control samples for the Pauciflora and Poplar sites – were considered as extreme high and low outliers, respectively, and were not included in the data analysis.

5.2.2 - Statistical Analysis

We used generalized linear models (function 'lm', package 'stats'), R Core Team, 2016) to generate the potential production rate (the slope of the portion of the methane concentration curve prior to maximum concentration being reached). The potential production rate, maximum methane concentration reached, and the time until the peak concentration were compared between sites and treatments within sites using non-parametric multivariate ANOVA (function 'adonis', package 'vegan', Oksanen et al. 2016). The significance of these differences was evaluated using Tukey's Honest Studentized Difference method (function 'TukeyHSD', package 'stats', and function 'multcompLetters4', package 'multcompView' (Graves et al. 2015) on the results of a multivariate ANOVA (function 'manova', package 'stats'). The influence of site environmental factors on potential methane production was determined by correlation test (using Kendall's tau statistic as the data were non-normal) between the derived values described above and site environmental factors (functions 'cor' and 'cor.test', package 'stats', REF). All statistical analyses were carried out using the software program R (R Core Team, 2015).

5.3 - Results

5.3.1 - Natural variation in potential methane production

The slope of the portion of the methane concentration curve prior to peak concentration was used as a measure of potential methane production rate; peak methane concentration and time to peak methane concentration were also used as measures of overall methane production potential. These results are summarized below as mean methane concentration curves separated by site and treatment within site (Figure 5-1).

Overall, methane production differed significantly by site ($F = 182.82$, $p=0.001$) and by treatment within site ($F=28.63$, $p=0.001$), as did maximum methane concentration reached

(F=66.62, p=0.001 and F=12.84, p=0.001 for site and treatment within site respectively). The time until peak concentration differed significantly by site (F=7.11, p=0.008) but not by treatment within site (F=2.69, p=0.057) (Table 5-1).

Table 5-1: Results of nonparametric multivariate ANOVA on parameters of methane production curve until maximum methane concentration was reached. Parent factors are separated from nested factors by a colon. Significant results are bolded.

Variable and model	d.f	F-value	p-value
CH_4 prod. rate			
Site	2	181.82	**0.001**
Site:Treat	6	28.63	**0.001**
Residuals	16		
Max. CH_4 conc.			
Site	2	66.62	**0.001**
Site:Treat	6	12.84	**0.001**
Residuals	16		
Time to max. conc.			
Site	2	7.11	**0.008**
Site:Treat	6	2.69	0.057
Residuals	16		

Mean natural potential methane production rate was higher at Poplar (104.8 µg CH_4 g dry peat $^{-1}$ d^{-1}) than at Pauciflora (48.4 µg CH_4 g dry peat $^{-1}$ d^{-1}) but not significantly so (p=0.774); both were significantly (p_{pop}<0.0001, p_{pau}=0.0004) greater than Saline's rate (3.8 µg CH_4 g dry peat $^{-1}$ d^{-1}). Natural mean peak methane concentration was significantly (p=0.044) higher in Poplar (3815.5 ppm) than in Pauciflora (433.9 ppm), which was not significantly greater (p=0.305) than Saline's peak concentration (129.3 ppm). Finally, Poplar's mean time to maximum concentration (38 days) was not significantly greater (p=0.971) than Saline (30 days), which was not significantly greater (p=0.088) than Pauciflora's (10.5 days). However, Poplar's time to peak was significantly (p=0.024) greater than Pauciflora's (Table 5-2).

5.3.2 - Effects of contaminants on methane production potential

Addition of OSPW and salt-amended OSPW decreased Pauciflora's mean methane production rate to 38.3 µg CH_4 g dry peat $^{-1}$ d^{-1} (79% of control) and 21.0 µg CH_4 g dry peat $^{-1}$ d^{-1} (43% of control), respectively, and increased peak concentration to 861.0 ppm (198% of control) and 581.9 ppm (143% of control), respectively. None of these changes were significantly different from the control values.

