CARBON SOURCES, MICROBIAL COMMUNITY PRODUCTION, AND RESPIRATION IN CONSTRUCTED WETLANDS OF THE ALBERTA, CANADA OIL SANDS MINING AREA

by

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ABSTRACT

Carbon sources, microbial community production and respiration were determined in 9 wetlands in northeastern Alberta subject to oil sands mining. A stable isotope mixing model estimated the proportion of carbon sources (primary production, sediment and petroleum) assimilated by microbes. The proportions of petroleum-derived carbon from oil sands process material (OSPM)-affected wetlands ranged from 62-97%. Bacterioplankton production was quantified by monitoring ^{3}H-leucine incorporation into bacterial proteins. Production and methanogenesis were inhibited by salinity and sulphate, respectively. Amending wetland sediments with topsoil, a reclamation strategy, did not affect bacterial production, or stimulate decomposition. Unvegetated wetland sediments were small net exporters of C (15.59 mg/m^2/d) and do not appear to be on a trajectory to becoming net sinks in these early stages of development. Overall, microbial functional processes in OSPM-affected wetlands are markedly different from processes in reference constructed wetlands of equivalent age and do not correspond to those in a natural wetland.

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CHAPTER I:
GENERAL INTRODUCTION

Microorganisms play a fundamental role in aquatic ecosystems in that they are the primary consumers of allochthonously contributed and autochthonously produced organic carbon. They have the potential to supply much of the carbon and energy to organisms at higher trophic levels. Microorganisms are not readily visible to the naked eye, and include viruses, bacteria, algae, protozoa and fungi (Sigee 2005). In this thesis I operationally define microorganisms and microbial processes as being dominated by bacteria, although viruses, protozoans, and fungi were not excluded in the study, except in chapter 3 where microbes strictly refers to bacteria.

Bacteria in aquatic environments can act as mediators of nutrient release from detrital materials (Berman et al. 1987). If large quantities of bacteria are consumed by microbial grazers then a significant portion of the nutrients in bacterial cells may be recycled within an aquatic food web (Berman et al. 1987). The carbon and other major elements supplied from primary producers have also been demonstrated to pass through the "microbial loop" to higher trophic levels (Pomeroy 1974, Azam et al. 1983, Berman et al. 1987). Microbial dominance in at least one aquatic ecosystem's metabolism, the ocean, is well established (Landry 2002).

In heterotrophic systems, such as soil (Coleman 1995) and estuaries (Findlay et al. 1992), microbes represent the trophic base of the ecosystem and. they can be more productive than primary producers (Hall et al. 1998). Bacteria are often associated with particulate detritus, such as in lotic ecosystems (Meyer 1994), which allows bacterial biomass to be consumed by organisms much larger than the cells themselves, such as protozoans and invertebrate larvae (Hall 1998).

It has become increasingly clear over the past 3 decades that microbes play a much larger role in marine and freshwater systems than previously thought (Legendre et al. 1995). During most of the 20th century, microbes were believed to be important in the turnover

of nitrogen and phosphorus, but not to be significant components of the flux of carbon in the marine ecosystem (Pomeroy 2001). Pomeroy (2001) combined the ranges of bacterial production reported by Ducklow (1992) and the ranges of bacterial growth efficiencies reported by Jahnke and Craven (1995), and determined that most marine primary production is actually utilized in the microbial loop. Analyses of food webs that omit bacterial processes are likely missing a significant portion of the flux of energy or carbon. The objectives of my research are to identify carbon sources that support the microbial community and to determine the relative importance of microbial production, biomass and respiration in newly constructed wetlands of varying ages and condition in the boreal region of northern Alberta subject to oil sands mining.

Microbial Use of Carbon in Natural Wetlands

Microbial communities may utilize a variety of carbon sources in wetlands. Primary production in natural wetlands is generally dominated by vascular plants, compared to phytoplankton (Moran et al. 1988). The bulk of primary production in wetlands enters the detrital pool. The microbial community is thought to be the primary group of organisms that utilize vascular plant detritus (Moran et al. 1988). Algae are also important sources of carbon that support the microbial community in wetlands. Hart and Lovvorn (2003) reported that microalgae rather than macrophytes provided most organic matter for these food webs in saline wetlands.

The ultimate fate of primary production in wetlands is less understood. The fate of primary production may be largely respiratory (i.e., CO_2 and CH_4) losses from the microbial community, with little biomass supporting production at higher levels. In contrast, primary production may be support the production of higher trophic organisms, in addition to the microbial community (Moran et al. 1988).

Wetland Reclamation

Anthropogenic impacts on wetlands result in their alteration and destruction. Impacts are caused by hydrological alteration, sedimentation, filling in of water bodies, eutrophication and pollution, peat mining, and mineral extractions (Geense 2004). Estimates suggest that about 50% of the world's wetlands have disappeared in the last few decades (Geense 2004). Wetlands are often created or restored to mitigate the loss of wetland function caused by these anthropogenically-induced changes to the landscape (Hunter and Faulkner 2001). Various reclamation strategies have been tested. Brown and Odum (1988) reported that spreading organic topsoil from a donor wetland onto a reclaimed wetland is a reliable rehabilitation technique. Erwin and Best (1985) reported that topsoil additions to freshwater marshes reclaimed from phosphate mining had higher macrophyte species richness and cover values than the overburden areas that were naturally revegetated. Topsoiling also encouraged the accelerated establishment of late successional plants, which could compete with aggressive weedy species, such as *Typha latifolia* (Erwin and Best 1985). A standard practice in the restoration of Canadian peatlands is the application of straw mulch, which promotes the re-colonization of *Sphagnum* moss (Waddington et al. 2003).

Oil Sands Mining

One of the largest reserves of hydrocarbons in the world is in the Athabasca Basin in northeastern Alberta, Canada (Holowenko et al. 2002). The oil sands industry currently produces over 120 million barrels of a light sweet crude oil annually, mainly through open pit mining. Production is expected to increase within the next decade to as much as 400 million barrels per year (Holowekno et al. 2002).

Bitumen is removed from the oil sands using the Clark Hot Water Extraction method. The extraction process uses a combination of hot water, steam, and caustic soda (NaOH) to separate the bitumen from the oil-bearing sand (The Fine Tails Fundamentals Consortium (FTFC) 1995). For each m^3 of oil sand processed, about 3 m^3 water is

required and that means 4 m^3 of fluid tailings are produced. The oil sands companies do not release any extraction wastes from their leases, so the process-affected waters and fluid tailings (oil sands process materials – OSPM) are contained on site, primarily in large settling ponds (Holowenko et al. 2002). By 1991, the volume of water in tailings ponds had reached approximately 310 x 10^6 m^3 (MacKinnon 1993) and is expected to reach 10^9 m^3 by 2025 on the Syncrude Canada Ltd. lease (Rogers et al. 1999). Tailings in this case are an aqueous suspension of sand, silt, clay, residual bitumen, and naphtha at a pH between 8 and 9 (FTFC 1995).

In settling basins, tailings undergo a settling and dewatering process. During this process the tailings are referred to as mature fine tailings (MFT) (Leung et al. 2001). The fine sediments in the suspension have a slow sedimentation rate. Oil sands companies mix fresh tailings with sands and gypsum ($CaSO_4.2H_20$), which flocculates the clays and speeds the tailings dewatering process. The resulting materials are called consolidated tailings (CT) (Fedorak et al. 2003). When a CT slurry settles, it releases water quickly, reaching 62-70% solids within a few hours. The released water or "process water" and sediment pore waters contain high concentrations of sulphate (>1000 mg/L), which is derived from gypsum (Fedorak et al. 2003). MFT, CT, process waters and tailings pore waters contain high levels of salts (i.e. the total ion count in Syncrude's Mildred Lake Settling Basin is >2000 mg/L). Process waters and MFT contain high levels of dissolved organics (naphthenic acids 70-100 mg/L) (Leung et al. 2001). Naphthenic acids are derived from the bitumen, but can also occur naturally when oil is eroded by local streams (FTFC 1995). The naphthenic acids in the freshly produced process water are acutely toxic to aquatic organisms. For example, 100% of fathead minnows (*Pimephales promelas*) exposed to fresh tailings pond water died in less than 48 h (Lai et al. 1996).

The great diversity of microorganisms on Earth contains vast genetic resources for solutions to cleaning up the environment (Madigan et al. 2003 p. 8). In aquatic environments, the microbial degradation process is the major mechanism for removal of spilled oil (Benka-Coker 1997), which can potentially be dangerous to flora and fauna. Microbial degradation may play an important role in removing the 2% of residual

bitumen found in Syncrude's fine tailings (FTFC 1995). Microbes degrade naphthenic acids via an aerobic breakdown pathway (Hadwin et al. 2005; Videla 2006). Herman et al. (1994) indicated that sediment-associated microorganisms were capable of degrading only some naphthenic acids, excluding those with alkyl substitutions on the rings since they are more recalcitrant. Laboratory microcosm studies (Nix & Martin 1992; Lai et al. 1996) indicated that aerobic conditions, moderate temperature and appropriate phosphorus concentrations were necessary for significant naphthenic acid degradation to occur. Thus, other carbon sources, besides primary production, are present in oil sands affected wetlands that may support the microbial community and, potentially, higher trophic levels. Anthropogenic carbon sources include bitumen and associated naphthenic acids.

Athabasca Wetlands

Twenty to forty percent of the boreal zone of which the oil sands region is a part is naturally composed of wetlands (Albert Environment 1998). They include bogs, fens, and marshes, with bog and fen peatlands being the characteristic wetland type in this boreal forest region. Over 90% of these wetlands are peat forming areas (Oil Sands Wetlands Working Group ((OSWWG) 2000). Wetlands are recognized as integral components of natural landscapes since they can enhance environmental quality by increasing biodiversity, protecting and improving water quality, flood control and carbon sequestration (Zedler 2005). In addition, wetlands provide key economic resources and heritage values, including values associated with traditional land use (OSSWG 2000).

Extensive disturbance of natural landscapes, including wetland areas, occurs during oil sands mining. The area to be mined is deforested. The organic topsoil layer is removed and stockpiled for later use in reclamation. Next, the overburden is removed (a layer of sand, clay and other materials that may be up to 100 m thick) to expose the underlying oil sands. The Environmental Protection and Enhancement Act (EPEA) and the Alberta Department of Environment require oil sands operators to reclaim disturbed land to a state of equivalent land capability. This means the reclaimed landscape must be able to

support land uses after reclamation similar to the ability that existed prior to the activities conducted on that land, although the land uses will not likely be the same (OSSWG 2000). The constructed wetlands may evolve towards peatlands, but it will occur over a long period (OSWWG 2000) since it took a few thousand years for northern peatlands to develop following the end of the last glaciation (Harden et al. 1992). Post-mining conditions will not be conductive to the formation of vegetative communities typically associated with peatland communities (OSWWG 2000). The presence of salts in oil-sands process waters (OSPW), used in wetland construction, will affect the ability of some vegetation to colonize these constructed wetlands. Some mosses are unable to establish viable communities in areas where saline conditions occur (Vitt et al. 1993). Based on these factors, which influence wetland formation and the predicted future characteristics of the reclaimed landscape, it is not feasible to reclaim peatlands in the short term (OSWWG 2000).

Post-mining landscape reclamation efforts are being implemented following the large scale removal of bitumen during oil sands mining of the Athabasca deposit. One approach focuses on constructing wetlands and utilizing mining by-products in the design. This strategy relies heavily on the ability of aquatic bacteria to metabolize hydrocarbon residues in pond water overlying tailings, thereby allowing biological colonization to proceed (EVS Consultants 1992). Young OSPW-affected wetlands (those 3-5 years old) supported fewer families of aquatic invertebrates than equivalent wetlands that were unaffected by oil sands mine process materials. Aquatic invertebrate richness had become equivalent after 7 years (Leonhardt 2003). This lag possibly represents the period of time needed for microbial activity to detoxify naphthenic acids and other materials enough to facilitate macrophyte establishment (Cooper 2002) and subsequent colonization by zoobenthos (L. Barr, M.Sc., University of Windsor, in prep).

Macrophyte establishment and the food web that depends on macrophytes require a suitable organic substrate (Irwin and Best 1985). Therefore, one reclamation tool designed to improve constructed wetland quality and speed the successional process is the addition of overburden or peat amendments to wetland sediments (Turetsky et al.

2002). Overburden is composed of surface (<1 m) topsoil, clay and includes vegetation. It is removed prior to mining so as to expose the oil-rich sands and is stockpiled for later use in reclamation (FTFC 1995). Replacement of naturally peat-dominated wetlands takes many years (Harden et al. 1992) since the accumulation of organic matter depends on hydrological, chemical and biotic factors that result in a decrease in organic matter decomposition relative to primary production (OSWWG 2000). Amending constructed wetlands with stockpiled overburden is thought to accelerate succession and the development of a natural community. Organic matter amendments increase moisture and nutrient supply and retention, and provide structure for aiding in vegetation establishment (OSSWG 2000).

Thesis Objectives

Several Athabasca oil sands mining partners (Syncrude Canada Ltd., Suncor Energy Ltd., Canadian Natural Resources Ltd. and Albian Sands) have worked with a consortium of university researchers (University of Alberta, University of Guelph, University of Saskatchewan, Univeristy of Waterloo and University of Windsor) to form an integrated, collaborative group to improve understanding of the effects of tailings and mine process waters on the biological makeup and carbon dynamics of wetland communities. This collaborative research initiative is entitled, *Carbon flow, Food web dynamics & Reclamation strategies in Athabasca oil sands Wetlands* (CFRAW; Ciborowski et al. 2006). CFRAW proposes to document how soft tails and process waters in constructed wetlands modify the maturation process leading to natural conditions in a fully reclaimed landscape.

This thesis represents part of this collaborative effort to understand energy flow in natural and constructed wetlands. The goal of my research is to evaluate microbial activity in a suite of young and older, reference and oil sands affected wetlands. More specifically I will estimate a) microbial carbon sources using stable isotope analysis, b) microbial production, and c) respiration losses (CO_2 and CH_4 evolution) in wetlands of contrasting reclamation type, age and organic base in the Athabasca oil sands region. Overall, this

research will help to deduce the rate at which carbon moves through the microbial compartment by measuring accumulation (production) and by tracking the losses (as respiration) that occur during the transfer between compartments. Furthermore, by examining stable isotope signatures, we will be able to determine the sources of the carbon that contribute to microbial production.

This thesis is composed of 5 chapters. The first chapter (General Introduction) reviews background information relevant to the study. The review includes the concept of the microbial component of the food web, its pertinence to aquatic ecosystems and relevance in this study. An overview of the oil sands mining process is given, followed by wetland reclamation strategies. Chapter 2 focuses on ascertaining the dominant energy sources used by the microbial community in wetlands of contrasting physico-chemical properties. In Chapter 3, I quantify secondary production of pelagic microorganisms inhabiting constructed wetlands. Chapter 4 focuses on quantifying carbon losses through microbial respiration. Chapter 5 is a discussion that synthesizes the pertinent conclusions drawn in chapters 2, 3 and 4. This discussion assesses variation among wetlands in the sources of energy at the base of the aquatic food web, the rate of carbon loss from the microbial food web and the relative contribution of microbial biomass to higher trophic organisms. The chapter concludes with a theoretical assessment of the likely effectiveness of existing reclamations strategies from a microbial perspective and makes recommendations for future research needed to resolve present uncertainties.

CHAPTER II:
DETERMING ENERGY FLOW AND TROPHIC STRUCTURE IN THE MICROBIAL COMMUNITY OF CONSTRUCTED WETLANDS: A STABLE ISOTOPE APPROACH

Abstract

Reclamation efforts are being implemented following the large scale removal of bitumen during oil sands mining of the Athabasca Basin in northeastern Alberta, Canada. One approach focuses on constructing wetlands, an integral component of the natural landscape, and utilizing mining by-products in the design. Adding peat amendments is one strategy believed to improve wetland quality. Analyzing stable isotope signatures of microbial biofilms was used to monitor microbial assimilation of carbon sources of natural and anthropogenic origins in oil sands-affected constructed wetlands and to link microbial processes to trophic levels higher in the aquatic food web. Stable carbon and nitrogen isotopic composition of samples of microbial biofilm, phytoplankton, metaphyton, macrophytes, sediment, and dissolved inorganic carbon (DIC) were determined in 9 wetlands of varying ages and physico-chemical properties. Stable isotope compositions of dissolved organic carbon (DOC) and macroinvertebrates derived from previous studies in the same wetlands were also incorporated into this extensive food web study. A concentration-weighted linear stable isotope mixing model was used to estimate the proportions of 3 potential carbon sources (primary production, sediment, and oil sands mine-process material sources) assimilated in the microbial community in oil sands constructed wetlands. The concentration-weighted stable isotope mixing model produced credible results in most of the OSPM-affected wetlands (Test Pond 9, 4-m CT Wetland and Natural Wetland) since the composition of microbial biofilm (mixture) carbon fell within the triangular space enclosed by lines connecting the 3 potential carbon sources. According to the mixing model the calculated proportions of carbon assimilated in microbial biofilm from OSPM-affected wetlands, Test Pond 9, 4-m CT Wetland and Natural Wetland were 68%, 62% and 97% petroleum, confirming that carbon sources of mine-process origin (OSPM) were incorporated into microbial biomass in OSPM-affected wetlands. Results from all the reference wetlands were problematic since a plot of constituent carbon sources for microbial biofilm fell outside of the mixing triangle,

implying nonsensical negative biomass estimates for one carbon source of either primary production, anthropogenic or sediment carbon origin. Mean±SD microbial biofilm $\delta^{15}N$ values in all reference wetlands (-4.08±1.4 to -0.10±0.7‰) were more negative than any of their potential sources (2.63±0.05 to 12.12±0.01‰) indicating that a nitrogen source common to reference wetlands may have been overlooked or that nitrification may play an important role in nitrogen dynamics in reference wetlands. Mean±SD $\delta^{13}C$ measurements of DIC (-9.3±0.2 to -0.6±0.4‰) indicate that a significant amount of DIC originated from microbial respiration and organic matter decomposition. $\delta^{15}N$ enriched values (3.23±1.56 to 11.63±5.95‰) characteristic of volatization and denitrification were quantified in microbes and other wetland biota in OSPM-affected constructed wetlands. The $\delta^{15}N$ enrichment appears to be the result of nitrogenous compounds introduced via either the bitumen extraction process or wastewater effluent disposal into mine process waters, which would later be used in wetland construction. Evidence was found for the transfer of carbon and nitrogen assimilated by microbes to higher trophic-level organisms (small chironomids and *Daphnia*). Microbial assimilation of petroleum indicates that OSPM-associated carbon sources can fuel the microbial component of the food web in oil sands constructed wetlands and the potential exists for this carbon to support higher trophic levels.

Introduction

Defining major energy flows is critical in understanding the effects of oil sands processed material (OSPM) and reclamation treatments on aquatic food web dynamics. This information will elucidate the importance of primary production, dissolved inorganic carbon (DIC), a reclamation treatment (peat amendment), and petroleum in constructed wetlands. For the purpose of this thesis, petroleum refers to bitumen and naphthenic acids (NAs). Additionally, since petroleum constituents are made up of hydrocarbons, some which may be toxic to biota (MacKinnon and Boerger 1986; Lai et al. 1996; Rogers et al. 2002; Dixon et al. 2003) but are a potential source of assimilable carbon (Herman et al. 1994, Holowenko et al. 2002, Lai et al. 1996), it is especially important to be able to

assess the bioavailability of these materials and their potential for incorporation into the food web.

Both stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes have been used extensively to study elemental cycling and food chain structure (DeNiro and Epstein 1978; DeNiro and Epstein 1981; France and Schlaepfer 2000; Mitchell et al. 1996; Peterson & Fry, 1987). Some studies have used stable isotopes to examine the effects of anthropogenic materials on the aquatic food web. Coffin et al. (1997) examined bacterial, algal and consumer organism assimilation of fertilizer nitrogen and oil following the Exxon Valdez oil spill in Prince William Sound, Alaska using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes. Lake et al. (2001) examined trophic position in fish and mussels affected by residential development in Rhode Island, USA using stable nitrogen isotope ratios ($\delta^{15}N$). Wayland and Hobson (2001) traced the movement of nutrients from sewage and pulp-mill effluent in algae, insects and tree swallows in Alberta and Saskatchewan rivers using ratios of stable carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulfur ($\delta^{34}S$) isotopes. These studies demonstrate the value of using stable isotopes to trace natural and anthropogenic carbon and nitrogen sources into aquatic food webs.

Recently, new approaches have made it possible to elucidate the importance of primary production, detritus and anthropogenic sources of organic matter to pelagic bacterial production (Coffin et al. 1989, 1990; Kelly et al. 1998; Kritzberg et al. 2004). Stable carbon isotope values of bacteria were determined using bacterial concentrates (bioassays) that were grown in 0.2-μm filtered water samples for short-term incubations (Coffin et al. 1989). Subsequent research developed the extraction of nucleic acids from concentrated particulate organic matter in the water column for $\delta^{13}C$ analysis of bacteria (Coffin et al. 1990). Values of bacterial $\delta^{13}C$ measured by the bioassay technique were similar to the nucleic acid extraction method (Coffin et al. 1990) and thus the bioassay method has been subsequently used by other researchers (Cifuentes et al. 1996; Kelley et al. 1998; Kritzberg et al. 2004). Complementing these isotopic approaches for examining bacteria are analytical methods for measuring $\delta^{13}C$ values for dissolved inorganic carbon (Miyajima et al. 1995). With these methods the fate of carbon and energy can be traced

through the bacterial community. This study aims to improve methodology for stable isotope analysis of bacterial communities in aquatic environments by presenting a novel approach for studying carbon flow dynamics in microbial biofilm.

In nature, populations of microbial cells live and interact with other populations of cells in microbial communities. These communities may consist of free-swimming cells in aquatic environments, but often form biofilms on living or nonliving surfaces (Madigan et al. 2003). Sigee (2005) defined a biofilm as "a community of microorganisms which is attached to an exposed surface." Jackson et al. (2001) defined biofilms in natural waters as "complex heterogeneous structures composed of bacteria, algae, and other microorganisms within an intracellular matrix." In natural waters, the majority of bacteria in freshwater are found growing as biofilms on the surfaces of submerged substrata, such as macrophytes and sediment (Jackson et al. 2001). Microbes rapidly colonize substrates. When a surface is submerged it immediately becomes available to the microbial community, which usually colonizes the substrate within hours (Costerton et al. 1987). For the purpose of this study, biofilm refers to a community dominated by heterotrophic bacteria and excluding photosynthetic microorganisms.

Stable Isotope Mixing Model

Stable isotopes act as natural labels by integrating the contributions of multiple sources to a mixture (Phillips and Koch 2002). For example, stable isotope analysis is commonly used to elucidate the relative contributions of different food sources to a consumer (Hobson 1999). Isotopic ratios are determined for a consumer and each of its potential food sources (Phillips 2001). The similarity of the ratios between the consumer and individual food sources (after correcting for fractionation caused by digestion and assimilation) represents the relative importance of each source to the consumer's diet (DeNiro and Epstein 1978). A concentration-weighted linear mixing model, "assumes that for each element [i.e. C or N], a source's contribution is proportional to the contributed mass times the elemental concentration in that source (Phillips and Koch 2002)." This model is appropriate for scenarios where elemental concentrations vary substantially among sources (Phillips and Koch 2002). Simultaneous use of two isotopes

(i.e. $\delta^{13}C$ and $\delta^{15}N$) allows the contributions of three sources to be estimated (Phillips and Koch 2002). The approach works only if all important dietary sources have been measured and if there are no more than three important sources (Phillips and 2002). The method is most effective when food sources differ substantially in isotopic composition, but show low variance (Phillips and Gregg 2001). A key assumption in this model is that C and N isotopes from all carbon sources are completely homogenized into the consumer's body before biomass is synthesized (Phillips and Koch 2002). Finally, in a bivariate plot of the two isotopes of interest, the composition of the mixture must fall within the triangular area created by drawing lines that connect the points representing the three food sources. Failure of the mixture's signature to fall within the mixing triangle indicates that either: (1) an important source was overlooked, (2) an incorrect stable isotope value was used, or (3) a mixing model assumption was violated (Phillips and Koch 2002).

This study reports on the use of stable isotopes to monitor microbial assimilation of carbon in oil sands constructed wetlands and to link microbial processes to higher trophic levels in the aquatic food web. The objectives were to: (1) examine microbial assimilation of primary production, sediment organic matter (SOM), DIC, DOC and petroleum in oil sands constructed wetlands; (2) determine the dominant carbon and nitrogen sources assimilated by microbial communities; (3) assess if petroleum could be assimilated by microbes and, thus be potentially available to higher trophic-level organisms; (4) determine the effectiveness of a new approach which examines carbon flow dynamics in microbial communities; and (5) to examine the contrast in microbial carbon dynamics between young vs. older wetlands, those with and without additional peat materials, and the nature of materials between reference and OSPM-affected wetlands.

Materials and Methods

Site Description

Fort McMurray (56.66° N 111.21° W), Alberta, is the location of two of the largest oil sands mining and refinery operations in the world, Suncor Energy Inc. and Syncrude Canada Ltd. (Fig. 2.1). Significant growth is expected within the oil sand industry, with mines created by companies such as Albian Sands and Canadian Natural Resources Ltd., becoming newly established. Companies are removing the oil sands using open-pit mining operations (Fedorak et al. 2002). By the year 2023, mined areas are expected to exceed 1,406 km^2 (Cooper and Lee 2003). Wetlands are expected to make up 20 to 40 percent of the final reclamation landscape in the Athabasca oil sands area.

The Fort McMurray region is located in the Central Mixed Wood Natural Subregion and is rich in biological and geological diversity (Vitt et al. 1996). The area is covered by mixed spruce forests and bogs and fens, which make up a significant component of the landscape (Vitt 1996). Generally, the terrain is composed of flat to rolling composites of clay till to alluvial sands. The diverse area is appropriate for various wildlife habitats (CEMA 2003). The climate is boreal cold temperate (Mulligan and Gignac 2001).

Study Design

This project assessed several classes of 'constructed' wetland defined *a priori* and differing in reclamation type (reference vs. oil sands mine-process material affected), age (young vs. older), and organic base (poor vs. rich) (Table 2.1). Oil sands mine-process material affected (OSPM) wetlands were constructed utilizing tailings and/or process waters. For the purpose of this study, oil sands process material (OSPM) refers to fine tailings and process water that was released during the sedimentation of CT and MFT. The 4m-CT wetland was constructed with CT and sand sediments and capped with process waters. Natural Wetland, Mike's Pond, and Test Pond 9 were capped with or receive input from process waters (Appendix 2.1 – Wetland Formation). All wetlands

Figure 2.1 – Map showing the location of the Athatbasca oil sands deposit near Fort McMurray, Alberta (modified from http://images.google.com/).

occurred within the lease areas of a mining company in areas that had previously been mined. Reference wetlands formed in either natural or excavated depressions, but received minimal oil sands mining effluent. One control wetland was also included in the study. The control was a beaver pond formed in a stream channel that existed on the mining lease site prior to mining companies' establishment. It showed no evidence of being affected by oil sands development activity.

The substrates of some constructed wetlands were augmented with a 30-cm thick layer of topsoil materials ("peat") that had been collected and stockpiled during the development phase of the mine. Such wetlands were considered to have a "rich organic base". Those built on a clay base but receiving process water (rich in salts and naphthenic acids) were on a 'poor organic base'. Wetlands aged less than 7 years were considered "young wetlands". Wetlands aged 7 years or older and ranging up to approximately 30 years were designated "older wetlands". The age distinction was based on previous work by Leonhardt (2003) who determined that zoobenthic richness and abundance reached asymptotes in reference wetlands aged 5 years and older. Richness, but not abundance, was significantly lower in young OSPM affected wetlands than in equally young reference wetlands. After examining additional zoobenthic community data from the same wetlands (Whelley 1999) a more conservative value was chosen, 7 years, to distinguish between age classes.

Water chemistry parameters in the study wetlands during July 2005 ranged from 7.6 to 9.1 for pH, 245 to 4,660 μS for electrical conductivity, 0.1 to 2.5 ppt for salinity, 16.1 to 20.4 ^{o}C for temperature and 5.3 to 11.5 mol/L for DO (Appendix 2.2). NAs measurements ranged from 1.2 to 30.9 mg/L (Appendix 2.5).

Field Sampling Protocol

An attempt was made to ascertain the $\delta^{13}C$ and $\delta^{15}N$ values of microbial communities in Fort McMurray wetlands by isolating the microbial component by filtering 3-L microbial bioassays on GF/F filters as described by Coffin et al. (1989). The quantity of microbial

TABLE 2.1: Wetland Classification Scheme

Wetland	Code	Status	Age Category	Organic Base	Lease Area	Age in 2004	Surface Area ($m.^2$)	Approx. Water Depth (m)	Oil Sand Process Material	Current Substrate Characteristics
Mike's Pond	MP	OSPM	Young	Poor	Syncrude	12		3.5	Process Water	Silty-clay
4m Consolidated Tailings Demonstration Pond	4-m CT	OSPM	Young	Rich	Suncor	7		0.5	CT and process water	Peat surface layer above CT & sand mixture
Test Pond 9	TP9	OSPM	Older	Poor	Syncrude	11		*0.7	Process Water	Clay
Natural Wetland	NW	OSPM	Older	Rich	Suncor	18	12,700	0.6	Process Water	Silty/Sandy sediment with large organic component
Canadian Natural Resources Ltd. Wetland	CNRL	Reference	Young	Poor	CNRL	0	~7,500	0.7	N/A	Sand
Peat Pond	PP	Reference	Young	Rich	Syncrude	3		*0.7	N/A	Peat
Shallow Wetland	SW	Reference	Older	Poor	Syncrude	12		0.6	N/A	Clay with fine detritus surface layer
High Sulphate	HS	Reference	Older	Rich	Suncor	20	2,193	0.6	N/A	Silty-sandy sediment with large organic component
South West Sands Beaver Pond (Control)	BP	Control	Older	Rich	Syncrude			0.7	N/A	Clay with detrital component

* Maximum depth in this pond was somewhat deeper; however, measurement refers to sampling depth near shoreline.
Blank = unknown/not measured

carbon and nitrogen was below detection limits, which implies that the microbial component (<1 μm) was either not prevalent, was associated with larger particles or the microbial stable C and N isotopic values were diluted by the GF/F filters. This failed pilot study in 2004 led to the development of the biofilm technique later implemented in 2005.

(a) Biofilm Collection:

A series of polyvinyl pipes designed to collect heterotrophic microbial biofilm was driven into the wetland substrate to extend up into the water column. The pipes were constructed so as to allow water flow around pipes, thereby facilitating the attachment of free-floating microbes and development of a biofilm. However, the pipes were also constructed so as to eliminate sunlight and hence development of photosynthetic microbial populations.

A 180-m long section was marked along the shoreline perimeter of each of the 9 study wetlands. Five locations were selected along the marked shoreline with reference to a random number table. At each location a series of 3, 1-m long polyvinyl chloride (PVC) and corrugated metal pipes was installed in the submergent zone (0.5-1 m water depth) (Fig. 2.2). A 3.8-cm outer diameter PVC pipe was surrounded by a 7.6-cm outer diameter PVC pipe with holes along the long axis. Then a 12.7-cm outer diameter metal corrugated pipe with holes along the long axis was placed around both pipes. The holes in the exterior two pipes allowed water to circulate while preventing sunlight from penetrating to the interior 1.5" pipe, hence inhibiting the development of photosynthetic microbial populations. An opaque black plastic cap covered the surface of the pipes, also omitting light. The pipe design allowed colonization of heterotrophic microbes, while inhibiting autotrophic microbial establishment.

Pipes were installed in the wetlands between July 4 and 13, 2005. They were left in situ for 30 - 35 d, and biofilm was harvested between August 8 and 11, 2005. Biofilm was separated into "subsurface" and "epibenthic". Subsurface biofilm was collected from the

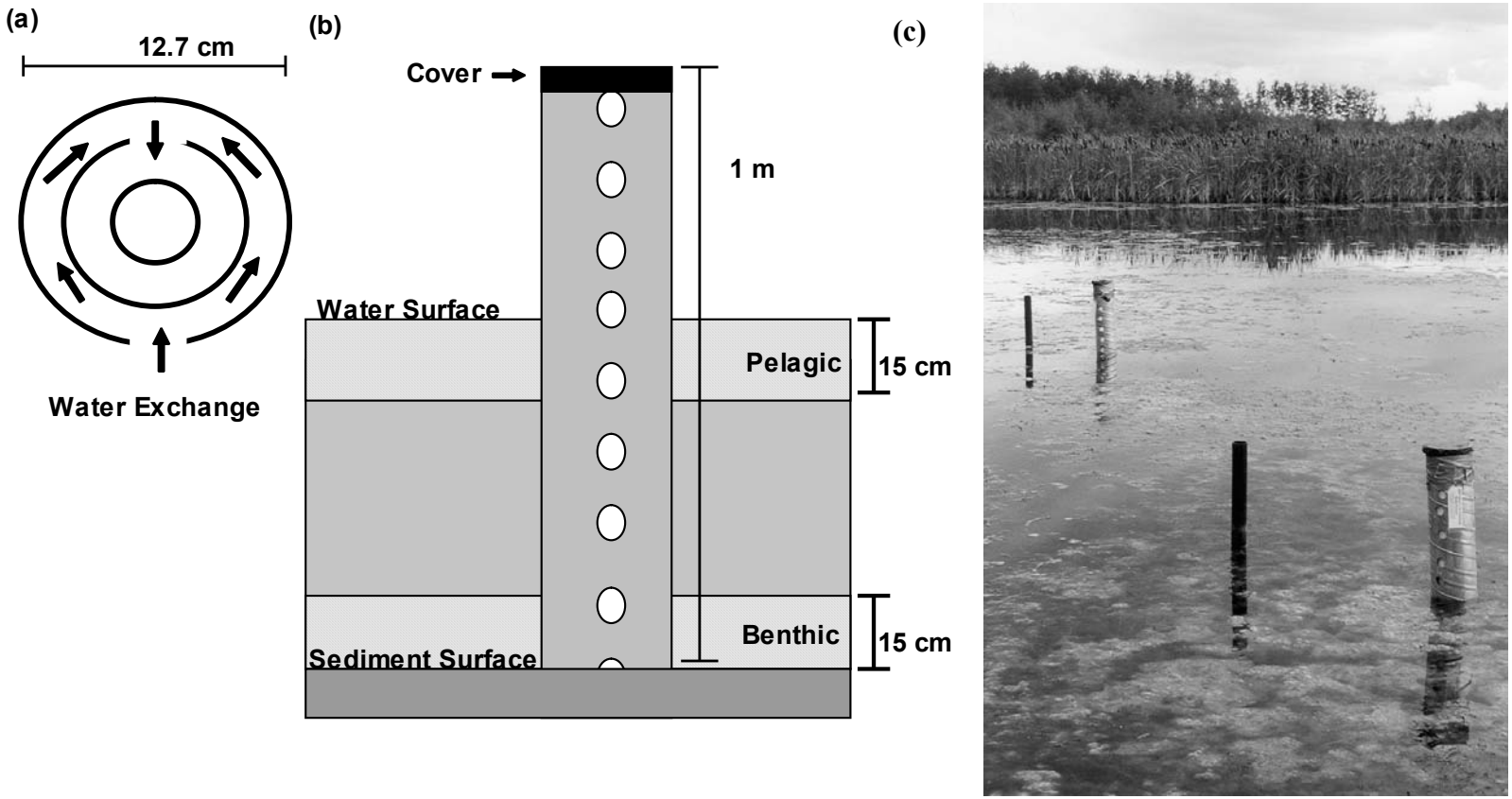


Figure 2.2 – Diagram illustrating (a) the plan view and (b) the profile view of the pipe design for collecting heterotrophic microbial biofilm. (c) A photograph of the set up.

top 15 cm of the submerged portion of the 3.8-cm pipe. Epibenthic biofilm was collected from the bottom 15 cm of the submerged portion of the 3.8-cm pipe, near the sediment water interface. Biofilm was scraped off of the innermost 3.8-cm pipe using vinyl gloved fingers. Gloves were changed after each sample collection. The biofilm was transferred to 15-mL polypropylene tubes and remained in a cooler on ice for less than 2 h before being stored in the laboratory freezer at -4^{o}C. The samples remained frozen until their preparation for stable isotope analysis.

Additional biofilm was scraped from docks and buoys that had been in place for several years in Test Pond 9 and Peat Pond at the surface of the water column. This mature biofilm contained both heterotrophic and autotrophic microbes and would be compared to young heterotrophic biofilm collected from pipes.

All potential microbial carbon sources were collected between June 28th and August 4th, 2005. Duplicate samples of algae (metaphyton), sediment, and particulate organic matter (POM) were collected from each wetland. All observed emergent and submergent macrophytes, including live stems and leaves, were collected and identified for each wetland. For each macrophyte species, several individuals were collected within a wetland and pooled together for analyses. Floating algae was scooped up from the surface of the water column using a sieve (180μm). A 6-cm diameter tube was used to collect the top 10 cm of wetland sediment. Sediment represents detrital carbon accumulating in the sediment from all sources (i.e., allochthonous inputs, decomposing macrophytes and phytoplankton). Petroleum (i.e. bitumen) was scraped from the surface of rocks and sediment (clay) in Test Pond 9. All macrophytes, algae, sediment and petroleum were stored in plastic bags and remained in a cooler on ice prior to storage in the laboratory freezer at -4^{o}C. Macrophytes and algae were rinsed in distilled water prior to freezer storage. Suspended particulate organic matter was used as a proxy for stable carbon and nitrogen isotope values in phytoplankton, as the phytoplankton cannot easily be separated from other components of the particulate organic matter (Coffin et al. 1997). Phytoplankton was collected from wetlands by filtering aliquots of up to 20 L of water on

47- mm diameter glass fiber filter (Whatman GF/C, ashed at 500^{o}C for 4h). The filters were frozen immediately and stored at -4^{o}C.

(b) Dissolved Inorganic Carbon

Total dissolved inorganic carbon ($\sum CO_2$) was determined from wetland water samples using a headspace method combined with GC/C/IRMS (gas chromatograph/combustion furnace/isotope-ratio mass spectrometer) described by Miyajima et al. (1995). Water samples were collected in airtight glass serum bottles (inner volume 125 mL) between June 24 and July 8, 2005 from 10 cm below the surface in the submergent aquatic plant zone (0.5-1 m depth) at 3 randomly selected locations. Each bottle was sealed with a rubber septum and contained no air bubbles. Samples were immediately transported back to the lab, usually within 1h after collection. A head space was created inside each sample at constant temperature (23^{o}C) by removing 10 mL of water with a 60-mL gas-tight plastic syringe while the same volume of ultrapure helium gas was injected into the serum bottle with a different 60-mL gas-tight syringe (Fig. 2.3). The sample was then acidified with 6.0 N CO_2-free HCl solution (final concentration 50 mM), which was injected via syringe into the serum bottle and thoroughly mixed by vigorous hand shaking. Final concentration (50 mM) was sufficient to quantitatively convert all carbonate and bicarbonate anions into aqueous CO_2. Subsamples of water were removed to confirm that samples were sufficiently acidified to achieve pH 2. CO_2-free HCl solution was prepared by bubbling ultrapure helium gas through a 6.0 N HCl solution for at least 3 h. Sample bottles were left upside down in the dark for 48 h, during which time the aqueous CO_2 gradually equilibrated with the headspace gas. The sample bottles required storage prior to analysis, and so 0.5 mL of 10% $HgCl_2$ solution was injected into each bottle as a fixative, while the same volume of sample water was removed with another syringe. Sample bottle tops were covered with paraffin and electrical tape to prevent diffusion of atmospheric CO_2 through the septa.

Figure 2.3 – Withdrawing a portion of wetland water and replacing with ultrapure helium using gas-tight syringes.

Measurements of temperature (^{o}C), pH, dissolved oxygen concentration (DO) (mg/L), salinity (ppt) and conductivity (µS) were taken simultaneously with stable isotope sample collection.

Stable Isotope Analysis

The materials analyzed for stable isotope signatures included microbial biofilm, macrophytes, sediments, phytoplankton, algae, petroleum and $\sum CO_2$. In the laboratory all biomass samples, were dried (60^{o}C) for 48 h, ground into powder using a mortar and pestle, and were stored in glass vials prior to isotopic analysis. The organic portion of macrophytes, algae and sediment was isolated by acidifying samples with 0.5 M HCl to remove carbonates and then redrying at 60^{o}C for 48 h. The HCl procedure was repeated once for all samples. If bubbles were visible in samples during the second HCl treatment, an additional treatment was applied to select samples until no bubbles were present. Aliquot portions (1-30 mg dry mass) of the samples were placed in tin cups.