Poplar methane production rates under OSPW and salt-amended OSPW contamination were 7.4 µg CH_4 g dry peat $^{-1}$ d^{-1} (7% of control rate) and 2.8 µg CH_4 g dry peat $^{-1}$ d^{-1} (2.6% of control), respectively; peak methane concentrations under the same contaminants were 271.8 ppm (7% of control) and 108 ppm (2.8% of control), respectively. OSPW contamination caused a significant decrease of both methane production rate (p=0.0002) and peak concentration (p=0.003), but salt-amended OSPW responses were not significantly different (p_{rate}= 0.173, p_{conc}=0.413) from the response to OSPW alone.

Saline methane production rates under OSPW and salt-amended OSPW was 0.29 µg CH_4 g dry peat $^{-1}$ d^{-1} (7.6% of control) and 0.13 µg CH_4 g dry peat $^{-1}$ d^{-1} (3.4% of control), respectively; peak concentrations were 14.6 ppm (11.2% of control) and 10.3 ppm (8% control), respectively. As with Poplar, contamination with OSPW significantly decreased both rate (p<0.0001) and peak concentration (p= 0.013) below natural levels, but the responses to salt-amended OSPW were not significantly different (p_{rate}= 0.752, p_{conc}=0.999) from the response provoked by OSPW alone (Table 5-2).

Table 5-2: Means (standard error) of values derived from analysis of methane concentration time series. Methane production was calculated from the period until maximum methane concentration is reached. Maximum methane concentration is given in parts per million, time until maximum concentration in days. Treatments within a column that share a letter code are not significantly different, as determined by Tukey's Honest Studentized Difference test on the results of a multivariate ANOVA.

Site and treatment	Methane production (µg CH_4 g dry peat $^{-1}$ d^{-1})	Max. CH_4 conc. (ppm)	Approximate time to max conc. (d)
Pauciflora			
Control	48.4(7.8)^{ab}	433.9(59.0)^{bcd}	10.5(3.5)^{b}
OSPW	38.3(12.0)^{ab}	861.0(328.8)^{ab}	24.3(7.1)^{ab}
OSPW+NaCl	21.0(1.3)^{b}	581.9(116.9)^{abc}	32.3(5.7)^{a}
Saline			
Control	3.8(0.4)^{d}	129.3(43.3)^{cd}	30.0(8.0)^{ab}
OSPW	0.29(0.009)^{e}	14.6(3.1)^{e}	38.0(0)^{a}
OSPW+NaCl	0.13(0.001)^{e}	10.3(0.9)^{e}	40.7(1.3)^{a}
Poplar			
Control	104.8(25.8)^{a}	3815.5(590.7)^{a}	38.0(0)^{a}
OSPW	7.4(1.2)^{cd}	271.8(55.4)^{bcd}	37.3(4.7)^{a}
OSPW+NaCl	2.8(0.9)^{d}	108.2(42.0)^{d}	37.3(4.7)^{a}

The time taken to reach this maximum methane concentration, conversely, did not vary significantly with respect to treatment in Poplar and Saline samples, though the control Saline samples took less time to reach maximum methane concentration than OSPW or OSPW+NaCl, albeit not significantly so. There was an overall trend towards a lower time to maximum CH_4 concentration along with increasing contamination in the Pauciflora samples, but only the difference between the control and the OSPW+NaCl samples was relevant (Table 5-2).