Biomass and $\sum CO_2$ samples were sent to the University of Waterloo isotope facility for analyses. Microbial biofilm, macrophytes, sediments, phytoplankton, algae, and petroleum samples were analyzed isotopically for $\delta^{13}C$ or $\delta^{15}N$ and for %C and %N using an Isochrom Continuous Flow Stable Isotope Mass Spectrometer (Micromass) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108) that converts carbon and nitrogen to CO_2 and N_2 gas for mass spectral analysis. $\sum CO_2$ samples were analyzed isotopically for $\delta^{13}C$ of CO_2 using the same Isochrom Continuous Flow Stable Isotope Mass Spectrometer.

Stable carbon and nitrogen isotope ratios are reported according to the standard formula:

$$\delta X = [(R_{sample}/ R_{standard}) - 1] \times 10^{3}, \text{ per mil,}$$

where δX is either $\delta^{13}C$ or $\delta^{15}N$ and R is either $^{13}C/ ^{12}C$ or $^{15}N/ ^{14}N$. All values were reported relative to the conventional Pee Dee Belemnite standard (PDB). Results were

corrected using internal lab reference materials (nitrogen standards: ammonium sulphate; carbon standards: sugar, cellulose and graphite) corrected using international standards. Increase in δ value indicates that a sample is enriched in the heavier isotope relative to the PDB standard and a decrease in δ value indicates the sample is depleted in the heavier isotope. The analytical precision of the measurement for $\delta^{13}C$ and $\delta^{15}N$ was ±0.2‰ and ±0.3‰, respectively.

Stable Isotope Mixing Model

Two isotopic signatures, $\delta^{13}C$ and $\delta^{15}N$, were used to determine the proportional contributions of three sources (X, Y, Z) to a mixture (M), microbial biofilm, in each constructed wetlands (n = 9) (Phillips and Koch 2002). Let $f_{X,B}$, $f_{Y,B}$, and $f_{Z,B}$ represent the fractions of assimilated biomass (B subscript) of sources X,Y, and Z, in microbial biofilm. Then let $f_{X,C}$, $f_{Y,C}$, $f_{Z,C}$, $f_{X,N}$, $f_{Y,N}$, and $f_{Z,N}$ similarly represent the fractions of assimilated C (C subscript) or N (N subscript) of the individual sources in the mixture. Mass balance equations are as follows:

$$\delta^{13}C_M = f_{X,C}\delta^{13}C_X' + f_{Y,C}\delta^{13}C_Y' + f_{Z,C}\delta^{13}C_Z' \quad (1)$$
$$\delta^{15}N_M = f_{X,N}\delta^{15}N_X' + f_{Y,N}\delta^{15}N_Y' + f_{Z,N}\delta^{15}N_Z' \quad (2)$$

where $\delta^{13}C_X$ and $\delta^{15}N_X$ represent the C and N isotopic signatures for source X, and similarly for sources Y and Z and the mixture M. Isotopic signatures for the sources have been corrected for trophic fractionation as designated by the prime (') symbol. The source fractional contributions for C, N and biomass are constrained to sum to 1:

$$1 = f_{X,C} + f_{Y,C} + f_{Z,C} \quad (3)$$
$$1 = f_{X,N}, f_{Y,N}, \text{ and } f_{Z,N} \quad (4)$$
$$1 = f_{X,B} + f_{Y,B} + f_{Z,B} \quad (5)$$

The model assumes that for each element, the contribution of a food source to a consumer is proportional to the assimilated biomass times the elemental concentration in that source. So, letting $[C]_X$, $[C]_Y$, $[C]_Z$, $[N]_X$, $[N]_Y$, and $[N]_Z$ represent the C and N concentrations in food sources X,Y and Z, then:

$$Fx,c = \frac{f_{X,B}[C]_X}{f_{X,B}[C]_X + f_{Y,B}[C]_Y + f_{Z,B}[C]_Z} \quad (6)$$

$$F_Y,c = \frac{f_{Y,B}[C]_Y}{f_{X,B}[C]_X + f_{Y,B}[C]_Y + f_{Z,B}[C]_Z} \quad (7)$$

$$F_Z,c = \frac{f_{Z,B}[C]_Z}{f_{X,B}[C]_X + f_{Y,B}[C]_Y + f_{Z,B}[C]_Z} \quad (8)$$

The latter equation (equation 8) is not independent of the previous equations, 6 and 7, because $f_{Z,C}$ is completely dependent on the values of $f_{X,C}$ and $f_{Y,C}$ and can be determined by subtraction. Likewise for N:

$$Fx,_N = \frac{f_{X,B}[N]_X}{f_{X,B}[N]_X + f_{Y,B}[N]_Y + f_{Z,B}[N]_Z} \quad (9)$$

$$F_{Y,N} = \frac{f_{Y,B}[N]_Y}{f_{X,B}[N]_X + f_{Y,B}[N]_Y + f_{Z,B}[N]_Z} \quad (10)$$

$$F_{Z,N} = \frac{f_{Z,B}[N]_Z}{f_{X,B}[N]_X + f_{Y,B}[N]_Y + f_{Z,B}[N]_Z} \quad (11)$$

As was the case for C, the later equation (equation 11) is not independent of the previous two equations because $f_{Z,N}$ is completely dependent on the values of $f_{X,N}$ and $f_{Y,N}$ and can be determined by subtraction.

Equations 1-7 and 9, 10 represent a set of 9 independent equations in 9 unknowns (the f variables). This can be reduced to a set of 3 equations to solve for the source fractional contributions for assimilated biomass ($f_{X,B}$, $f_{Y,B}$, and $f_{Z,B}$), which can be substituted into the original equations to calculate the source contributions for C and N. Substituting equation 6-8 into equation 1 and rearranging terms yields:

$$(\delta^{13}C'_X - \delta^{13}C_M)\,[C]_X f_{X,B} + (\delta^{13}C'_Y - \delta^{13}C_M)\,[C]_Y f_{Y,B} + (\delta^{13}C'_Z - \delta^{13}C_M)\,[C]_Z f_{Z,B} = 0 \quad (12)$$

Similarly, substituting equations 9, 10, 11 into equation 2 and rearranging terms gives:

$$(\delta^{13}N'_X - \delta^{13}N_M)\,[N]_X f_{X,B} + (\delta^{13}N'_Y - \delta^{13}N_M)\,[N]_Y f_{Y,B} + (\delta^{13}N'_Z - \delta^{13}N_M)\,[N]_Z f_{Z,B} = 0 \quad (13)$$

Reiterating equation 9:

$$f_{X,B} + f_{Y,B} + f_{Z,B} = 1 \quad (14)$$

gives a system of three equation (12-14) in three unknowns ($f_{X,B}$, $f_{Y,B}$, and $f_{Z,B}$). In matrix algebra notation equations 12-14 can be written:

$AF=B$

$$A = \begin{bmatrix} (\delta^{13}C'_X - \delta^{13}C_M)[C_X] & (\delta^{13}C'_Y - \delta^{13}C_M)[C_Y] & (\delta^{13}C'_Z - \delta^{13}C_M)[C_Z] \\ (\delta^{13}N'_X - \delta^{13}N_M)[N_X] & (\delta^{13}N'_Y - \delta^{13}N_M)[N_Y] & (\delta^{13}N'_Z - \delta^{13}N_M)[N_Z] \\ 1 & 1 & 1 \end{bmatrix}$$

$$F = \begin{bmatrix} f_{X,B} \\ f_{Y,B} \\ f_{Z,B} \end{bmatrix}$$

$$B = \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix}$$

To solve for $f_{X,B}$, $f_{Y,B}$, and $f_{Z,B}$ (vector F), both sides of the matrix equation are pre-multiplied by the inverse of A to give: $F=A^{-1}B$. These values for $f_{X,B}$, $f_{Y,B}$, $f_{Z,B}$ can then be inserted into equation 6, 7 and 8 to solve for $f_{X,C}$, $f_{Y,C}$ and $f_{Z,C}$, and in equations 9, 10 and 11 to solve for $f_{X,N}$, $f_{Y,N}$, and $f_{Z,N}$.

A computer program (IsoSource) was used to perform these calculations and produce dietary mixing triangle graphs (Fig. 2.4) and was available at http://www.epa.gov/wed/pages/models/stableIsotopes/isotopes.htm.

A surplus of sources is commonly found in environmental studies using stable isotope analysis (Phillips et al. 2005). One common method of dealing with stable isotope

mixing model limitations for systems with more than 2 or 3 sources is to assume that source signatures that are closest to (i.e., most similar to) the mixture provide the greatest contribution (Phillips and Gregg 2003) and to omit sources that do not contribute significantly to the mixing model (Phillips and Newsome 2005). Another approach is to combine sources to simplify the analysis and estimate source contribution (Phillips and Newsome 2005). The sources must be logically related (Phillips and Newsome 2005).

Multiple primary production items (macrophytes, phytoplankton and metaphyton) were pooled together within a wetland to fit the two isotope-three source mixing model. All macrophytes were pooled within a wetland. In some cases, $\delta^{13}C$ and $\delta^{15}N$ values for macrophytes were pooled with metaphyton and/or phytoplankton when mean macrophyte $\delta^{13}C$ and $\delta^{15}N$ values were similar to mean metaphyton and/or phytoplankton $\delta^{13}C$ and $\delta^{15}N$. One example where primary production items were analyzed separately in the stable isotope mixing model was in Peat Pond. An algae bloom was observed in Peat Pond during July 2005. The algae had a distinctive rod shape (~ 5mm x 1mm) and stable isotopic signatures ($\delta^{13}C$ -31.12; $\delta^{15}N$ 2.82) that were also distinct from macrophytes, metaphyton and phytoplankton (filtered to exclude the rods) ($\delta^{13}C$ -24.32±5.47; $\delta^{15}N$ 6.29±2.10) (Table 2.2). In the stable isotope analysis the distinct algae, herein referred to as "rod algae", was analyzed as a separate carbon source from the pooled macrophytes, metaphyton and phytoplankton.

Petroleum was a potential source for the microbial community in OSPM-affected wetlands only. In some cases, sediment could not be used in the mixing model as a potential source of carbon to the microbial community since the total nitrogen percentage in sediments was below detection level (CNRL, MP and Natural Wetland). In those cases, sediment would unlikely be a potential carbon source since nutrient levels in sediment were low. In some cases it may have been more appropriate to separate some primary production items. For example, stable carbon isotopic values of emergent and submergent vegetation were significantly different in some wetlands (*see below*). However, mixing model applications are limited to solving contributions of n+1 sources when n isotopes are used (Phillips 2001). To increase the number of potential carbon

sources available to the microbial community from 3 to 4, an additional stable isotope (e.g., sulphur) should have been incorporated. However, there was not enough microbial biomass left to quantify $\delta^{34}S$. Future studies examining the carbon source to microbial communities in oil sands constructed wetlands should collect more microbial biofilm (>10 mg dry mass per wetland) so that 3 stable isotopes and 4 potential carbon sources may be examined.

Statistical Analysis

Parametric statistical tests (Statistica 6.0, Statsoft, Inc. 1984-2001) were used to evaluate data since microbial biofilm $\delta^{13}C$ and $\delta^{15}N$ values did not depart significantly from a normal distribution (Kolmogorov-Smironov test, $p > 0.05$). Differences in stable isotope signatures of biofilm were expected to be much greater among sample locations than between subsurface and epibenthic biofilms at one location. Therefore a paired-comparison t-test was used to test the significance of differences in $\delta^{13}C$ and $\delta^{15}N$ between subsurface and epibenthic biofilms.

A concentration-weight linear stable isotope mixing model, which was based on a standard mathematical solution for three unknowns using three equations, estimated the proportion of potential carbon sources (primary production, sediment, and anthropogenic sources) assimilated in the microbial community in oil sands constructed wetlands. The Independent Samples t-test was used to test the significance of differences in $\delta^{13}C$ between biofilm and DOC. Three-way factorial analysis of variance (ANOVA) was used to test whether the $\delta^{13}C$ and $\delta^{15}N$ values of microbial biofilm and $\sum CO_2$ were significantly influenced by age class, organic base, and/or reclamation type. Regression analysis was used to test whether there was a significant relationship between the $\delta^{13}C$ and $\delta^{15}N$ biofilm and $\sum CO_2$ values and water chemistry parameters. The significance level adopted for statistical tests were $\alpha = 0.05$.

Results & Discussion

Interpretation of Discrimination Factors

An isotope discrimination factor is the change in the stable isotope ratio (i.e. $\delta^{13}C$) during assimilation between food or carbon source and the consumer's diet (Phillips and Koch 2002) due to the uneven distribution of isotopes among and within compounds (Farquhar et al. 1989) or trophic levels. Isotope discrimination factors for $\delta^{13}C$ (1‰) and $\delta^{15}N$ (3.4‰) between trophic levels have been assigned from classical stable isotope studies. Deniro and Epstein (1978) reported that animal tissues are on average enriched in $\delta^{13}C$ by 1‰ relative to their dietary carbon, which ranged from -0.6 to 2.7 ‰. Comparing $\delta^{15}N$ values between consumers and their dietary nitrogen source shows an approximate 3.4‰ increase with each increase in trophic level (Minagawa and Wada 1984). However, the 3.4‰ value is also an average of a distribution of $\delta^{15}N$ values across trophic levels. Minawaga and Wada (1984) determined nitrogen isotope discrimination ranges from 1.3 to 5.3‰. Similarly, Peterson and Fry (1987) describe 3 to 5 ‰ enrichment in animal $\delta^{15}N$ ratios relative to dietary nitrogen. In spite of the known variations between trophic levels, most studies have adopted the 1‰ and 3.4‰ isotope discrimination values for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Several lab studies provide evidence that the carbon isotope discrimination factor commonly used in food web studies actually deviates in microorganisms relative to diet. Blair et al. (1985) measured 0.6‰ depletion in *Escherichia coli* ^{13}C relative to its carbon source, glucose. Coffin et al. (1990) observed a 2.3‰ $\delta^{13}C$ enrichment in total cell carbon from *Pseudomonas aeruginosa* cultures relative to its carbon substrate. Hullar et al. (1996) found that the enrichment in ^{13}C between bacterial biomass and DOC derived from tidal marsh vegetation varied from 0.4‰ to 2.8‰, with a 1.4‰ average. Hall et al. (1999) examined carbon isotope fractionation during aerobic degradation of organic contaminants, phenol and benzoate, by *Pseudomonas putida* and *Rhadococcus* sp. I_1. The ^{13}C enrichment values between *Pseudomonas putida* and phenol, *Pseudomonas putida* and benzoate, and *Rhadococcus* sp. I_1 and phenol were as much as 2.5‰, 2.3‰,

and 12.4‰ (4.4‰ for the majority of the experiment), respectively. However, it has been theorized that in natural environments large isotope fractionations may cancel each other out and not be observed because of species diversity or substrate dominance by a particular compound (Macko and Estep 1984).

Videla (2006, University of Waterloo, pers. comm.) used stable isotopes to trace degradation of a commercially manufactured naphthenic acid (-27.1‰) extract by bacteria collected from mine process waters in the Fort McMurray area during August 2005. Bacterial $\delta^{13}C$ was enriched by 0.5 to 3.3‰ relative to diet, with a 1.8‰ average enrichment. The carbon isotope discrimination factor derived from Videla (2006) was deemed most appropriate to use in this study since microbial communities, diet items present and study period were similar in both studies. The nitrogen isotope discrimination factor used in the current study was derived from Minagawa and Wada (1984). Discrimination factors used in this study for $\delta^{13}C$ and $\delta^{15}N$ were +1.8‰ and +3.4‰, respectively.

Tracing Microbial Diet Composition

The basal carbon sources potentially available to microbes were primary production (phytoplankton, metaphyton, and macrophytes), sediment, DOC, and petroleum in the oil sands constructed wetlands. Phytoplankton $\delta^{13}C$ and $\delta^{15}N$ ranged from -33.40 to -20.11‰ and -0.84 to 23.65‰, respectively. Metaphyton $\delta^{13}C$ and $\delta^{15}N$ values ranged from -32.44 to -12.26‰ and -0.81 to 24.18‰, respectively. Algae $\delta^{13}C$ values can measure -45‰ (Rau 1978; Rau 1980; Rounick and Winterbourn 1986 cited in Peterson and Fry 1987). Macrophyte $\delta^{13}C$ and $\delta^{15}N$ ranged from -33.2 to -12.7‰ and -1.96 to 23.08‰, respectively. Sediment $\delta^{13}C$ and $\delta^{15}N$ ranged from -29.90 to -26.35‰ (outlier -90.21‰; see below) and -1.84 and 6.75‰, respectively. Mean (±SD) carbon isotope ratios of DIC measured in the wetland pelagic zone ranged from -9.3±0.2 to -0.6±0.4 ‰. Mean (±SD) petroleum $\delta^{13}C$ and $\delta^{15}N$ values were -29.±0.06‰ and 1.95±0.02‰, respectively. Similarly, the $\delta^{13}C$ of naphthenic acids in Syncrude's Mildred Lake Settling basin was -29‰ (P. Videla 2006, Syncrude Canada Ltd., pers. comm.).

The isotopic ratio of microbial biofilm was monitored to identify natural and OSPM-derived carbon sources assimilated by microbes in oil sands constructed wetlands. Mean (±SE, n=37) microbial biofilm $\delta^{13}C$ and $\delta^{15}N$ values ranged from -32.0±1.7 to -22.7±2.9‰ and -4.1±1.4 to 11.6±6.0‰, respectively (Fig. 2.4). Similar results were obtained by Cifuentes et al. (1996) in a bacterial stable isotope study conducted in a mangrove estuary, in Ecuador. The bacteria $\delta^{13}C$ and $\delta^{15}N$ ranged from -26.7 to -23.1‰ and 4.4 to 9.3‰, respectively, reflecting the importance of detritus from mangroves and other allochthonous sources. Conversely, Waichmen (1996) determined the $\delta^{13}C$ of bacterial produced CO_2 to vary between -27.7 and -13.5‰ in a floodplain lake in the central Amazon. According to Blair et al (1985) fractionation exists during aerobic metabolism of heterotrophic bacteria whereby pyruvate is converted to Acetyl-CoA and the CO_2 released during this reaction is depleted in ^{13}C. Additional fractionation occurs during the Kreb cycle producing CO_2 depleted in ^{13}C (Blair et al. 1985). The total CO_2 fractionation is 3‰ (Blair et al. 1985). Considering a fractionation of 3‰ by bacterial respiration (Blair et al. 1985) and that C_4 plants $\delta^{13}C$ values range from –6 to –23 $^{o}/_{oo}$ (Faure 1998), bacterial cell $\delta^{13}C$ values ranging from -24.7 to -10.5‰ reflected that they consumed carbon mainly derived from C_4 macrophytes (Waichmen 1996).

The C:N ratios of microbial biofilm ranged from 8.3 to 13.4. These values are similar to 10±2, which are characteristic C:N ratios for microbial biomass (Flanagan et al. 2005) (Appendix 2.3).

DOC can be produced by benthic algae, phytoplankton, macrophytes and petroleum in the study wetlands. Since microbial metabolism often depends on dissolved rather than particulate organic matter (Peterson et al. 1994) I expected biofilm $\delta^{13}C$ values to mirror DOC values. However, the conservative nature of $\delta^{13}C$ for DOC (-28.7‰ to -26. 7‰) (Videla 2006) in these wetlands does not explain the variation observed in C isotope values of microbial biofilm (-34.34‰ to -20.46‰) (Appendix 2.3). Similarly, Kelley et al. (1998) presented $\delta^{13}C$ values of bacteria that were considerably depleted in comparison to DOC (-24.7 to -19.6) in the Gulf of Mexico, with values as low as -33‰ suggesting that the microbes used an alternative carbon source. Consequently, microbial

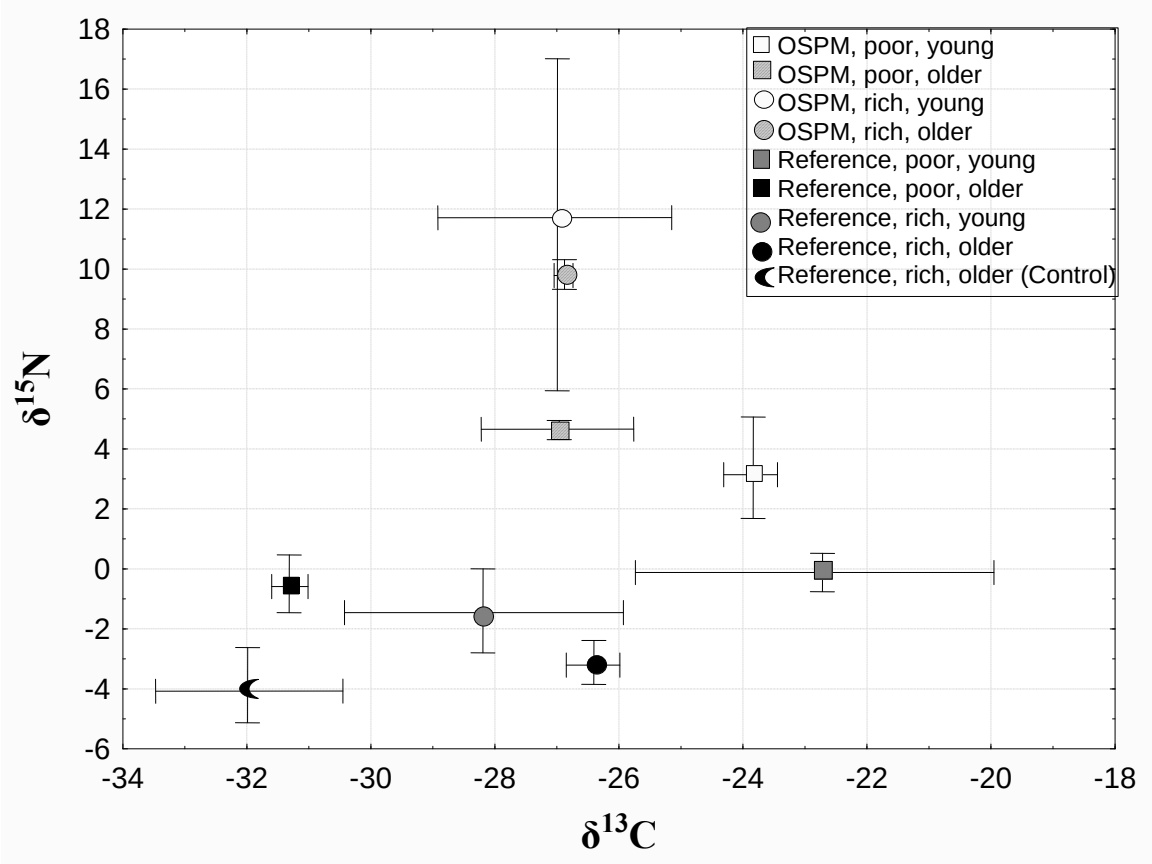


Figure 2.4 – $\delta^{13}C$ & $\delta^{15}N$ values of microbial biofilm that developed in varying classes of wetlands. Open symbols represent OSPM-affected wetlands; filled symbols are reference wetlands. Wetlands that have organic-poor sediments have square symbols; organic-rich wetlands are circles or crescents. Open or grey symbols represent young wetlands; crosshatched or black symbols represent samples from older wetlands.

$\delta^{13}C$ and $\delta^{15}N$ were compared to each potential carbon source (phytoplankton, metaphyton, macrophytes, sediment, and petroleum) within each of the wetlands to further explain the variation inherent in microbial $\delta^{13}C$.

Stable Isotope Mixing Models

$\delta^{13}C$ and $\delta^{15}N$ values of subsurface and epibenthic biofilm were compared within a location in each wetland. Subsurface and epibenthic biofilms $\delta^{13}C$ values were not significantly different from each other in any of the wetlands (paired-comparison t-test; mean±SE difference in $\delta^{13}C$: 0.37±0.53, n =13, $p>0.05$). In contrast, subsurface and epibenthic biofilms $\delta^{15}N$ values were significantly different from each other (paired-comparison t-test; mean±SE difference in $\delta^{15}N$: -0.71±0.31, n =13, $p<0.05$). Subsurface and epibenthic biofilm $\delta^{15}N$ were highly correlated (Fig. 2.5; simple linear regression; $R^2 = 0.97$, $p < 0.0001$) indicating that an enriched subsurface $\delta^{15}N$ value was paralleled by an enriched epibenthic $\delta^{15}N$ value at a wetland location and conversely a depleted subsurface $\delta^{15}N$ value was paralleled by a depleted epibenthic $\delta^{15}N$ value at a wetland location. Consequently, subsurface and epibenthic biofilm data were pooled together in each wetland for further determination of microbial carbon sources (Table 2.2).

Microbial biofilm $\delta^{13}C$ and $\delta^{15}N$ were compared with potential carbon sources (primary production, sediment and anthropogenic sources) within each wetland using a concentration-weight linear stable isotope mixing model to estimate the proportion of potential carbon sources assimilated by the microbial community in oil sands constructed wetlands.

OSPM-affected Wetlands

According to the mixing model the calculated proportion of carbon in Mike's Pond microbial biofilm was 100% phytoplankton (Table 2.2; Fig. 2.6a). Heterotrophic bacteria are known to consume DOM of planktonic origin and convert it to particulate biomass (Van Mooy et al. 2001). However, results from Mike's pond are problematic since

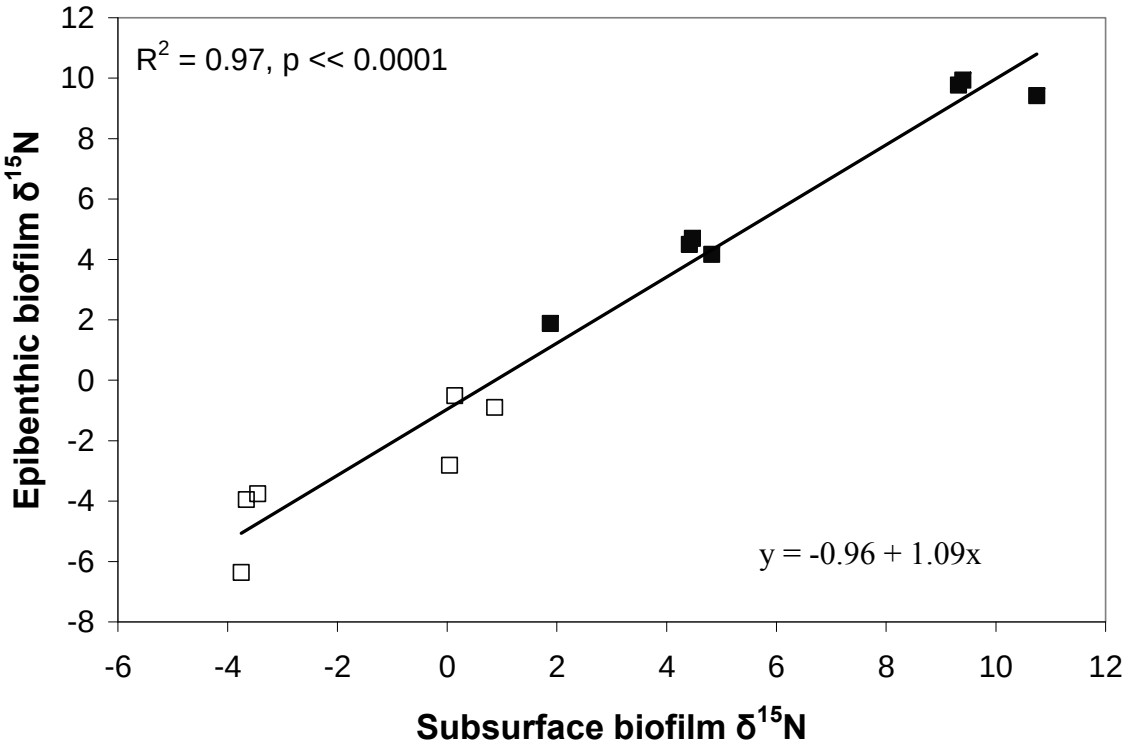


Fig. 2.5 – Subsurface biofilm $\delta^{15}N$ versus epibenthic biofilm $\delta^{15}N$. Each data point refers to a single location within a reference (□) or an OSPM-affected (■) wetland.

Table 2.2 – Data used for the concentration-dependent stable isotope mixing model in each wetland.

Wetland	Item	Source	Mixture	$\delta^{13}C'$ *	$\delta^{15}N'$ *	%C	%N	Fraction of biomass assimilated by source X,Y or Z mixture ($f_{X,B}$, $f_{Y,B}$ or $f_{Z,B}$)
MP	Phytoplankton	X		-21.99±0.26	7.96±0.87	1.58±0.01	0.13±0.01	1.00
MP	Petroleum	Y		-27.25±0.06	5.35±0.02	14.38±2.56	0.13±0.03	0.00
MP	Macrophytes	Z		-21.36±7.71	17.27±4.17	34.59±14.68	1.68±3.00	0.00
MP	Microbial biofilm		M	-23.80±0.48	3.20±1.56	5.61±0.37	0.48±0.03	
TP9	Macrophytes/ phytoplankton/ metaphyton	X		-24.31±3.42	11.21±3.25	27.63±15.00	1.38±0.77	0.00
TP9	Sediment	Y		-25.54±1.39	4.05±0.65	6.37±0.32	0.25±0.04	0.32
TP9	Petroleum	Z		-27.25±0.06	5.35±0.02	14.38±2.56	0.13±0.03	0.68
TP9	Microbial biofilm		M	-26.97±1.45	4.65±0.45	11.39±3.10	0.90±0.26	
4-m CT	Macrophytes	X		-26.11±2.10	20.12±4.90	36.76±12.85	2.38±1.03	0.04
4-m CT	Sediment	Y		-26.56±1.55	7.33±3.05	7.99±2.11	0.19±0.12	0.34
4-m CT	Petroleum	Z		-27.25±0.06	5.35±0.02	14.38±2.56	0.13±0.03	0.62
4-m CT	Microbial biofilm		M	-26.98±1.91	11.63±5.95	13.78±5.35	1.23±0.92	
NW	Macrophytes	X		-18.21±5.11	15.04±6.36	35.79±7.03	2.35±0.73	0.00
NW	Metaphyton/ Phytoplankton	Y		-19.90±1.50	19.67±5.06	17.60±15.61	1.42±0.90	0.04
NW	Petroleum	Z		-27.25±0.06	5.35±0.02	14.3±2.56	0.13±0.03	0.96
NW	Microbial biofilm		M	-26.9±0.2	9.77±0.5	14.38±1.13	0.13±0.16	
CNRL	Macrophytes	X		-24.82±2.89	7.34±1.55	39.58±5.55	2.14±0.87	-0.14
CNRL	Metaphyton	Y		-10.73±0.38	2.63±0.05	10.87±0.39	0.69±0.07	0.03
CNRL	Phytoplankton	Z		-27.68±0.78	6.30±1.37	2.98±0.38	0.31±0.03	0.11
CNRL	Microbial		M	-22.72±2.95	-0.10±0.67	13.16±1.01	1.16±0.13	

	Biofilm							
SW	Metaphyton/ phytoplankton	X		-25.26±2.40	3.89±0.79	19.33±20.32	1.17±1.08	0.17
SW	Sediment	Y		-26.07±0.04	4.51±0.14	2.96±0.44	0.23±0.04	0.97
SW	Macrophytes	Z		-24.47±4.41	6.19±1.80	37.78±6.22	2.21±0.65	-0.13
SW	Microbial biofilm		M	-31.32±0.30	-0.52±1.16	14.61±3.19	1.5±0.56	
PP	Sediment	X		-26.44±0.83	4.08±0.30	10.47±6.25	0.36±0.39	1.13
PP	Rod algae	Y		-31.12	2.82	42.61	8.11	0.02
PP	Macrophytes/ phytoplankton/ metaphyton	Z		-24.32±5.47	6.29±2.10	32.68±15.12	2.41±1.23	-0.15
PP	Microbial biofilm		M	-28.20±2.43	-1.41±1.43	18.34±3.88	2.32±0.94	
HS	Phytoplankton	X		-22.48±1.37	2.86±0.42	3.96±0.43	0.33±0.07	1.09
HS	Sediment	Y		-57.51±43.70	4.15±3.66	16.06±18.68	0.34±0.39	0.01
HS	Macrophytes/m etaphyton	Z		-23.33±3.38	4.14±0.21	44.94±1.98	3.11±0.83	-0.10
HS	Microbial biofilm		M	-26.41±0.37	-3.16±0.73	17.82±2.18	1.99±0.40	
BP	Macrophytes	X		-27.69±2.05	8.25±1.26	40.2±5.06	2.68±0.73	-0.06
BP	Sediment	Y		-26.77±0.25	9.64±0.72	2.00±0.72	0.07±0.02	0.74
BP	Phytoplankton	Z		-28.71±0.16	12.12±0.01	1.73±0.33	0.22±0.03	0.31
BP	Microbial biofilm		M	-31.96±1.66	-4.08±1.38	14.89±2.21	1.42±0.19	

* = Isotopic signatures for the sources have been corrected for trophic fractionation (C: +1.8‰; N: +3.4‰)

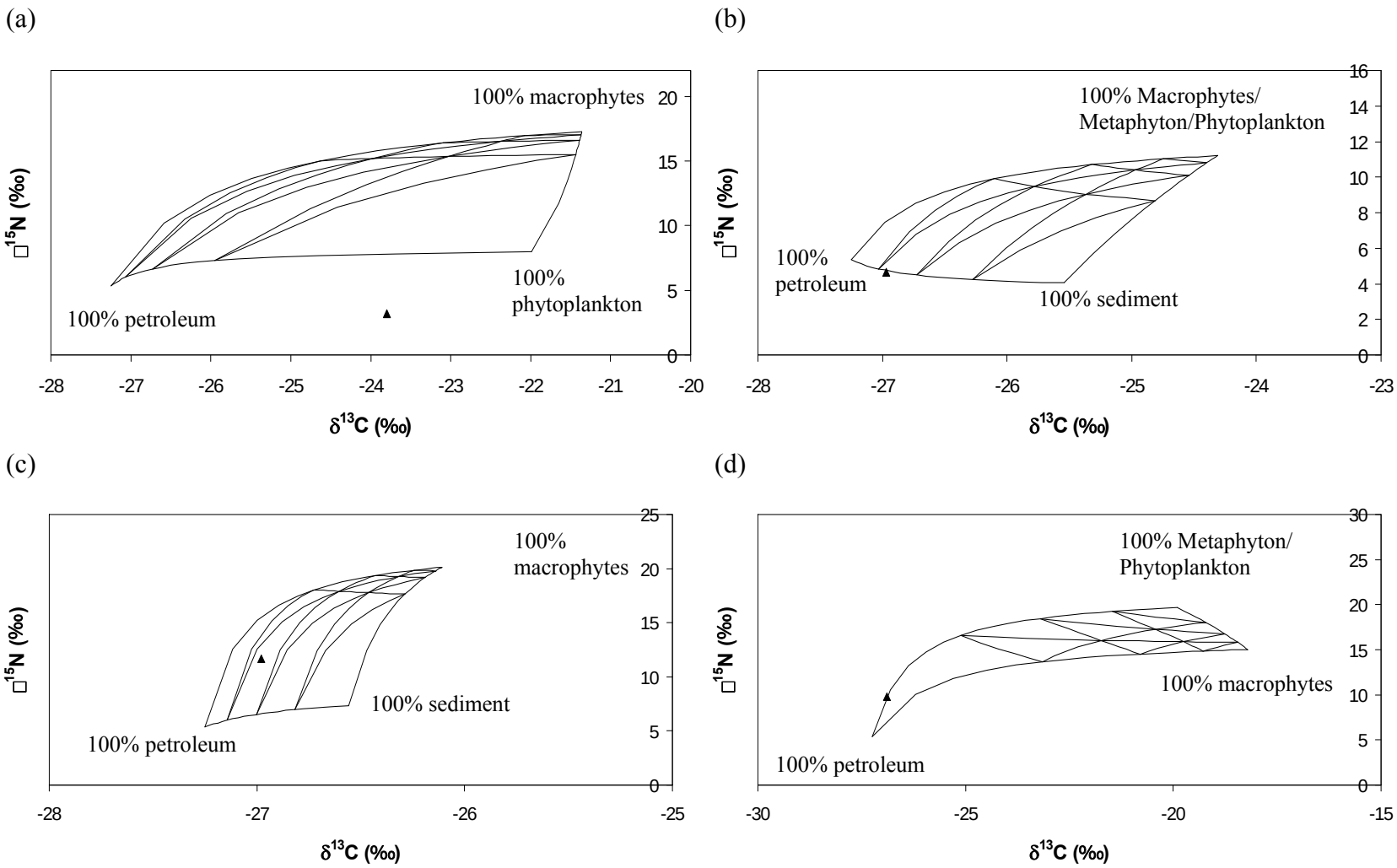


Figure 2.6 – Dietary mixing triangles for microbial biofilm in OSPM-affected wetlands (a) Mike's Pond, (b) Test Pond 9, (c) 4-m CT Wetland, and (d) Natural Wetland. Mean (±SD) microbial biofilm is represented by ▲. Isotopic values for pure sources at triangles vertices have been corrected for trophic fractionation (Table 2.2). Lines within the triangles are "iso-diet" lines along which the proportion of one dietary component is invariant. Iso-diet lines increase from 0% on one side of the triangle opposing a vertex to 100% at the vertex (Phillips and Koch 2002). Note that scale of x and y-axes vary among graphs.

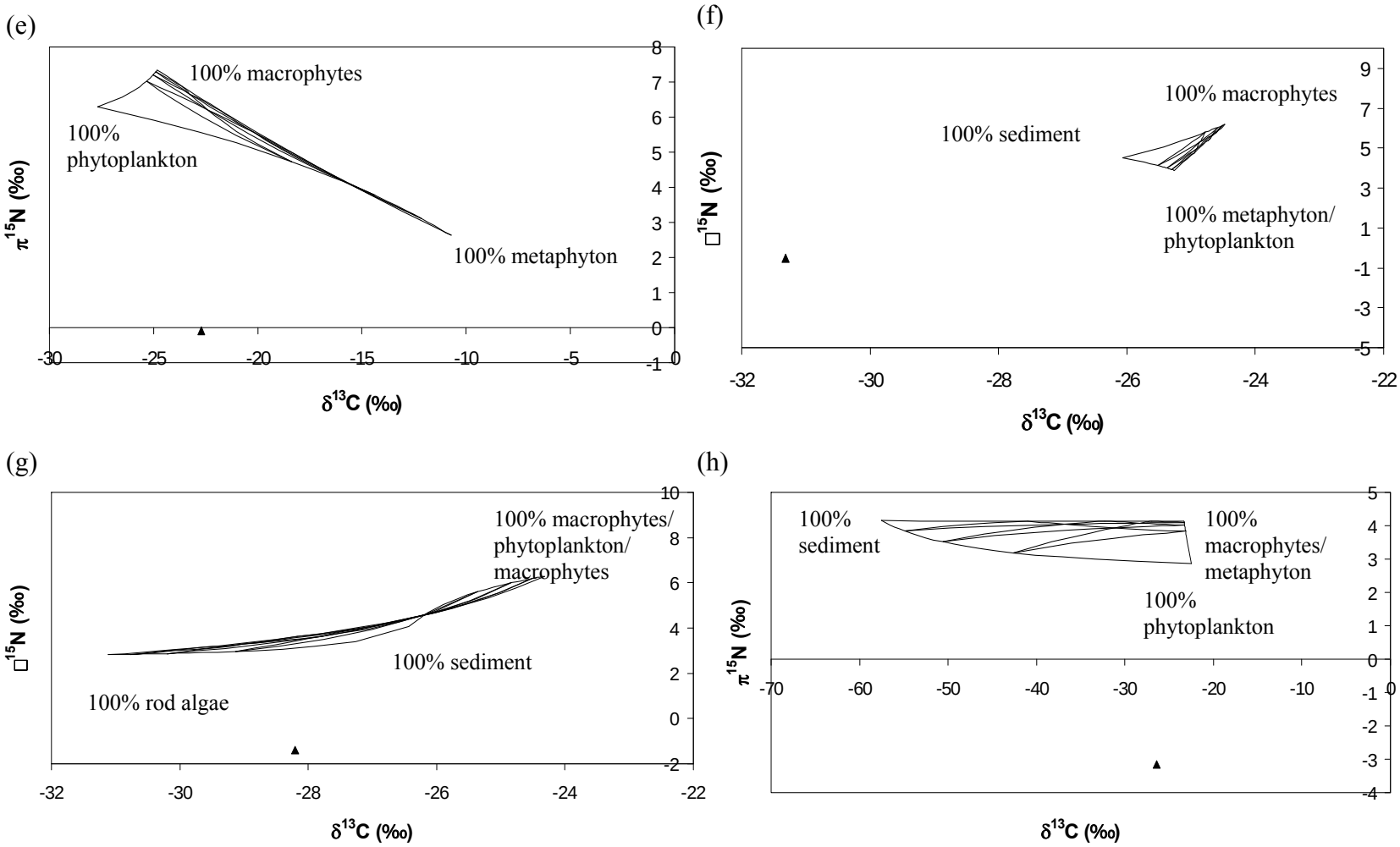


Figure 2.6 – Dietary mixing triangles for microbial biofilm in reference wetlands: (e) CNRL Wetland, (f) Shallow Wetland, (g) Peat Pond, and (h) High Sulphate Wetland. Mean (±SD) microbial biofilm is represented by ▲. Isotopic values for pure sources at triangles vertices have been corrected for trophic fractionation (Table 2.2). Lines within the triangles are "iso-diet" lines along which the proportion of one dietary component is invariant. Iso-diet lines increase from 0% on one side of the triangle opposing a vertex to 100% at the vertex (Phillips and Koch 2002). Note that scale of x and y-axes varies between graphs.