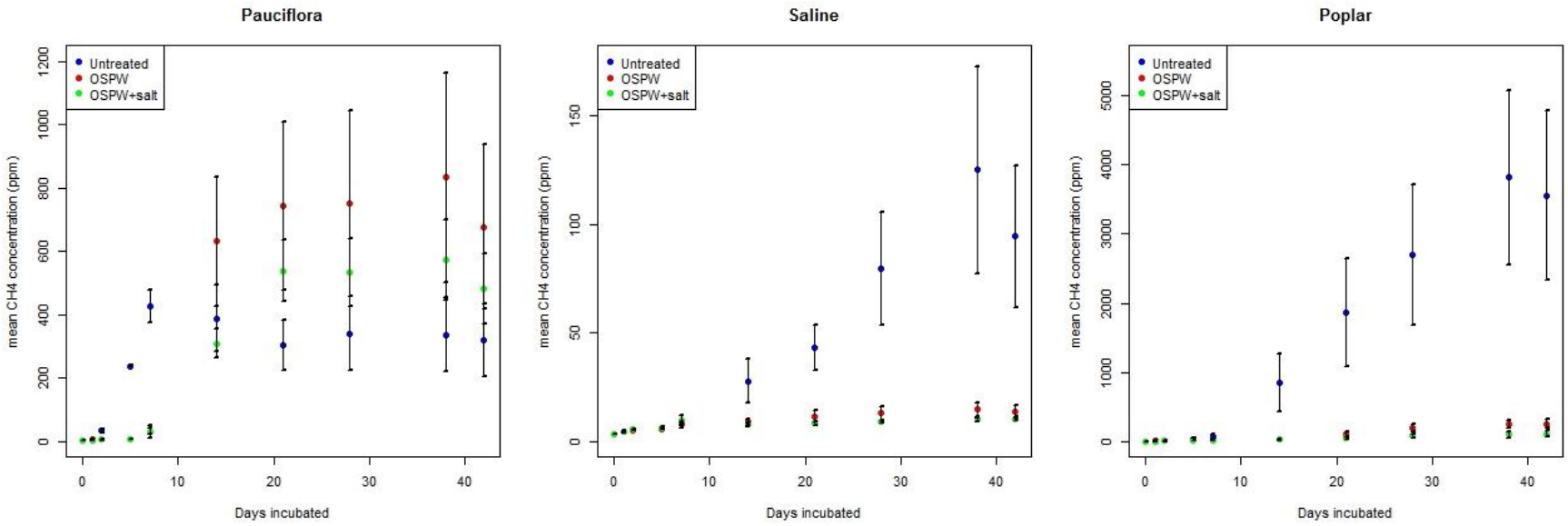

Figure 5-1: Mean and standard error of dilution-corrected methane concentration over time, separated by site. Y-axis scales are different across sites to allow for easier readability.

Table 5-3: Correlation between edaphic and vegetation factors and parameters of methane production. EC = electrical conductivity, WT= water table depth.

	CH4 production rate		**Maximum CH4 concentration**		**Time to peak concentration**	
	Correlation	p-value	Correlation	p-value	Correlation	p-value
pH	-0.176	0.52	-0.156	0.52	0.462	0.047
EC	-0.656	0.198	-0.616	0.198	0.351	0.206
Soil moisture	-0.4	0.01	-0.424	0.01	-0.073	0.856
WT depth	0.656	0.198	0.616	0.198	-0.351	0.206
Tree cover%	0.138	0.052	0.168	0.052	0.331	0.194
Canopy cover%	0.4	0.01	0.424	0.01	0.073	0.856
Vasc. richness	0.4	0.01	0.424	0.01	0.073	0.856
Moss richness	0.656	0.198	0.616	0.198	-0.351	0.206

5.3.3 - Effect of environmental factors on methane production potential

Only four environmental factors were significantly correlated to any of the measures of methane production potential we used– pH, soil moisture, canopy cover, and vascular plant richness. Soil moisture was found to be moderately negatively correlated with both production rate and maximum concentration reached, while both canopy cover and vascular richness were found to be moderately positively correlated to production rate and maximum concentration. pH was found to be significantly moderately positively correlated with the time taken to reach peak concentration (Table 5-3).

5.4 - Discussion

5.4.1 - Effect of OSPW/ OSPW + NaCl contamination on methane production potential

The three sites varied in their response to OSPW contamination more or less along the lines of nutrient availability. In the two rich fens (Poplar and Saline), mean methane production rate fell to 7% of the natural rate after exposure to OSPW, and peak methane concentration fell to 7% and 11% of natural values in Poplar and Saline, respectively, on exposure to OSPW. While the addition of salt further diminished both methane production rate and peak methane concentration in both Poplar and Saline, in neither case was the difference significant. It is therefore impossible to tell whether the depression of methanogenic activity at Poplar under OSPW is due to the high salt (NaCl) concentration of the water, naphthenic acid toxicity, the toxicity of some other contaminant entirely or potentially the introduction of an alternative electron acceptor. In the case of the Saline fen, however, the significant depression of methanogenesis suggests high

sodium concentrations are unlikely to be the cause, given that the microbial community is likely already adapted to high-salinity conditions.