(i)

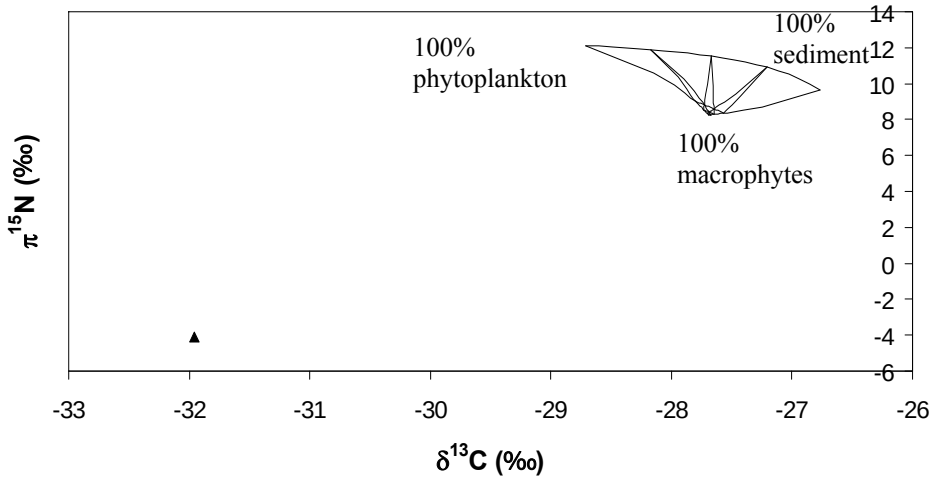


Figure 2.6 – Dietary mixing triangles for microbial biofilm in reference wetland: (i) Beaver Pond. Mean (±SD) microbial biofilm is represented by ▲. Isotopic values for pure sources are at triangle vertices. Isotopic values for pure sources at triangles vertices have been corrected for trophic fractionation (Table 2.2). Lines within the triangles are "iso-diet" lines along which the proportion of one dietary component is invariant. Iso-diet lines increase from 0% on one side of the triangle opposing a vertex to 100% at the vertex (Phillips and Koch 2002). Note that scale of x and y-axes varies between graphs.

microbial biofilm fell outside of the mixing triangle, suggesting that microbes were using another carbon source that was not examined during this study. This source would have to be much depleted in $\delta^{15}N$ if it were to balance the mixing model equations. Mike's Pond microbial biofilm $\delta^{13}C$ (-23.8±0.5‰) and $\delta^{15}N$ (3.2±1.6‰) was not significantly different from phytoplankton (-23.50±0.48‰ C and 4.49±0.87‰ N) $\delta^{13}C$ (Independent samples t-test; C: n=6, t = -0.88, p>0.05; N: n=6, t = -1.02, p>0.05). The similarity in isotopes between the two groups suggests that phytoplankton is probably still an important carbon source for the microbial community in Mike's Pond. However, the exact proportion of carbon derived from phytoplankton can not yet be determined without incorporating the unknown source in the mixing model.

According to the mixing model the calculated proportion of carbon in Test Pond 9 microbial biofilm was 68% petroleum and 32% sediment (Table 2.2; Fig. 2.6b). In Test Pond 9 NAs are present in oil sands process waters (OSPW) that caps this wetland and these NAs are bioavailable to the microbial community (Videla 2006). Similary, Coffin et al. (1997) reported that bacteria assimilated petroleum oil in laboratory microcosm studies using stable carbon and nitrogen isotopes. A moderate proportion of the carbon in microbial biofilm was derived from Test Pond 9 sediment. Perhaps the microbial community assimilated organic matter leached into the water column from Test Pond 9 sediments. Furthermore, microbial biofilm ($\delta^{13}C$ -27.0±1.5‰) was similar to DOC ($\delta^{13}C$ -26.72‰) (Independent samples t-test; n=6, -0.16, p>0.05) (Videla 2006) suggesting that the microbial community in this wetland assimilates DOC, which contains a large proportion of naphthenic acids in this OSPM-affected wetland (Quagraine et al. 2005). Results suggest that microbes metabolize mine process-derived carbon sources.

According to the mixing model the calculated proportions of carbon in 4-m CT Wetland microbial biofilm were 62% petroleum, 34% sediment and 4% macrophytes (Table 2.2; Fig. 2.6c). The $\delta^{13}C$ signature of microbial biofilm in 4-m CT Wetland (-27.0±1.8‰) was not significantly different from DOC $\delta^{13}C$ (-27.22‰) (Videla 2006) (Independent samples t-test; n=3, t = 0.10, p>0.05). Similar to Test Pond 9, results suggest that microbes incorporate OSPW-derived carbon sources in 4-m CT Wetland. The inflow of

OSPW from an adjacent wetland, Pond 5, constantly replenishes OSPW and provides a continuous supply of NAs. DOC in estuaries can be produced by marsh grasses, mangroves, sea grasses, benthic algae and phytoplankton (Nixon 1980; Twilley 1985; Peterson et al. 1994). Since DOC often represents a mixture of carbon sources from an aquatic environment and can be derived from anthropogenic carbon sources (Quagraine et al. 2005) and primary production (i.e. macrophytes) (Nixon 1980; Twilley 1985; Peterson et al. 1994), then the apparent ability of the microbial community to assimilate DOC derived from petroleum (i.e., naphthenic acids), macrophytes and nutrients leached from wetland sediments is not unprecedented.

According to the mixing model, the calculated proportion of carbon in Natural Wetland microbial biofilm was 96% petroleum and 4% algae (metaphyton and phytoplankton) (Table 2.2; Fig. 2.6d). DOC $\delta^{13}C$ (-27.9 ‰) (Videla 2006) in Natural Wetland was similar to biofilm $\delta^{13}C$ (-26.90±0.2‰), although the two means were significantly different from each other (Independent samples t-test; $n=7$, $t = 5.74$, $p<0.05$). Results suggest that the majority of carbon assimilated by the heterotrophic microbial community in Natural Wetland was derived from petroleum, likely from NAs present in OSPW that enter this wetland as dyke seepage from an adjacent tailings pond. They apparently also metabolized small proportions of carbon derived from primary production (metaphyton and phytoplankton).

Reference Wetlands

According to the mixing model the calculated proportion of carbon in CNRL Wetland microbial biofilm was 110% phytoplankton and 3% metaphyton (Table 2.2; Fig. 2.6e). Results from CNRL Wetland were problematic since the signature values for microbial biofilm fell outside of the mixing triangle (Fig. 2.6e). Microbial biofilm $\delta^{15}N$ (-0.10±0.67) was more depleted than any of its potential sources (phytoplankton, metaphyton and macrophytes) once sources were corrected for trophic fractionation (Table 2.2), indicating that an important nitrogen source within this wetland was not examined. I am not sure what the unknown carbon source may have been in this young (<7 y) reference wetland with poor organic sediments. CNRL wetland was the youngest

of all 9 study wetlands and formed opportunistically in 2004. This wetland was sparsely vegetated with diverse macrophytes and had no dominant species. A detritus base had not yet been established on the surface of the sandy substrate in CNRL. Algal production may contribute to microbial biomass. However, without including the unknown nitrogen source in the mixing model it is not possible to quantify how much algal production contributes to microbial biomass in CNRL Wetland.

According to the mixing model, the calculated proportion of carbon in Shallow Wetland microbial biofilm was 97% sediment and 17% algae (metaphyton and phytoplankton) (Table 2.2; Fig. 2.6f). Results from Shallow Wetland were problematic since microbial biofilm fell outside of the mixing triangle (Fig. 2.6f). Microbial biofilm $\delta^{13}C$ (-31.32±0.30) and $\delta^{15}N$ (-0.52±1.16) were more depleted than any of its potential sources (phytoplankton and metaphyton, macrophytes and sediment) once sources were corrected for trophic fractionation (Table 2.2) indicating that an important carbon source within this wetland was not examined. Nutrients leaching from sediments and algal production may have contributed to microbial biomass. However, without including the unknown carbon and nitrogen source in the mixing model it is not possible to quantify how much these sources contributed to microbial biomass in Shallow Wetland. Similarly, Kelley et al. (1998) reported that the stable carbon isotope values of the bacterial assemblage in the water column of the Gulf of Mexico were very depleted compared to terrestrial and phytoplankton carbon, the expected carbon sources. They inferred that the depleted carbon signature may have been attributed to hydrocarbon seeps in the Gulf, which were not examined, or to bacterial assimilation processes that fractionate carbon isotopes (i.e., nitrification (see below) or methane oxidation) (Kelley et al. 1998).

Methanotrophic bacteria oxidize methane to CO_2 (Wang et al. 1996) and during the process incorporate methane's carbon into biomass (Kelley et al. 1998). Carbon isotopes fractionate during methanotrophy resulting in the biomass and CO_2 being more depleted in ^{13}C than the original methane (Summons et al. 1994). Biomass typically becomes more negative with a 0-20‰ depletion (Summons et al. 1994, Jahnke et al. 1999). Thus, the isotopic composition of carbon incorporated into methanotrophic biomass can be very

^{13}C-depleted since $\delta^{13}C$ values for methane produced from anaerobic sediments typically range from -50 to -100‰ (Whiticar et al. 1986). Large methane emissions (11.95 mg $m^{-2}d^{-1}$) were measured from Shallow Wetlands sediments relative to the other study wetlands (Chapter 4). Therefore, methanotrophy could play an important role in Shallow Wetland. The more negative carbon isotope values observed in the Shallow Wetland microbial community may indicate that methanotrophs are part of the microbial community in this wetland. Perhaps the enclosed confines of the biofilm tubes selected for methanogenic bacteria and methanotrophs.

According to the mixing model the calculated proportion of carbon in Peat Pond microbial biofilm was 113% sediment and 2% rod algae (Table 2.2; Fig. 2.6g). Results from Peat Pond were problematic since microbial biofilm fell outside of the mixing triangle (Fig. 2.6g). Microbial biofilm $\delta^{15}N$ (-1.41±1.43) was more depleted than any of its potential sources (sediment, rod algae, and phytoplankton, metaphyton and macrophytes) once sources were corrected for trophic fractionation (Table 2.2) indicating that an important nitrogen source within this wetland was not examined (see below). The $\delta^{13}C$ of biofilm (-28.20±2.43‰) was not significantly different from DOC (-26.65‰) (Videla 2006) (Independent sample t-test; $n = 4$, $t = -0.55$, $p > 0.05$), sediment (-26.44±0.83‰)) (Independent sample t-test; $n = 5$, $t = 0.02$, $p > 0.05$) and rod algae (-31.13‰) (Independent samples t-test; $n = 4$, $t = 1.51$, $p>0.05$), when sources were corrected for trophic fractionation, suggesting that nutrients leached from sediments and rod algae may be contribute carbon to microbial community biomass in Peat Pond.

According to the mixing model the calculated proportion of carbon in High Sulphate Wetland microbial biofilm was 109% phytoplankton and 1% sediment (Table 2.2; Fig. 2.6h). Results from High Sulphate Wetland were again problematic since microbial biofilm fell outside of the mixing triangle (Fig. 2.6h). Microbial biofilm $\delta^{15}N$ (-3.16±0.73) was more depleted than any of its potential sources (sediment, phytoplankton, and metaphyton and macrophytes) once sources were corrected for trophic fractionation (Table 2.2) indicating that an important nitrogen source within this wetland was not examined (see below). Two sediment cores collected from High Sulphate Wetland had

carbon isotope values of -90.21‰ and -28.40‰, respectively. The large carbon isotope range between the 2 cores represents the heterogeneity of microbial activities within a wetland and the tremendous spatial variability of sediments. Large spatial variability within soils can occur on the micrometer scale because of the lack of mixing within the profile (Hopkins et al. 1998). The depleted value (-90.21‰) in one of the High Sulphate sediment samples almost certainly represents methane production via a CO_2 reduction pathway (Schlesinger 1997) and is likely responsible for the depleted biofilm $\delta^{13}C$ (–26.41±0.37‰).

According to the mixing model the calculated proportion of carbon in South West Sands Beaver Pond, the control wetland, microbial biofilm was 74% sediment and 31% phytoplankton (Table 2.2; Fig. 2.6i). As was observed for the other reference wetlands results from South West Sands Beaver Pond were problematic since microbial biofilm fell outside of the mixing triangle (Fig. 2.6i). Microbial biofilm $\delta^{13}C$ (-31.96±1.66) and $\delta^{15}N$ (-4.08±1.38) were more depleted than any of its potential sources (sediment, phytoplankton, and macrophytes) once sources were corrected for trophic fractionation (Table 2.2) suggesting that carbon and nitrogen from sources other than those examined supports production of the microbial assemblage within this control wetland (e.g. methane). The depleted carbon and nitrogen signatures may have been a reflection of a carbon source that was overlooked or to bacterial assimilation processes that fractionate carbon isotopes (i.e. nitrification (see below)). Large methane emissions (4.18 mg $m^{-2}d^{-1}$) were measured from Beaver Pond sediments relative to the other study wetlands (Chapter 4). The more negative carbon isotope values observed in the Beaver Pond microbial community may indicate that methanotrophs are part of the microbial community in this wetland.

Carbon Dynamics in Constructed Wetlands

According to the stable isotope mixing models in reference wetlands, metaphyton, phytoplankton and nutrients leached from sediments may possibly be important carbon sources to the microbial community. However, results from all the reference wetlands and Mike's Pond were problematic since microbial biofilm fell outside of the mixing

triangle, leading to nonsensical negative biomass estimates for primary production, OSPM-derived, and sediment carbon sources. Microbial biofilm $\delta^{15}N$ values in all reference wetlands were more negative than their potential sources (that were corrected for trophic fractionation, Table 2.2), indicating that a nitrogen source common to reference wetlands may have been overlooked. One explanation is nitrification, which is the biological oxidation of ammonia into nitrite followed by the oxidation of these nitrites into nitrates (Schlesinger 1997). Nitrification is carried out by nitrifying bacteria. Fractionation occurs when nitrifying bacteria (Bender 1971) incorporate DIC into biomass (Kelley et al. 1998). Minimal nitrogen fractionation occurs during nitrification in N-limited systems, and the $\delta^{15}N$ of nitrate is usually within a few per mil of the source total organic N content. Elevated ammonium concentrations may result in large fractionations occurring during nitrification (Hoch et al. 1992; Kendall 1998). Similarly, a large range of fractionations (-27 to 0‰) has been measured for nitrate and ammonium assimilation of N-bearing compounds by algae in aquatic environments (Fogel and Cifuentes 1993). The presence of NH_4^+ in reference wetlands (0.3 to 1.4 mg/L; n=3) suggests that nitrification may play an important role in nitrogen dynamics in reference wetlands. Nitrate concentrations were not measured in reference wetlands. Further stable isotope research in oil sands constructed reference wetlands should focus on nitrogen dynamics and nitrogen compounds (i.e. dissolved inorganic nitrogen (DIN)). An alternative explanation is that an assumption in this model was not met. Perhaps the C and N isotopes from all carbon sources were not completely homogenized when the microbial community assimilated them. It is possible that microbial communities utilized only a portion of bulk organic matter - presumably components with a more isotopically depleted signal, such as the lipid compounds. For example, lipids present in macrophytes tend to have $\delta^{13}C$ values more depleted than the bulk (Monson and Hayes 1982, Blair et al. 1985, Jahnke et al. 1995).

It is also possible that an unidentified energy source that is depleted in both $\delta^{13}C$ and $\delta^{15}N$ may be present in reference wetlands. If this is possible, then the same source may be present in OSPM-affected wetlands. If this were the case, then the C and N isotope values in OSPM-wetlands would both be depleted suggesting that the depleted $\delta^{13}C$ of

petroleum was not the only C source assimilated by bacteria in this class of wetlands. However, biofilm $\delta^{15}N$ values in OSPM-affected wetlands are much enriched. I believe that it is more plausible that methanogenesis and nitrogen dynamics (i.e. nitrification) in reference wetlands are responsible for the depleted $\delta^{13}C$ and $\delta^{15}N$ biofilm signatures. Furthermore, the signatures of the natural (mature) biofilm in Peat Pond and Test Pond 9 both fell outside of the mixing model triangles (Appendix 2.12). The young and mature biofilm in Peat Pond fell outside of the mixing model triangle suggesting that an unidentified energy source was overlooked in this reference wetland and that the pipe design did not alter the stable isotope signatures of the young biofilm, since the signatures were similar to natural biofilm.

The values of the concentration-weighted stable isotope mixing model results were credible in most of the OSPM-affected wetlands (Natural Wetland, 4-m CT Wetland and Test Pond 9) since microbial biofilm (mixture) fell within the triangular space enclosed by lines connecting the 3 potential carbon sources (Fig. 2.6b-d, respectively). Results from the OSPM-affected wetlands suggest that OSPM-derived carbon sources were available to the microbial community. Therefore, those carbon sources that were incorporated into microbial biomass in OSPM-affected wetlands may potentially be available to higher trophic organisms.

Most aquatic plants have or are assumed to have C_3 photosynthesis (Sharkey and Berry 1985). The $\delta^{13}C$ of C_3 plants typically ranges from -23 to -34‰ (Faure and Mensing 2005) depending on the amount of fractionation during photosynthesis. Signatures of plants become more negative than their atmospheric source because of isotope fractionation accompanying CO_2 uptake during photosynthesis (O'Leary 1988). Emergent and floating species can use atmospheric CO_2 (-8‰), whereas submergent vegetation is dependent on dissolved inorganic carbon (0‰) (Boschker and Middelburg 2002). C_3 plants use the RUBISCO enzyme to carboxylate ribulose biphosphate as the initial carbon fixation reaction, and the kinetics of this reaction impose a strong isotope discrimination upon $^{13}CO_2$ (Farquar et al. 1989) in favour of $^{12}CO_2$. The increased diffusion resistance of DIC in water may reduce fractionation and produce higher stable

carbon isotope ratios in submergent macrophytes (LaZerte and Szalados 1982; O'Leary 1988). The carbon isotope values of macrophytes in the study wetlands ranged from -33.2 to -12.7‰ and were similar to typical ranges for C_3 photosynthesis, excluding *Lemna minor* in Beaver Pond (Appendix 2.4). Similar $\delta^{13}C$ values of from -29 to -11‰ were measured by Hecky and Hesslein (1995) in temperate lakes in northwestern Ontario. Some macrophytes, notably submergent biota, had more positive carbon isotope ratios than the expected range for C_3 plants. In the 9 wetlands of the current study, there were highly significant differences among functional classes. Submergent plants (mean -23.5‰) had more positive carbon isotope ratios than emergent (mean -28.1‰) and floating plants (mean -33.4‰) (1-way ANOVA, $p<0.001$) (Fig. 2.7). Emergent and floating macrophytes had a more negative isotope ratio than submergent macrophytes because they use different forms of inorganic carbon (LaZerte and Szalados 1982).