The lack of significant change in time to peak concentration, lowered methanogenic rates, and lowered peak methane concentrations with contamination, in the Saline and Poplar sites appears to indicate inhibition of methanogenesis - less substrate overall is processed during the incubation, as represented by the significantly decreased peak concentration. In contrast, the increase in time to peak concentration and insignificant reduction in methanogenic and peak methane concentration with contamination in Pauciflora suggests that that methanogenesis in Pauciflora is not inhibited so much as it is slowed, as it seems that the microbial community simply takes longer to process the same overall quantity of substrate. However, as variability within production potential rates and peaks was quite high in all Pauciflora treatments (Figure 5-1), a repetition of this experiment with higher replication is needed to constrain the responses and reduce the variation within a given treatment and confirm this interpretation.

Overall, the low methane production potential and high susceptibility to OSPW contamination of the Saline site was contrary to initial expectations. We anticipated that methane production potential would be higher in rich fens than poor fens, as *Sphagnum*-dominated acid peatlands tend to have lower methane production potential than more neutral peatlands possessing vascular-plant dominated vegetation communities (Table 2-1). While methanogenesis can be temporarily suppressed in high-salinity situations (Denier van der Gon and Neue 1995; Edmonds et al. 2009) or under exposure to naphthenic acids (Holowenko et al. 2000, 2001), it is capable of recovery over time, indicating long term adaptability to these conditions. Furthermore, methanogenesis is possible even in very high-salinity conditions (Conrad et al. 1995; Liu and Boone 1991). The finding that the natural methane production potential of the Saline fen was

much lower than either Pauciflora or Poplar was therefore unexpected, as was the decrease in that potential upon the addition of OSPW and NaCl-amended OSPW. Given that methanogenesis was shown to be fairly resilient to the addition of 0.25 M salt in culture (Liu and Boone 1991) it may be possible that the high salinity level is not the primary cause of Saline's low methane production potential. A possible explanation is that relatively high levels of alternative terminal electron acceptors are present in the soil, which would explain the abnormally low natural production potential. A previous study of the hydrology and geochemistry of the Saline fen revealed the presence of SO_4^{2-} at concentrations of 28-3080 mg L^{-1} in the peat pore water (Wells & Price, 2015), and sulfate can suppress methane production through competitive inhibition (Dise and Verry 2001; Karhadkar et al. 1987; Lozanovska et al. 2016; Yavitt et al. 1987) and has been known to do so almost completely in bioreactors at concentrations between 800-2000 mg L^{-1} (Kroiss et al. 1985), well within the range of pore water sulfate concentrations found at the Saline site. It is therefore possible that methane production potential in the Saline fen is being suppressed by the presence of alternative electron acceptors.

The contrast between the very strong response of the Poplar microbial community to OSPW contamination and the comparative lack of response of the Pauciflora community may be explained by different mechanisms, predicated on the physicochemical differences between the sites. First, the Pauciflora and Poplar sites closely resemble the descriptions of the archetypal hydrogenotrophy- and acetoclasty-dominated peatlands. It is therefore likely that they differ in methanogenic pathway and preferred precursor. However, methanogens are invariably associated with a variety of syntrophic organisms that produce the necessary precursors (CO_2/H_2 or acetate), and a different community of syntrophs exists for each pathway. It is possible that the syntrophs of the Pauciflora site (which are likely to provide the precursors for hydrogenotrophic