One sample of floating macrophyte, *Lemna minor* collected from Beaver Pond, was unusually depleted in in $\delta^{13}C$ (-40.9‰) (Appendix 2.4). Aquatic photosynthesis can produce $\delta^{13}C$ of -37‰ if atmospheric CO_2 or DIC are in excess. Terrestrial plants reduce uptake of atmospheric CO_2 to conserve water losses and therefore, CO_2 is not often in excess (Hecky and Hesslein 1995). However, aquatic plants incorporating atmospheric CO_2 during photosynthesis likely do not conserve water and could potentially reach $\delta^{13}C$ values near those measured in *Lemna minor.* Although aqueous CO_2 is usually the preferred carbon substrate in aquatic plants because it requires no energy expenditure for uptake (Sharkey and Berry 1985). CO_2 in freshwater systems is seldom in equilibrium with the atmosphere (Cole et al. 1994). Since Beaver Pond is supersaturated with respect to atmospheric CO_2 (see chapter 4), *Lemna minor* probably achieved such negative $\delta^{13}C$ ratios using DIC during aquatic photosynthesis. Also, similar carbon and nitrogen stable isotope depletion were found in the microbial assemblage in Beaver Pond. Perhaps ^{15}N depleted nitrates derived from nitrifying bacteria can account for negative nitrogen isotopes in *Lemna minor*.

The 'young' microbial biofilm collected from colonization tubes had $\delta^{13}C$ (-26.97±1.45‰) and $\delta^{15}N$ (4.65±0.45‰) signatures that were not significantly different

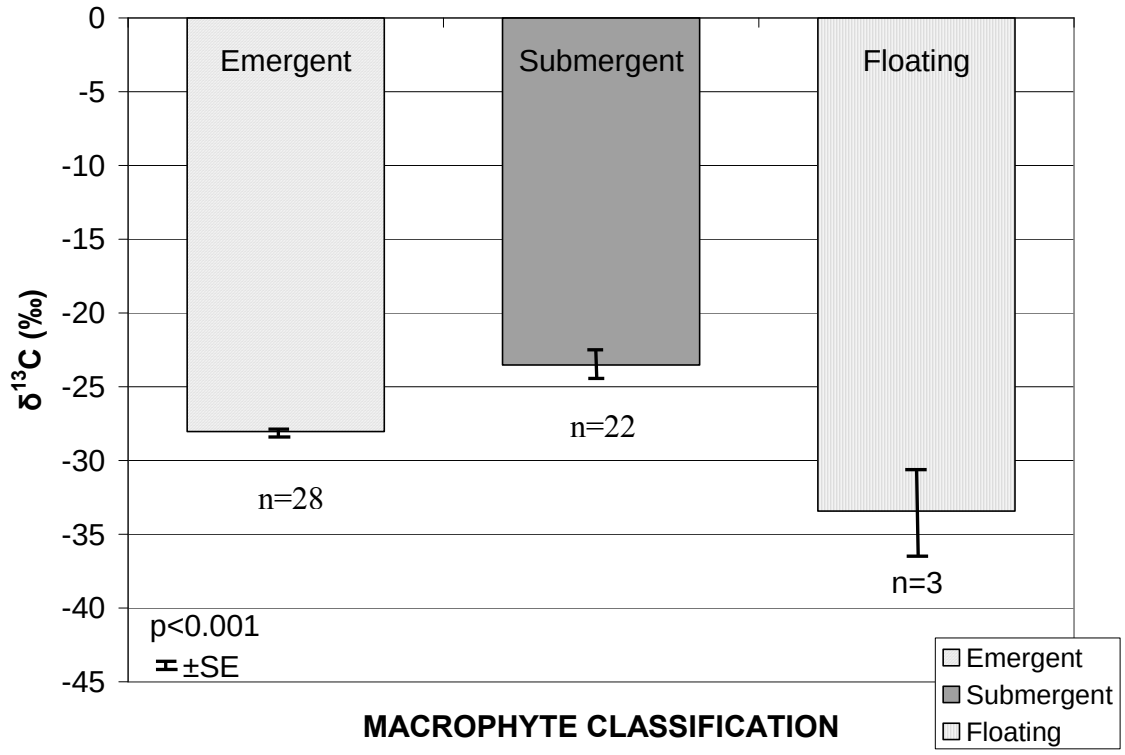


Figure 2.7 – Mean (±SE) $\delta^{13}C$ ratios emergent, submergent and floating-leaf classes of macrophytes collected from the all wetlands (all species from all wetlands pooled). Means were significantly different (1-Way ANOVA, $p < 0.001$)

from biofilm of indeterminate age ($\delta^{13}C$ -29.3‰; $\delta^{15}N$ 3.94‰) in Test Pond 9 (Independent samples t-test; $\delta^{13}C$: n=6, t=1.46, p >0.05, $\delta^{15}N$: n=6, t= 1.45 p>0.05) indicating that heterotrophic bacteria may dominate microbial biofilm in OSPM-affected wetlands. In contrast, young microbial biofilm $\delta^{13}C$ (-28.20±2.43‰) was significantly more depleted than mature biofilm ($\delta^{13}C$ -11.9‰) collected from Peat Pond (Independent samples t-test, n=4, t=-5.80 p<0.05).). Although, young microbial biofilm $\delta^{15}N$ (-1.41±1.43‰) was not significantly different than mature biofilm ($\delta^{15}N$ - 0.4‰) collected from Peat Pond (Independent samples t-test; n=4, t = -1.11, p<0.05).

The C & N isotope ratios of mature biofilm in Peat Pond seem to reflect an algal contribution ($\delta^{13}C$ -19.5‰, $\delta^{15}N$ 0.5‰). The mature biofilm communities appear to be different from young biofilm. The natural (mature) biofilm likely contains photosynthetic algae in both wetlands.

Nitrogen Dynamics in Constructed Wetlands

A 3-way factorial ANOVA determined there was no significant relationship between biofilm $\delta^{13}C$ and the main effects of wetland age class, organic base, or reclamation type (Table 2.3). There was no significant relationship between biofilm $\delta^{15}N$ and age (Table 2.4). But there was a significant relationship between biofilm $\delta^{15}N$ and reclamation type. OSPM-affected wetlands were enriched ($\delta^{15}N$ = 7.32‰) compared to reference wetlands (-$\delta^{15}N$= 1.85‰) (Table 2.4, Fig. 2.8). A significant interaction was observed with biofilm $\delta^{15}N$ between organic base and reclamation type (Fig. 2.8). Biofilm $\delta^{15}N$ in reference wetlands with poor organic sediments (-0.31‰) was depleted compared to OSPM wetlands with poor organic sediments (3.94‰). The biofilm $\delta^{15}N$ in reference wetlands with rich organic sediments (-2.88‰) was more depleted compared to wetlands with poor organic sediments, while in OSPM-affected wetlands with rich organic sediments (10.7‰) $\delta^{15}N$ becomes much more enriched.

The nitrogen isotope ratios of biofilm in most of the study wetlands remained approximately near 0‰ (Fig. 2.5). This suggests that plants, blue-green and other

Table 2.3 – A 3-way factorial ANOVA examining the relationship between biofilm $\delta^{13}C$ and wetland age class, organic base, and/or reclamation type.

Effect	**D.F.**	**Sum of Squares**	**Mean Square**	**F**	**p**
Reclamation type (R)	1	6.07	6.07	0.07	>0.05
Organic base (B)	1	5.50	5.50	0.61	>0.05
Age (A)	1	21.29	21.29	2.37	>0.05
R x B	1	0.01	0.01	0.00	>0.05
R x A	1	5.69	5.69	0.66	>0.05
B x A	1	15.62	15.62	1.74	>0.05
Discrepance	2	17.96	8.98		

Table 2.4 – A 3-way factorial ANOVA examining the relationship between biofilm $\delta^{15}N$ and wetland age class, organic base, and/or reclamation type.

Effect	**D.F.**	**Sum of Squares**	**Mean Square**	**F**	**p**
Reclamation type (R)	1	162.6	162.6	406.5	< 0.005*
Organic base (B)	1	11.1	11.1	27.8	<0.05*
Age (A)	1	1.3	1.3	3.3	>0.05
R x B	1	42.8	42.8	107.0	<0.01*
R x A	1	0.6	0.6	1.5	>0.05
B x A	1	3.4	3.4	8.5	>0.05
Discrepance	2	0.7	0.4		

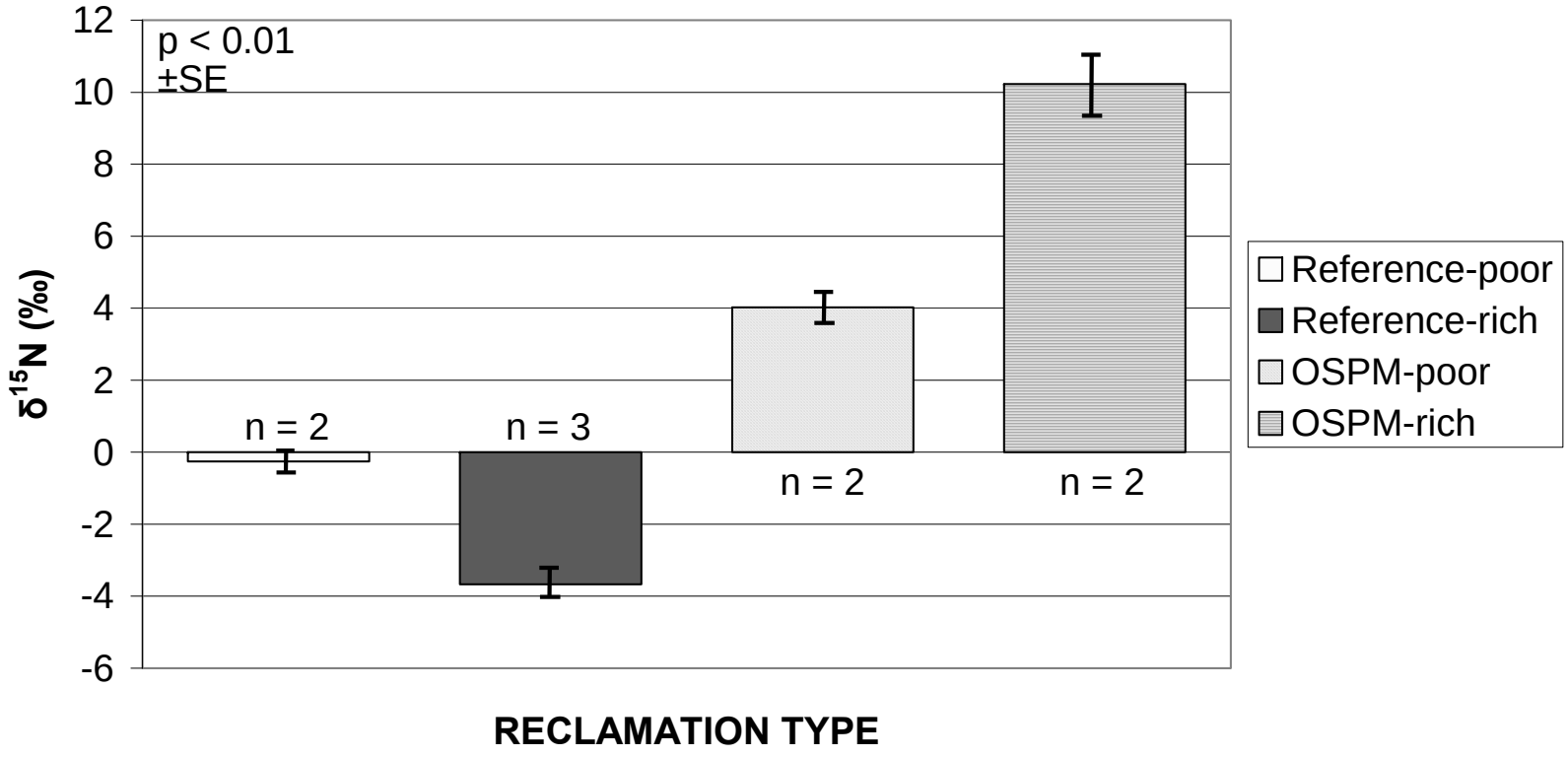


Figure 2.8 – Mean±SE nitrogen isotope values for microbial biofilm in reference and OSPM-affected wetlands with rich or poor organic bases.

bacteria that fix atmospheric N_2 ($\delta^{15}N$ = 0‰) (Kendall 1998) may contribute significantly to the nitrogen dynamics in the constructed wetlands. Wetlands are often considered to be nitrogen limited (Small 1972), consequently resulting in the microbial recycling of DIN and the prominence of N-fixing bacteria, such as cyanoprokaryotes (Mitsch & Gosselink 1990). The higher stable nitrogen isotope ratios measured in microbial communities (Fig. 2.8) and retained in other biota from OSPM-affected wetlands (Appendix 2.4 & 2.7), especially those with highly organic sediments, is likely the result of a large fractionation during N-mediated processes, such as denitrification and volatization. Similar increases in the $\delta^{15}N$ of estuarine biota, including macrophytes, algae, and suspended POM, have been noted as wastewater loads to estuaries increased (McClelland et al. 1997, McClelland and Valieal 1998; Ganshorn 2002).

Denitrification is the biological reduction of nitrates and nitrites to gaseous end-products, N_2 and N_2O (Hopkins et al. 1998). This microbially-mediated process causes the $\delta^{15}N$ of the residual nitrate to increase exponentially as nitrate decreases. Fractionations measured during denitrification typically range from -40 to -5‰ causing N_2 & N_2O to be ^{15}N-depleted. These ^{15}N-depleted gases escape the aquatic system, leaving behind $\delta^{15}N$ enriched nitrate (e.g., +15 to +30‰) (Kendall 1998).

During volatization, ammonia gas can be lost from the soil and return to the atmosphere. The ammonia gas produced has a lower $\delta^{15}N$. Consequently, volatization results in the increase of the nitrogen isotope signature in residual ammonia left in the wetland. Volatization commonly occurs in fertilized soils resulting in the organic matter having $\delta^{15}N$ values > 20‰ because of ammonia loss (Kendall 1998).

The ^{15}N enrichment in microbial biofilm collected from OSPM-affected sites seems indicative of denitrification and volatization. Amines are added to the oil sands ore during bitumen extraction (M. MacKinnon 2002, Syncrude Canada Ltd., pers. comm. cited in Ganshorn 2002), and these are likely metabolized by microbes during volatization leading to the elevated $\delta^{15}N$ signatures in OSPM-affected biota. Alternatively, wastewater enriched in NH_4^+ was added to some settling basins containing

process waters, such as Mildred Lake at Syncrude Canada Ltd., and may also be responsible for the ^{15}N enrichment. Perhaps after significant volitization and denitrification, wastewater inputs at Syncrude Canada Ltd. and Suncor Energy Inc. resulted in ^{15}N enriched process materials, which would later be used in the construction of the OSPM-affected wetlands. In 2001, total nitrogen content in Mildred Lake settling basin was 559 μg/L (M. MacKinnon 2006, pers. comm.). Ammonia and nitrate concentrations were both below detection limits. The low ammonia and nitrate levels in the Mildred Lake Settling Basin indicate that current N-processes are probably not the cause of the ^{15}N enrichment observed in the OSPM-affected wetlands. However, past N-processes stimulated by amine or wastewater additions may still be conserved in the process materials utilized by wetland biota. Ammonia concentrations in wetlands ranged from 0.2 to 1.7 mg/L (Appendix 2.5). Mean (±SD) ammonia concentrations in OSPM (0.6±0.7 mg/L) and reference wetlands (0.8±0.6 mg/L) were not significantly different (Independent samples t-test, n=7, t= -0.23, $p > 0.05$) suggesting that current ammonia levels are not responsible for differences in $\delta^{15}N$ between wetland reclamation types. Alternatively, nitrate was not measured in wetlands, but if nitrate levels are currently higher in OSPM wetlands compared to reference wetlands, volitization and nitrification may be play an important role in ^{15}N enrichment directly within the wetlands. The higher $\delta^{15}N$ values observed in the OSPM-affected wetlands that have been amended with highly organic sediments may indicate that nitrates leached from peat may also stimulate N dynamics.

Attempts to trace fixed N through the food web are complicated by the complex fractionations caused by a multitude of processes that affect the $\delta^{15}N$ ratio, such as mineralization, nitrification, immobilization, and denitrification. Isotopic fractionation during N transformations can obscure the source $\delta^{15}N$. Further research that focuses on the stable N isotopes of nitrate and ammonia is required to determine N sources and processes leading to ^{15}N enrichment in the biota of OSPM-affected wetlands. The dual isotope approach using both $\delta^{15}N$ and $\delta^{18}O$ offers great potential to identify changes to the N stable isotope via mineralization, nitrification, industrial effluent and atmospheric deposition (Silva et al. 2000).

Dissolved Inorganic Carbon

The stable carbon isotope ratios of dissolved inorganic carbon ($\sum CO_2 = [CO_{2\,(aq)}] + [HCO_3^-] + [CO_3^{2-}]$) reflects the internal biogeochemical dynamics of a freshwater system. The source of DIC ultimately determines the wide variations measured in the $\delta^{13}C$ ratios of components of the carbon cycle in fresh waters and include respired organic matter, (Hecky and Hesslein 1995, Miyajima et al. 1995; Peterson and Fry 1987) the interaction with atmospheric CO_2, and weathering of the geological setting (Helling et al. 2000; Miyajima et al. 1995; Peterson and Fry 1987).

The carbon isotope ratios of DIC measured in the wetland pelagic zone ranged from -9.3±0.2 to -0.6±0.4 ‰ and were comparable to those measured by Videla (2006) within the same wetlands during the same time period, supporting the validity of the results (Regression analysis: $DIC_{this\ study} = -1.13_{Videla\ 2006} + 0.91x$; $R^2 = 0.79$, $p<0.01$; t-test: slope ≠ 1, 0.91±0.176 (mean±SD), $t=0.51$, $p>0.05$) (Table 2.5). The variability (±SD) of $\delta^{13}C$ DIC within a wetland was relatively small for most wetlands indicating that the $\delta^{13}C$ of DIC is uniform throughout the wetland (Table 2.5). Similar ratios were observed in temperate lakes of Quebec (-6.9‰) and New York (-7.2‰) (LaZerte and Szalados 1982; Mitchell et al. 1996). $\delta^{13}C$ DIC in catchment waters generally range from -5 to -25‰ (Bullen and Kendall 1998)

No significant relationship existed between $\delta^{13}C$ DIC and water chemistry parameters (Dissolved oxygen, temperature, salinity, pH, & specific conductivity) or DIC concentration (Videla 2006) (Regression analysis, $p>0.05$).

The DIC $\delta^{13}C$ ratios were more negative than those of aqueous CO_2 (0‰) (Boschker and Middelburg 2002), except in Mike's Pond, indicating that microbial respiration and organic matter decomposition likely play an important role in the carbon cycle of these wetlands. There was a significant interaction between DIC $\delta^{13}C$ and reclamation type and organic base where DIC $\delta^{13}C$ in OSPM-affected wetlands with poor organic sediments (Mike's Pond and Test Pond 9) approached 0‰ whereas DIC $\delta^{13}C$ was more

TABLE 2.5: Stable carbon isotope ratios for DIC from (a) the current study, (b) Videla (2006), and (c) DIC concentrations from Videla (2006) collected in the study wetlands.