methanogenesis due to site conditions) proved more resilient to the contaminants present in OSPW than the Poplar syntrophic community (which likely provide precursors for acetoclastic methanogenesis for similar reasons). Second, the sites are quite distinct in terms of pH, nutrient availability, vegetation and litter composition; it is therefore possible that some property of the *Sphagnum*-dominated Pauciflora peat may have attenuated the toxic effects of NAs and Na^+ at that site, while the sedge-derived peat of Poplar proved unable to do so. However, (Rezanezhad et al. 2012b) found that rich fen sedge-derived peat was able to sorb a large proportion of the naphthenic acids and Na^+ applied to it in the form of OSPW (94% sorption of 43.5 mg L^{-1} NAs per kg peat and approximately 84% sorption of 382 mg L^{-1} Na^+ per kg of peat). In conjunction with the results of chapter 4 of this thesis, where Saline and to a lesser extent Pauciflora evinced a slight nutrient preference response but where no site displayed an overall SIR decrease in response to the NAs and Na^+ present in OSPW under aerobic conditions, this suggests that neither NAs nor Na^+ are the contaminants responsible for the significant decrease of anaerobic respiration potential in Saline and Pauciflora on exposure to OSPW. A likely candidate would be an alternate electron acceptor, which have been known to suppress methanogenic activity (Kadharkar et al. 1987; Yavitt and Lang 1990; Dise and Verry 2001; Lozanovska et al. 2016). Sulfate in particular is known to be present in some oil sands tailings, (Franklin et al. 2002) and is also present in the pore water of the Saline site (Wells and Price 2015). If OSPW contains an alternative electron acceptor, it would likely not have much influence on the thermodynamics of aerobic respiration (as oxygen is likely a better terminal electron acceptor than any found in OSPW), but would inhibit methanogenesis nonetheless, yielding results consistent with the findings of this chapter and Chapter 4.

5.4.2 - Drivers of methane production potential

Only four of the edaphic and biotic variables tested significantly influenced the parameters of methanogenesis in any way - canopy cover percentage and vascular plant species richness both moderately positively influenced potential methanogenic rate and maximum concentration reached, while soil moisture negatively influenced both of these variables, and pH moderately significantly positively influenced time taken to reach maximum concentration. Water table was not significantly correlated to methane production potential, in contrast to its influence on vegetation - Priede et al. (2016) found that water table was the single most important variable for the reestablishment of fen vegetation on cutover sites. The positive correlation of canopy cover and vascular species richness are consistent with previous research (Table 2-1) that indicates that vascular dominated vegetation compartments increase methanogenic potential both through the provision of more labile carbon than is provided by mosses and through rhizo-deposition of highly-labile root exudates much deeper into the soil than they would normally occur. Similarly, the positive correlation of pH with time taken to reach maximum methane concentration may reflect a tendency for higher-pH (and therefore more neutral) fens to provide higher-quality carbon input than acid fens and have a greater proportion of vascular vegetation, thus providing a greater pool of substrate for methanogenesis.

5.5 – Conclusions

Natural methane production potential was higher in the Poplar rich fen than in the poor Pauciflora fen, but Saline fen natural methane production potential was much lower than the other two sites, which was thought to be due to inhibition of methanogenic potential by some factor intrinsic to the site. This factor was thought not to be the high Na^+ concentration of the Saline pore water, as methanogenesis is known to adapt to high-salinity conditions over time.

Response to contamination varied with vegetation type - amendment with OSPW caused the methanogenic potential of both sedge-peat fens (Saline and Poplar fens) to be sharply curtailed over the short to middle term (i.e. our incubation period of 6 weeks). Neither community's methane production potential was significantly more adversely affected by OSPW amended with additional salt compared to amendment with OSPW alone, suggesting that the high sodium content of OSPW was not the primary inhibitory factor in Poplar as well as in Saline. The effect of OSPW amendment on the methane production potential of the *Sphagnum*-dominated Pauciflora poor fen was insignificant, and the apparent ability of the Pauciflora peat to attenuate the inhibitory effect of OSPW on methanogenesis at the other two sites is potentially of interest to creators and managers of constructed peatland sites. Unfortunately, the variance in the Pauciflora results was extremely large, and thus these conclusions are somewhat tentative.

In contrast to these results, there was little evidence of inhibition of aerobic response by contamination with OSPW in the Poplar site and no significant decrease in overall SIR at any site (Chapter 4). This discrepancy might be due to the extended incubation period compared to the SIR assay or the comparatively greater quantity of OSPW relative to the peat assayed, but neither of these explains why the Saline site, whose sedge vegetation is known to be less resilient to degradation than *Sphagnum* litter (Freeman et al. 2001), would have a natural methane production potential so much lower than that of the Pauciflora poor fen. Given that the Pauciflora and Saline aerobic respiration potentials were quite similar both at start of season and midseason, this discrepancy further suggested some Saline-intrinsic suppression of methanogenesis. Sulfate is present in the Saline pore water (Wells and Price 2015) at concentrations sufficient to inhibit methanogenesis. Inhibition of methanogenesis by a more favourable terminal electron acceptor would explain the discrepancy in methanogenic potential between Pauciflora and Saline without

contradicting the aerobic respiration results, as oxygen is a more favourable alternative electron acceptor than sulfate and thus sulfate's presence in pore water would not influence aerobic respiration.