Wetland Name	**Mean(±SD) DIC $\delta^{13}C$ (‰) (This study)**	**DIC $\delta^{13}C$ (‰) (Videla 2006)**	**DIC (ppm) Videla (2006)**
SW	-4.8±0.7	-5.1	39.8
TP9	-3.8±0.5	-3.0	131.1
CNRL	-8.0±0.1	-5.2	20.7
PP	-5.2±0.2	-5.5	34.5
4m-CT	-9.0±0.8	-6.5	133.8
MP	-0.6±0.4	-0.3	75.1
NW	-6.2±1.1	-6.2	115.6
HS	-7.8±1.9	-6.6	21.3
BP	-9.3±0.2	-10.4	55.2

Table 2.6 – A 3-way factorial ANOVA that examined the relationship between DIC $\delta^{13}C$ and wetland age class, organic base, and/or reclamation type

Effect	**D.F.**	**Sum of Squares**	**Mean Square**	**F**	**p**
Reclamation Type (R)	1	6.40	6.40	10.67	>0.05
Organic base (B)	1	18.09	18.09	30.15	<0.05*
Age (A)	1	0.04	0.04	0.07	>0.05
R*B	1	12.86	12.86	21.43	<0.05*
R*A	1	0.01	0.01	0.02	>0.05
B*A	1	0.05	0.05	0.08	>0.05
Discrepance	2	1.20	0.60		

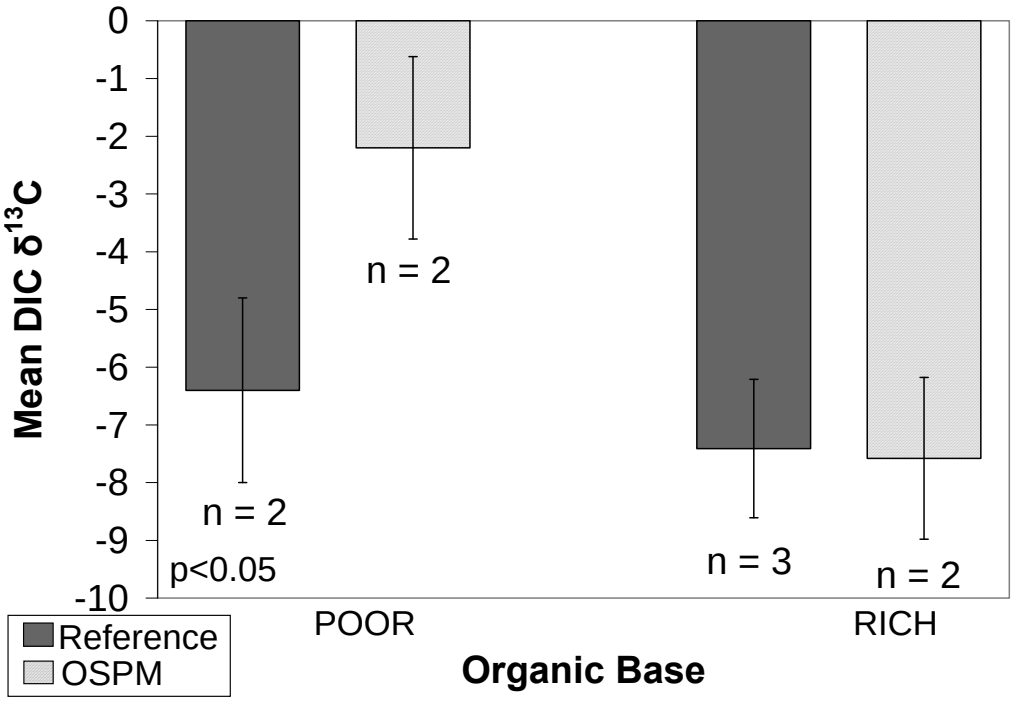


Figure 2.9 - Mean±SE DIC $\delta^{13}C$ in reference and OSPM-affected wetlands with rich or poor organic bases.

depleted in other wetland classes – OSPM wetlands with rich organic bases and reference wetlands with rich and poor organic sediments (Table 2.6, Fig. 2.9; 3-way factorial ANOVA, $p<0.05$). A greater proportion of DIC appears to be derived from microbial respiration and organic matter in most of the study wetlands, excluding OSPM wetlands with poor organic bases. DIC $\delta^{13}C$ ratios were influenced by the reclamation practice of adding peat amendments to wetland soils since the most depleted DIC $\delta^{13}C$ values were present in wetlands with rich organic bases. There was no significant difference between Mike's Pond DIC $\delta^{13}C$ and the $\delta^{13}C$ of aqueous CO_2 (0‰) (Independent samples t-test: $n=10$, $t = -1.44$, $p>0.05$). Test Pond 9 DIC $\delta^{13}C$ (-3.8±0.5‰) was the second closest value to aqueous CO_2. This indicates that the majority of inorganic carbon is derived from atmospheric CO_2 and microbial respiration and organic matter decomposition are minimal within OSPM- affected wetlands with poor organic bases. There was no relationship between DIC $\delta^{13}C$ and age (Table 2.6; 3-way factorial ANOVA, $p>0.05$).

The dissolution of carbonates (0±5‰) derived from sediments can also influence the wetland carbon cycle (Bullen and Kendall 1998), but DIC $\delta^{13}C$ ratios in sediment cores were not measured in the wetlands. Carbonate dissolution was probably not a major factor in at least half of the study wetlands (Peat Pond, High Sulphate, Beaver Pond, 4m-CT and Natural Wetland) because their sediment bases were composed of organic peat.

Technique Evaluation

Stable carbon isotope values of bacteria are commonly determined (Coffin et al. 1989, 1990; Kelly et al. 1998; Kritzberg et al. 2004) with the bacterial bioassay method which involves bacterial concentrates grown in 0.2-μm filtered water samples for short-term incubations (Coffin et al. 1989). An attempt was made to ascertain the $\delta^{13}C$ and $\delta^{15}N$ values of microbial communities in Fort McMurray wetlands by isolating the microbial component by filtering 3-L microbial bioassays on GF/F filters as described by Coffin et al. (1989). The quantity of microbial carbon and nitrogen was below detection limits, which implies that the microbial component (<1 μm) was either not prevalent, was associated with larger particles or the GF/F filters just diluted the microbial C and N

stable isotope signatures. This failed pilot study in 2004 led to the development of the biofilm technique later implemented in 2005.

The biofilm technique is a novel approach that can provide insight into the study of energy flow dynamics in an aquatic microbial community. It is especially useful for field studies for several reasons: (1) It involves the sampling of the natural microbial community; (2) It encompasses the whole assemblage and is not limited to assessment of a few species of bacteria, which is common practice in laboratory studies; (3) It provides a measure of actual activity at the time of sampling; (4) It takes into account potentially rapid changes in the microbial community in response to environmental factors, e.g. temperature, pH, water level fluctuations and wave action; and (5) A limited number of samples can provide an integrated view on organic matter cycling and microbial food-web relationships. The last point was especially valid for the study wetlands since not enough microbial biomass could be collected by the microbial bioassay method (Coffin et al. 1989), but enough microbial biomass for C and N isotope measurements could be obtained. Also, after the initial study setup (inserting tubes into wetland sediments and waiting biofilm development – 30 days) collecting microbial biofilm was rapid and easy compared to the bioassay method. Suspended particulate matter in the wetlands, especially in the process water of OSPM-affected wetlands, made filtering extremely slow and tedious. It sometimes took up to 6 hours to filter 3-L of wetland water through 0.2 μm filters. Considering 3 replicates per wetland and 9 wetlands in this study I found the biofilm technique I developed preferable to the bioassay method in terms efficiency and collecting sufficient microbial biomass.

The biofilm technique may be susceptible to error if POM is associated with biofilm. However, differences between microbial and phytoplankton $\delta^{13}C$ in the majority of study wetlands indicate that microbial biofilm does not contain a significant non-microbial component. Furthermore, the C:N ratios of microbial biofilm (8.3 to 13.4) were similar to the characteristic C:N ratio for microbial biomass (10±2) (Flanagan et al. 2005) (Appendix 2.3). One limitation to this methodology is that the pipes designed to collect heterotrophic microbial biofilm may alter the environment, leading to suspect stable

isotope C and N ratios for bacteria. However, holes in the 2 exterior pipes permitted water flow around the inner pipe, thereby facilitating the attachment of free-floating microbes and development of a biofilm which was later collected for stable isotope analysis. If conditions were anoxic enough, methanogenic bacteria could have developed and biofilm $\delta^{13}C$ may have been considerably depleted since $\delta^{13}C$ values for methane produced from anaerobic sediments typically range from -50 to -100‰ (Whiticar et al. 1986). In CNRL, a reference wetland, and Mike's Pond, an OSPM-affected wetland, microbial $\delta^{13}C$ values (-23.80±0.48‰ and -22.72±2.95‰, respectively) were considerably enriched compared to the other wetlands (Table 2.2) suggesting that methanogenesis did not impact microbial biofilm in these wetlands. Methanogenesis is inhibited by elevated salinity and sulphate concentrations, characteristic of OSPM-affected wetlands (chapter 4). However, CNRL, the reference wetland with low salinity (0.1 ppt) and likely low sulphate concentrations (not measured), showed no evidence of methanogenesis in microbial biofilm. If the environment was altered inside the pipes in which biofilm formed I would have expected much depleted biofilm $\delta^{13}C$ characteristic of methanogenesis, especially in reference wetlands where conditions do not appear to inhibit methane production (chapter 4).

Wetland Food Web

The microbial component of the food web is a major food resource for metazoans (Sherr and Sherr 1988) and can account for a significant amount of the biomass, respiration and nutrient recycling, and productivity in both marine and freshwater ecosystems (Azam et al. 1983; Cochrna-Stafira and von Ende 1998; Sherr and Sherr 1988). There is strong evidence that this also pertains to reference and oil sands-affected constructed wetlands in the Athabasca oil sands region.

Ganshorn (2002) used stable isotopes to assess the bioaccumulation potential of PAHs in benthic and pelagic macroinvertebrates in reference and OSPM-affected wetlands in the Athabasca Oil Sands. He determined that organisms in OSPM-affected wetlands had $\delta^{13}C$ signatures close to -30 ‰, suggesting a petroleum derived carbon source. In Natural

Wetland, small chironomids ($\delta^{13}C$: -28.9‰, $\delta^{15}N$: 15.6‰) (Ganshorn 2002), which commonly consume phytoplankton, protozoa and detritus, were nearly 2 trophic positions higher than the microbial community ($\delta^{13}C$ -26.9‰, $\delta^{15}N$ 9.8‰). My research in this OSPM wetland indicates that the microbial carbon and nitrogen derived from primary producers may be conserved in the higher organisms, protozoans and ultimately chironomids. Ganshorn (2002) indicated that macroinvertebrates in reference wetlands had dietary compositions reflecting autochthonously produced carbon. In High Sulphate Wetland, a reference wetland, *Daphnia* ($\delta^{13}C$ -25.9‰, $\delta^{15}N$ 2.64‰) (Ganshorn 2002) which commonly consume phytoplankton, protozoa and detritus were nearly 2 trophic positions higher than the microbial community ($\delta^{13}C$ -26.4‰, $\delta^{15}N$ -3.2‰). Therefore, the diet of microbes appears to be conserved in some higher trophic organisms. Since the $\delta^{13}C$ signature of the microbial community appears to be conserved in some higher trophic organisms, it is possible that OSPM-derived elements assimilated into microbial biomass, such as in Test Pond 9, may also be conserved in higher trophic levels.

Conclusions

1) According to the mixing model the calculated proportions of carbon assimilated in microbial biofilm from OSPM-affected wetlands, Test Pond 9, 4-m CT Wetland and Natural Wetland were 68%, 62% and 97% petroleum confirming that anthropogenic carbon sources were incorporated into microbial biomass in OSPM-affected wetlands.

2) Results from all the reference wetlands were problematic since microbial biofilm fell outside of the mixing triangle, leading to nonsensical negative biomass estimates for primary production, anthropogenic and sediment carbon sources. Mean microbial biofilm $\delta^{15}N$ values in all reference wetlands (-4.08±1.4 (mean±SD) to -0.10±0.7‰) were more negative than their potential sources (2.63±0.05 to 12.12±0.01‰) indicating that a nitrogen source common to reference wetlands may have been overlooked. Nitrification may play an important role in nitrogen dynamics in reference wetlands.

3) The mature biofilm communities were similar to young biofilm in an OSPM-affected wetland suggesting that heterotrophic bacteria may dominate microbial biofilm in these wetlands. In contrast, the mature biofilm communities appear to be different

from young biofilm in a reference wetland suggesting that the natural (mature) biofilm likely contains abundant photosynthetic algae in this reference wetland, Peat Pond.

4) $\delta^{15}N$ enriched values (3.23±1.56 to 11.63±5.95‰) characteristic of volatization and denitrification were quantified in microbes and other wetland biota in oil sands constructed wetlands. Either the addition of amines during the bitumen extraction procedure or the addition of ammonia-rich wastewaters to settling basins would stimulate denitrification and/or volitization in mine process waters, which were later used in wetland construction. Therefore, past microbial mediated nitrogen dynamics appear to still be conserved in wetland biota.

5) Further isotope studies are required in oil sands constructed wetlands where nitrogen transformation appear to obscure microbial $\delta^{15}N$ origins. A dual $\delta^{15}N$ and $\delta^{18}O$ approach offers the potential to identify changes to the N stable isotope via mineralization, nitrification, industrial effluent and atmospheric deposition.

6) The deviation of dissolved inorganic carbon $\delta^{13}C$ values (-9.3±0.2 to -0.6±0.4‰) from atmospheric origins (0‰) indicates that microbial respiration and organic matter decomposition play an important role in the majority of the study wetlands.

7) The novel stable isotope microbial biofilm technique used in this study is a useful procedure for monitoring carbon flow dynamics in the natural microbial communities of aquatic systems.

8) There is strong evidence that carbon assimilated into the cellular biomass of microbes is conserved in higher trophic organisms (small chironomids and *Daphnia)*. The results of this study indicate that the microbial component of the food web may be an important link to higher trophic levels in these wetland ecosystems.

9) Microbial assimilation of isotopes in Test Pond 9, 4-m CT Wetland and Natural Wetland is most consistent with a petroleum source of carbon. If this is true, it implies that oil sands mine-process derived carbon sources can fuel the microbial component of the food web in oil sands constructed wetlands. Therefore, the potential exists for OSPM-associated carbon sources to be incorporated into the biomass of higher trophic levels.

CHAPTER III:
PLANKTONIC BACTERIAL COMMUNITY PRODUCTION IN OIL SANDS-AFFECTED WETLANDS OF NORTHEASTERN ALBERTA, CANADA

Abstract

Planktonic bacterial community biomass and production were estimated in 9 constructed wetlands of varying ages and reclamation treatments. Bacterioplankton secondary production was quantified by monitoring the incorporation rate of ^{3}H-leucine into proteins in filtered water samples collected from each wetland in August 2004 and July and August 2005. Mean±SE bacterial production and biomass in the study wetlands ranged from 0.03±0.03 to 2.44±1.50 μg C L^{-1} h^{-1} and 1.13±1.43 to 6.04±2.22 μg C L^{-1}, respectively. Bacterial production was 5 times greater in reference wetlands than in oil sands process material (OSPM)-affected wetlands, indicating that compounds, possibly salinity, in the mine-process materials inhibited microbial activity. Low biomass measurements relative to those reported for other freshwater systems may reflect low dissolved organic matter (DOM) availability, top-down regulation, inhibition by viruses, or the size fraction examined. Elevated bacterial biomass in young reference wetlands (<7 years) compared to the other study wetlands suggests that initial reference wetland conditions were more favourable for the natural bacterial community, perhaps as a result of elevated algal production or predation limitation in older reference wetlands older (≥ 7 years). Biomass in older (≥ 7 years) OSPM wetlands was 2.2 times greater than in younger (< 7 years) OSPM wetlands and 3.2 times higher than in older reference wetlands. Perhaps naphthenic acids (NAs) in young wetlands are acutely toxic, whereas NAs in the older wetlands permits bacterial persistence (possibly tolerant species), albeit at lower levels of growth than occurs in reference wetlands of equivalent age. DOC was weakly positively correlated with biomass, suggesting that carbon availability may limit bacterioplankton community. Overall, bacterioplankton biomass in all the study wetlands (3.10±1.93 μg C L^{-1}; ^{n}=8) was higher than quantified in an unconstructed reference wetland (1.13±1.43 μg C L^{-1}; n=1), indicating that bacterial numbers are not atypical in the oil sands affected wetlands. Amending wetlands sediments with peat, as a

reclamation strategy, does not appear to affect bacterioplankton community production and biomass and or the potential carbon transfer to higher levels in the aquatic food webs.

Introduction

Modification and decomposition of oil sands mining by-products by microorganisms may stimulate the cycling of elements within the aquatic food web. The measurement of bacterial secondary production is therefore important in elucidating bacterial carbon cycling and the role anthropogenic carbon sources may play in what is referred to as "the microbial loop" (Azam 1983) in oil sands-affected constructed wetlands.

Bacteria are numerically dominant biota and thus constitute an important component of aquatic systems (Cole 1982, Pace and Cole 1996). They are characterized by high cell counts and the capacity for rapid rates of reproduction (Sigee 2005). Bacterial productivity, defined as "the intrinsic rate of increase in biomass in a [bacterial] population", is an important component of biomass formation and carbon transfer in freshwater microbial food webs (Sigee 2005). Dissolved organic matter (DOM) that would otherwise be lost from the food web because of excretion, exudation, and diffusion can be recovered by microbial uptake and metabolism in the microbial loop (Azam 1983).

Bacterial biomass production is commonly measured indirectly using the [^{3}H]-leucine method (Bernard et al. 2000, Cole and Pace 1995; Daniel et al. 2005, Ducklow 2000, Jorgensen 1992a, Jorgensen 1992b, Kirchman et al. 1985). One of the reasons this method has been widely adapted by microbial ecologists is that it is specific for heterotrophic bacteria (Kirchman 2001) because heterotrophic bacteria outcompete autotrophic organisms (i.e. phytoplankton and cyanobacteria) for nanomolar concentrations of ^{3}H-leucine added during experiments (Kirchman et al. 1985; Buesing and Gessner 2003). Furthermore, protein turnover, which could lead to overestimates of bacterial production, is thought to be negligible in prokaryotes (Kirchman 2001). Kirchman et al. (1986) did not find substantial protein turnover rates in bacteria from an aquatic environment. Finally, ^{3}H-leucine additions do not appear to change protein synthesis rates (Simon and Azam 1989; Buesing and Gessner 2003).

The [^{3}H]-leucine method monitors the rate of incorporation of a radiotracer into proteins thereby quantifying protein synthesis rates, which are then converted to production rates using empirically-derived conversion factors (Ducklow 2000). A conversion factor is a multiplication factor (bacterial cells/pmol) that lets one convert μg of radiolabelled leucine taken up by bacterial proteins into the estimated amount of carbon (or biomass) taken up by a known biomass of bacteria. Preliminary saturation studies determine the concentration at which extracellular ^{3}H-leucine additions inhibit de novo synthesis of leucine by bacterial communities in aquatic environments (Kirchman et al. 1985). Isotope dilution is the contribution of unlabeled leucine derived from the water column or synthesized during incubation that increases the amount of leucine beyond what was added by the investigator (Cole and Pace 1995). Bacterial carbon production would be underestimated if intracellular isotope dilution was not accounted for (Jorgensensen 1992b). Saturation studies add increasing concentrations of ^{3}H-leucine and note the concentration at which ^{3}H-leucine incorporation reaches a maximum (Kirchman 2001). Rates of protein synthesis, and thus bacterial production, would be underestimated if biosynthesis of leucine by the bacterial community occurred in natural waters (Kirchman et al. 1985).

The biomass of bacterial populations in aquatic ecosystems is commonly estimated by the 4', 6-diamidino-2-phenylindole (DAPI) direct count method (Porter and Feig 1980; Friedrich et al. 1999; Kuehn et al. 2000; Klammer et al. 2002; Muylaert et al. 2003) using abundance and biomass conversion factors (i.e. Bratbak 1985; Lee and Fuhrman 1987; Simon and Azam 1989). Microbial ecologists widely use this approach because the fluorescent DAPI stain distinguishes between bacteria and nonliving particles, such as sediment and organic debris, because it is highly specific for DNA (Porter and Feig 1980). When excited with a light at a wavelength >390 nm, the DNA-DAPI complex fluoresces a bright blue, whereas unbound DAPI and DAPI bound to non-DNA material may fluoresce a weak yellow (Porter and Feig 1980).

Estimates of microbial production are a measure of microbial activity (Kirchman 2001). They can also characterize the importance of the microbial loop since bacterial carbon

production is a reflection of the richness (i.e., nutritive value) of DOM available to microbes and an index of the suitability of the environment (Billen et al. 1990; Ducklow 2000) for the microbial loop. In the case of the heterotrophic bacterial community, richness can be related to the flux of available organic matter resources (Billen et al. 1990), which can originate from natural and/or anthropogenic origins. Organic matter can be taken up by bacteria at a rate that approximates bacterial production (Billen at al. 1990). Therefore, the rate of bacterial utilization of organic matter or bacterial production can be considered a good index of richness of dissolved organic matter and the suitability of the environment (Billen et al. 1990) for the heterotrophic bacterial community.

The objectives of the study were to

(1) determine the concentration of extracellular ^{3}H-leucine required to repress leucine biosynthesis in wetland bacterial communities (Study 1 – leucine saturation);
(2) quantify a conversion factor (bacterial cells/pmol) that relates ^{3}H-leucine incorporation to bacterial production in the Athabasca wetlands (Study 2 – estimating conversion factors); and
(3) assess the ecological suitability of reclaimed wetlands with varying reclamation treatments and of different ages for the microbial loop by examining the rate of incorporation of ^{3}H-leucine into heterotrophic bacterial proteins as a measure of bacterial secondary production and the activity of the microbial loop in the wetland water column (Study 3 – determining bacterial production).