While OSPW appears to heavily suppress the methane production potential of sedge-derived peat, in the long term, if the inhibition of methanogenesis is indeed due to an alternative electron acceptor, this problem may resolve itself in constructed sites as the alternative electron acceptor pool is exhausted in the anaerobic portion of the soil column. This relies on the maintenance of a stable water table, however, as any re-exposure to aerobic conditions will re-oxidize the alternate electron acceptor and resume suppression of methanogenesis. The maintenance of methanogenesis result may not initially appear favourable, as methane is a much more powerful greenhouse gas than CO_2, but methanogenesis is a much less efficient – and therefore slower - means of carbon breakdown than remineralization coupled to reduction of nitrate or sulfate. Thus, while it may not be favourable for greenhouse gas balance purposes, it will slow the degradation of anoxic peat, allowing for more stable long-term peat accumulation.

The apparent ability of *Sphagnum* peat to attenuate this effect, however, may prove to be of considerable use in the creation of these reconstructed fens. Further studies integrating microbial community composition and activity would be needed to further understand the contrasting responses of the different sites. The most useful subsequent experiment would be a repetition of the incubation study, but with a greater number of replicates so as to confirm or disprove any contaminant-attenuating properties of Pauciflora's peat. Other further studies might test whether Pauciflora's resistance to suppression of methane production potential by OSPW is due to some property of the microbial community or the peat itself via a sterilized-peat reciprocal inoculation experiment, as in Preston and Basiliko (2015). In such an experiment, microbial community

samples are taken from each site, and inoculated into peat samples from each site that have been sterilized via gamma radiation before methane production potential is tested for all combinations. In this case, one further treatment would be applied – treatment with either Milli-Q water (as a control) or with OSPW. Given the inability of the sedge-peat samples to provide the same resistance, it is unclear whether Pauciflora's resistance to OSPW contamination stems from a resilient microbial community or some chemical or physical property of the peat itself. A further understanding of this mechanism might prove important in the choice of whether or not to use *Sphagnum*-derived peat in future constructed sites. If attenuation of methanogenesis inhibition is a function of the microbial community, it may well continue unless the community is disturbed by some other factor (e.g. long-term toxic effects of other components of OSPW). On the other hand, if physical or chemical immobilization by peat is the source of this attenuation and *Sphagnum* peat is used in site construction, methanogenesis may suddenly decrease at a future time when the peat reaches its sorptive capacity for the inhibitory agent. In either case, however, if OSPW is present in reconstructed fen sites, the choice of substrate peat used appears to be crucial in determining their initial methane production potential, at least in the short term.

Chapter 6 - Conclusion

The sites studied differed from each other in terms of natural carbon cycling potential under both aerobic and anaerobic conditions, and these differences were not consistent between aerobic and anaerobic conditions. Natural aerobic carbon cycling potential was not significantly different between Pauciflora and Saline overall, while both sites had significantly greater carbon cycling potential than Poplar at start of season, but did not differ significantly at midseason. Overall aerobic carbon cycling potential was significantly lower at midseason than in start of season in Saline and Pauciflora, but was not significantly different by sampling date in Poplar. Poplar's low carbon cycling potential was possibly due to phosphorus limitation, indicated by a Poplar-specific substrate preference for low molecular weight organic acids, which have been implicated in mobilization of phosphate in soil systems. In contrast, anaerobic carbon cycling potential was significantly higher in Poplar than it was in Pauciflora, both of which had significantly greater methanogenic potential than Saline. Saline's low anaerobic carbon cycling potential might be explained by the presence of inhibitory quantities of sulfate, a more favourable terminal electron acceptor than carbon, in Saline pore water.

The effect of application of OSPW likewise differed by site and between aerobic and anaerobic conditions. Overall aerobic carbon-cycling potential was not significantly influenced by the addition of OSPW in any site, irrespective of sampling date. Saline and to a lesser extent Poplar displayed substrate preference changes when exposed to OSPW, specifically a slight preference for saccharide substrates. This tendency was stronger in Saline than in Pauciflora. In contrast, anaerobic carbon cycling potential in both Saline and Poplar was significantly and heavily inhibited by the addition of OSPW, while Pauciflora potential was not significantly changed.

Finally, the environmental drivers behind carbon cycling potential also varied between aerobic and anaerobic respiration, though less than with response to OSPW or carbon cycling potential. Vascular plant species richness and canopy cover were good predictors of both aerobic and anaerobic carbon-cycling potential; aerobic carbon-cycling potential was also well predicted by moss species richness, while anaerobic potential was significantly negatively correlated with soil moisture percentage.

Priede et al. (2016) found that spontaneous revegetation of cutover peatland sites with fen species was most strongly correlated to water table level first and foremost, and pH second, largely as a control on what kind of community (rich vs. poor fen species) might develop. In contrast, in this study neither water table nor pH were significant predictors of aerobic or anaerobic carbon-cycling potential. If the conditions for spontaneous revegetation on cutover peatlands are similar to the conditions for facilitated revegetation in constructed peatlands, this means that optimizing for pre-disturbance function of either the plant or microbial community may work at cross purposes to restoring the proper function of the other, or at least may not benefit the other compartment save indirectly.

Preston and Basiliko (2015) found that under normal circumstances, the microbial communities from a wide variety of peatland ecosystems would converge towards common structure and function when inoculated into the same environment. This assumption did not hold true, however, when the inoculated microbes came from a highly polluted environment, instead producing distinct patterns of structure and function. By extension, it seems logical that being in these polluted environments shaped these communities such that they would diverge in structure and function from other inocula even given a common substrate. It is, however, unclear whether site-specific conditions would shape the change in a microbial community's response to these

pollutants. Reciprocal-inoculation experiments as in Preston and Basiliko (2015), with the addition of a further OSPW treatment, would aid our understanding of the system, as any microbial community introduced into these reconstructed sites will likely diverge significantly from their state in their source fens due to the contaminants present therein. Knowledge of whether or not the environmental conditions of a peat donor site will yield a microbial community better equipped to deal with the contaminants present in a reconstructed site will be important for informed decision-making in the construction of such sites.

Similarly, Rezanezhad et al. (2012a) demonstrated that sedge peat itself was able to attenuate the inhibitory effect of OSPW contaminants on aerobic carbon cycling potential by both physically and chemically sequestering naphthenic acids and Na^+. However, the results of Chapter 5 indicate that sedge peat appears unable to provide the same attenuatory effect for anaerobic carbon cycling, while the anaerobic carbon cycling potential of the *Sphagnum*-derived peat of Pauciflora was not significantly affected by addition of OSPW. Given that the physical and chemical characteristics of sedge peat that enabled it to immobilize naphthenic acids and Na^+ were ineffective against the inhibition of anaerobic respiration by OSPW, it is unclear whether Pauciflora's resistance comes from a resilient microbial community or some chemical or physical property of the peat that enables it to sequester the contaminant responsible. The nature of Pauciflora's resistance to inhibition of methanogenesis by OSPW is of immediate interest in informing the choice of whether to use *Sphagnum-derived* donor-peat. Microbial resilience to OSPW-derived contaminants might provide lasting attenuation of inhibition of methanogenic function, while if peat sorption is the source of resistance, it might indicate that such attenuation would be only temporary, as it would cease when the peat reached sorptive capacity for the inhibitory compound.

Depending on the answers to the above questions, and how the hydrological conditions and vegetation community of the site develop, the GHG emissions from these constructed peatlands may vary significantly in amplitude and in composition (CO_2 vs CH_4) from their natural counterparts. We propose that field measurements will be necessary to fully understand the impact of contaminant on GHG emissions *in situ*, and should be tied in with detailed analyses of microbial communities and edaphic conditions.

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