To our knowledge, bacterioplankton production has only been used in a few studies to measure the effects of mine-derived compounds on the bacterial community in aquatic systems (Klammer et al. 2002), and no studies of this nature have been carried out in wetland systems.

Methods

Field Collection

For the saturation study, one 20-L water sample was collected from one randomly selected location near the shoreline in each of two wetlands (Mike's Pond, an OSPM-affected wetland and High Sulphate, a reference wetland) on August 10, 2004 (Table 2.1). For the conversion factor study, one 20-L water sample was collected from one randomly selected location near the shoreline in the same wetlands on July 22, 2005 (Table 2.1). For the bacterial production study, triplicate 20-L water samples were collected from randomly selected locations near the shoreline in each of the 9 wetlands differing in reclamation type, age and organic base to determine bacterial production between July 28 and August 1, 2005 (Table 2.1).

Water samples were collected in 20-L carboys approximately 10.0-15.0 cm below the water surface and from the open water, pondward to the emergent vegetation. Carboys were placed mouth downward approximately half-way beneath the water surface. The cap was removed and the carboys were slowly rotated 180 degrees until the neck was parallel with the water surface. As the carboy filled the container was rotated upward. When full, the carboy was capped under water. The approximate water depth at which 20-L water samples were collected in wetlands varied between 0.5 and 1.0 m. Samples were transferred to the laboratory and processed within 2 h of collection. Contemporaneously with the water sampling, environmental variables including specific conductivity, pH, dissolved oxygen content (DO) and temperature were measured *in situ* with standard instruments (YSI). Additional abiotic and nutrient variables (naphthenic acids (NAs), NH_4, SO_4, CO_3, HCO_3, $CaCO_3$, total cations, total anions, and the following ions: Na, K, Mg, Ca, F, Cl, Al, B, Ba, Fe, Mn, No, Ni, Se, Si, Sr, Ti, V, Zn, and Zr were determined from 1- L water samples collected separately from 20-L samples (Appendix 2.5). Water samples were stored at 4^{o}C until they were sent to the Syncrude Canada Analytical Research Lab in Edmonton, Alberta for determinations of NAs, NH_4, SO_4, CO_3, HCO_3, $CaCO_3$ and ions (MacKinnon 2005, Syncrude Canada Ltd., Pers. Comm.).

The NAs, NH_4, and most ions (i.e. Na^+, Ca^{2+}, Mg, 2^+, and Fe) were determined using standard industry methods (methods in Syncrude 1995). NAs were obtained from water samples by performing an acid-base extraction and determined by FT-IR spectroscopy (Holowenko et al. 2001). Water was analyzed for chloride, sulphate and ammonium by ion chromatography and for bicarbonate by titration with 0.5 HCl (Holowenko et al. 2000). Cations were analyzed by an Inductively Coupled Argon Plasma Atomic Emission spectrometer (Holowenko et al. 2000).

Leucine saturation and conversion factors were determined in only two wetlands each as it was impracticable to determine these measurements in all 9 study wetlands. I assume that quantities of radioisotopes added maximized incorporation rates were comparable among wetlands. Furthermore, I assume that the mean of the 2 empirically derived conversion factors is appropriate to apply to the 7 wetlands for which conversion factors were not estimated. It is possible that using the mean conversion factor may lead to invalid bacterial production estimates. However, the similarity between conversion factors in contrasting wetlands, Peat Pond which is a young reference wetland with rich organic sediment (1.03×10^5 cells/pmol) and Shallow Wetland, an older reference wetland with poor organic sediments (2.01×10^4 cells/pmol), and the similarity of conversion factors with similar aquatic environments (see discussion) indicates that using the mean conversion factor is appropriate.

Table 3.1 – The number and volume of samples collected in each study for quantifying leucine incorporation.

Study	**Wetlands Sampled**	**No. Wetlands Sampled**	**No. 20-L Carboys collected per wetland**	**No. 1-mL subsamples per wetland, excluding controls**	**No. 1-mL controls per wetland**
Study 1: Leucine Saturation	Mike's Pond; High Sulphate	2	1	18	3
Study 2: Estimating Conversion Factors	Peat Pond; Shallow Wetland	2	1	8	1
Study 3: Determining Bacterial Production	Mike's Pond; 4m-CT; Test Pond 9; Natural Wetland; CNRL; Peat Pond; Shallow Wetland; High Sulphate; Beaver Pond	9	3	6	1

Study 1: ^{3}H Leucine Saturation

One L of water from the single, 20-L sample collected in each wetland (n=2) was filtered through 1.0-µm pore size membrane filters (25 mm diameter) to remove eukaryotes and other organic debris (Table 3.1). To save time, the entire 20-L sample was not filtered since only 1-mL subsamples were required for the next step in the procedure. Saturation curves of leucine incorporation into protein were determined by adding increasing amounts of L-[4,5-^{3}H(N)]-Leucine (59.5 Ci/mmol; PerkinElmer Life Sciences, Inc., Boston, MA) to 1-mL wetland water samples (Kirchman et al. 1984) (Fig. 3.1). Duplicate 1-mL subsamples for each of 9 ^{3}H-leucine concentrations tested (10, 15, 20, 25, 30, 35, 40, 45 and 50 nM) per wetland were removed from the filtrate. Tritiated leucine concentrations (10-50 nM) were added to separate subsamples and incubated for 1 h at ambient temperatures. Incubations were stopped after 1 h by adding enough formaldehyde (100% formalin solution) to obtain a 5% final concentration. Triplicate controls per wetland were 1-mL aliquots from the filtrate to which formaldehyde (5% final concentration) was added immediately after the addition of tritiated leucine. The formaldehyde-preserved samples were stored in darkness at room temperature for approximately two months until ^{3}H-labeled proteins were extracted (See Recovery of ^{3}H-labeled Proteins).

Study 2: Estimating Conversion Factors

Conversion factors were calculated so that ^{3}H-Leu incorporation rates could be related to microbial production (i.e., rates of carbon fixation per unit time). They were calculated by comparing the increase in bacteria biomass over time with the rate of incorporation of tritiated leucine (Kirchman and Ducklow 1993) (Fig. 3.2, Table 3.2 & 3.3; Appendix 3.1 & 3.2). To obtain an absolute measure of bacterial biomass growth, processes that lead to bacterial biomass loss, such as grazing and viruses, were eliminated. The majority of grazers were assumed to have been removed by filtering 100 mL of water from the 20-L water sample through 1.0-µm membrane filters per wetland. A 900- mL aliquot of water from the 20-L water sample was filtered once through 0.2-µm membrane filters per

wetland. The potential for bacterial destruction by viruses was reduced by diluting an inoculum of 100 mL of 1.0 μm-filtered water with 900 mL of 0.2 μm-filtered wetland water per wetland. A total of only one out of 20 L of wetland water was filtered to save time since only 1-mL subsamples of filtrate were required in the following steps. Duplicate 1-mL subsamples were withdrawn at 0, 4, 8, and 24 h from the diluted water per wetland to measure leucine incorporation rates and between-sample variability (Table 3.1). Simultaneously, duplicate 10-mL subsamples were taken at each time point per wetland and preserved (5% formalin v/v) for later determination of bacterial abundance. At each time point ^{3}H-Leu was added to duplicate 1-mL subsamples at a concentration that maximized leucine incorporation, as determined in study 1 (final concentration 40 nM; Fig. 12). The incubation was stopped after 1 h by adding enough formaldehyde (100% formalin solution) to obtain a 5% final concentration. One control per wetland was created by removing a 1-mL subsample from the 1- L diluted wetland water at time 0 h to which formaldehyde (5% final concentration) was added immediately after the addition of tritiated leucine. The formaldehyde-preserved samples were stored in darkness at room temperature for approximately one month until ^{3}H-labeled proteins were extracted (See below).

Conversion factors (CF) were calculated using the equation

$CF = \mu N(0)/v(0)$ (Kirchman 1983) (Table 3.2)

where μ is the specific growth rate of the bacteria community(cells/mL/h) determined from the slope of the logarithmic plot of abundance (cells/mL) vs. time (h) (Fig. 3.2), N(0) is the initial bacterial abundance and v(0) is the initial incorporation rate (pmol/mL/h) (Table 3.3).

Study 3: Determining Bacterial Production

Leucine incorporation rates (pmol/mL/h) were measured according to a method modified from Kirchman and Ducklow (1993). One L of water from the 20-L water samples

collected at triplicate locations in each wetland was filtered through 1.0-μm membrane filters to ensure that measured biological activities were due to bacterial processes rather than to eukaryotic dynamics (Table 3.1; Appendix 3.3). For each sampling location, two 1.00-mL replicate samples were withdrawn from each filtered sample for determination of bacterial biomass production and between-sample variability. In addition, a 10.0-mL subsample was taken from each filtered sample (3 per wetland) and preserved (5% formaldehyde final concentration) for later determination of microbial abundance and between-sample variability. ^{3}H-Leu was added to all 1-mL subsamples (6 per wetland) at a concentration that maximized leucine incorporation, as determined in study 1 (final concentration 40 nM; Fig. 12). The incubation was stopped after 1 h by adding enough formaldehyde (100% formalin solution) to obtain a 5% final concentration. Nine controls (one per wetland) were 1-mL subsamples taken from 1.0-μm-filtered water in the first replicate in each wetland to which formaldehyde (5% final concentration) was added immediately after the addition of tritiated leucine.

The formaldehyde-preserved samples were stored in darkness at room temperature for approximately one month until ^{3}H-labeled proteins were extracted (see below). Leucine incorporation rates (pmol/mL/h) were then converted to bacterial biomass production (Table 3.4) with the empirically-derived conversion factors (6.14×10^{4} cells/pmol) from study 2 (see below) and a standard conversion factor which converts cell numbers to biomass assuming a cellular carbon content of 20 fg of C cell^{-1} (Lee and Fuhrman 1987). This value is a standard conversion factor and has been commonly applied to natural bacterial assemblages (Karner and Herndl 1992; Hoppe et al. 1993; Cambell et al. 1994; Biddanda et al. 2001).

Recovery of ^{3}H-labeled Proteins

Labeled protein from samples was extracted by heating each vial containing a sample to 80^{o}C in a water bath for 15 min, and then cooling to room temperature on ice. The resultant precipitated extract was filtered through cellulose nitrate membrane filters (0.2-μm, 25 mm diameter, Whatman), washed twice with 3 mL cold 5% trichloroacetic acid

(TCA), and then twice with 2 mL cold 80% ethanol. The filter was placed in a 20-mL uncapped scintillation vial, and allowed to dry for 30 min at 70^{o}C. 0.5 mL of ethyl acetate was added to dissolve the filter and thus increase ^{3}H counting efficiency. Ten mL of Scintiverse® scintillation cocktail was added, the scintillation vial capped and the subsample was radioassayed. Radioactivity was measured for 10 min per sample, using a liquid scintillation analyzer (Packard Tri-Carb 2900TR). Counts per minute (cpm) were converted to disintegrations per minute (dpm) with appropriate quench corrections established separately from the investigated water samples (Appendix 3.4).

Study 4: Estimating Microbial Abundance using Microscopy

Bacterial abundances in samples prepared for studies 2 & 3 were enumerated by the 4', 6-diamidino-2-phenylindole (DAPI) direct count method (Porter and Feig 1980) (Table 3.4; Appendix 3.5). Briefly, cells from a 1.00-mL aliquot of each 10.0-mL sample were stained with DAPI, filtered onto 0.2-µm black membrane filters and counted using epifluorescence microscopy. The cells were counted at 670X magnification on a Zeiss epifluorescent microscope. At least 100 cells were counted on each filter. Cell numbers were converted to biomass assuming a cellular carbon content of 20 fg of C cell^{-1} (Lee and Fuhrman 1987). This value is a standard conversion factor and has been commonly applied to natural bacterial assemblages (Karner and Herndl 1992; Hoppe et al. 1993; Cambell et al. 1994; Biddanda et al. 2001). I applied a constant factor rather than a volume-dependent one since Lee and Fuhrman (1987) determined only comparatively small variation in carbon content over relatively wide range in volume.

Statistical Analysis

Principal Components Analysis (PCA) (Statistica 6.0) was used to combine environmental, abiotic and nutrient variables (pH, specific conductivity, salinity, temperature, DO, NAs, DOC, NH_4^+, Na^+, K^+, Mg^+, Ca^+, Cl^-, SO_4^-, HCO_3^-, B^+, Fe^+, Si^+, Sr^+) into indices of salts, abiotic factors, and DOC availability, which explained 34.8%, 24.8%, and 30.3% of the total variability, respectively. Any environmental, abiotic and

nutrient variables that did not conform to a normal distribution (Kolmogorov-Smironov test, $p > 0.05$) were log transformed to remove heteroscedasticity. Some parameters were not measured in CNRL (NAs, pH, NH_4^+, Na^+, K^+, Mg^+, Ca^+, Cl^-, SO_4^-, HCO_3^-, B^+, Fe^+, Si^+, Sr^+) and Shallow wetlands (NH_4^+, Na^+, K^+, Mg^+, Ca^+, Cl^-, SO_4^-, HCO_3^-, B^+, Fe^+, Si^+, Sr^+). The mean of these parameters in other reference wetlands was used in the PCA analysis for CNRL and Shallow wetlands. There were more variables than cases, so only 8 variables (pH, conductivity, salinity, temperature, DO, NAs, DOC and Fe) were used in the first PCA since they were deemed most likely to affect productivity. The other variables were included in additional PCA to see if they loaded onto factors determined in the primary PCA (salts, abiotic factors and DOC availability) (Fig. 3.5 to 3.7).

Parametric statistical tests (Statistica 6.0) were used to evaluate the results of experiments since bacterial production, abundance and biomass values conformed to a normal distribution (Kolmogorov-Smironov test, $p > 0.05$). Three-way factorial analyses of variance (ANOVA) were used to test whether bacterial measures (production, abundance and biomass) were significantly influenced by wetland age class (young vs. older), organic base class (unamended vs. enriched with carbon sediments), and/or reclamation type (OSPM-affected vs. unaffected). Error sum of squares and the three-way interaction sum of squares were pooled and called discrepance. Each treatment mean square was divided by discrepance, thereby increasing the power of this unreplicated ANOVA. Production was log-transformed to remove heteroscedasticity. Biomass and abundance were not log transformed since they conformed to a normal distribution (Kolmogorov-Smironov test, $p > 0.05$). The mean of the 6 replicates per wetland was used in the ANOVAs.

Simple linear regression analyses were used to estimate the relationship between dependent variables (bacterial production, abundance and biomass) and independent variables (salts, abiotic factors, and DOC) separately. No transformations were necessary since salts, abiotic factors, and DOC conformed to a normal distribution (Kolmogorov-Smironov test, $p > 0.05$). The significance level adopted for statistical tests was $\alpha = 0.05$.

Results

Physico-chemical characteristics

Detailed physico-chemical measurements of each study wetland at the end of July 2005 are provided in Appendix 2.2. Surface water temperatures ranged from 16.1 to 20.4^{o} C. The pH in these wetlands varied between 7.3 and 9.0. DO values were between 5.3 and 11.3 mg/L. Salinities ranged from 0.2 to 2.5 ppt, and conductivities ranged from 245 to 4660 µS. The values measured in OSPM wetlands ranged from 1330 to 4660 µS, whereas reference wetlands conductivities varied between 245 and 2910. The concentrations of DOC were between 23.0 and 73.2 ppm (Videla 2006). The range of covariates among wetland classes was consistent with other studies performed in the same wetlands (Ganshorn 2002; Leonhardt 2003).

Study 1: ^{3}H Leucine Saturation

The concentration of extracellular ^{3}H-leucine required to repress leucine biosynthesis in wetland bacterial communities was measured in High Sulphate and Mike's Pond. Initially there were duplicate subsamples for each of the 9 tested concentrations per wetland (Total=18 replicates/wetland). However, upon transport of samples from Fort McMurray, to Windsor, Ontario some sample vials opened and were lost. The remaining vials totaled 9 replicates per wetland.

The concentrations of leucine at which rates of leucine incorporation into bacterial protein became asymptotic (i.e., maximum rate of uptake) occurred at similar concentrations in the two wetlands (Fig. 3.1). The asymptotic rate of leucine uptake was approximately 220 pmol/L/h. The asymptotes were reached at leucine concentrations of approximately 35 and 40 nM in High Sulphate and Mike's Pond, respectively. Asymptotes were clearly defined in both wetlands and the maximum rate of leucine uptake into bacterial proteins in both wetlands was established with the just the remaining

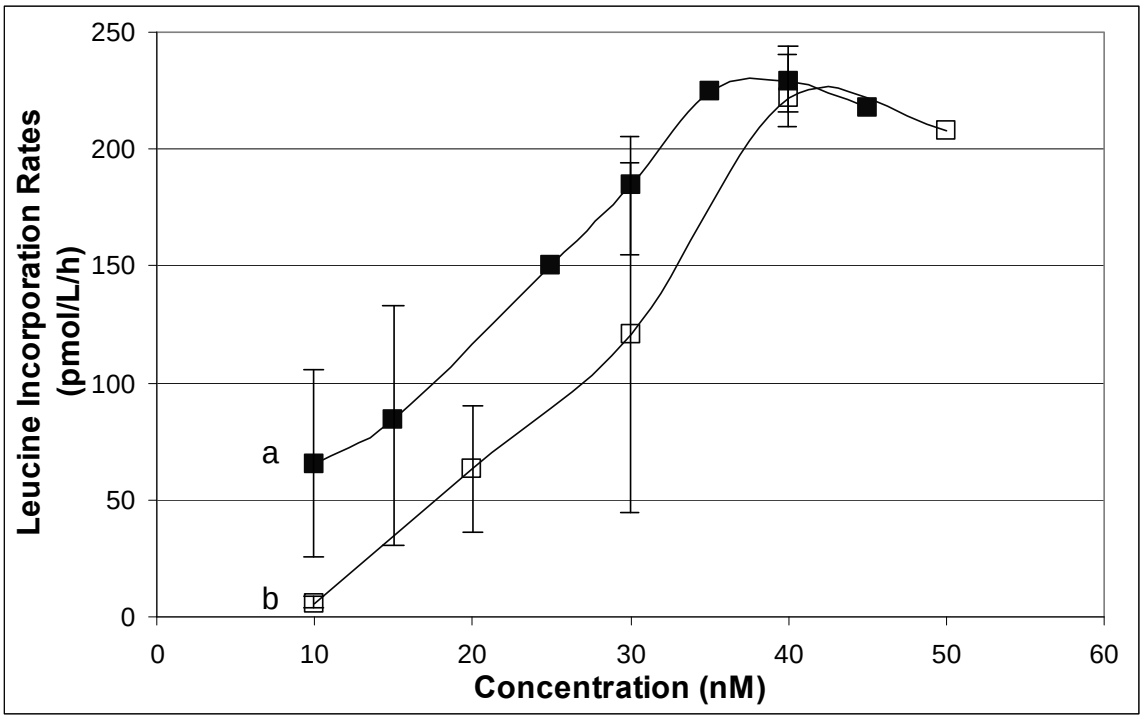


Figure 3.1 – Incorporation of ^{3}H-leucine into bacterial proteins from High Sulphate Wetland (■) and Mike's Pond (□) as a function of concentration (standard errors [±1 SE] of duplicates are given where applicable). A smoothed line was drawn through the means by Microsoft Excel.

samples. Therefore, loss of some samples did not appear to compromise the results.

Study 2: Conversion Factors

Conversion factors (CF) were calculated using the equation

$$CF = \mu N(0)/v(0) \qquad \text{(Kirchman 1983)}$$

where μ is the specific growth rate of the bacteria community(cells/mL/h) determined from the slope of the logarithmic plot of abundance (cells/mL) vs. time (h) (Fig. 3.2), N(0) is the initial bacterial abundance and v(0) is the initial incorporation rate (pmol/mL/h) (Table 3.3). The measured conversion factors in Mike's Pond and High Sulphate were 1.03×10^5 cells/pmol and 2.01×10^4 cells/pmol, respectively (Table 3.2). A mean of the 2 conversion factors, 6.14×10^4 cells/pmol, was used to convert leucine incorporation to bacterial production in the other study wetlands.

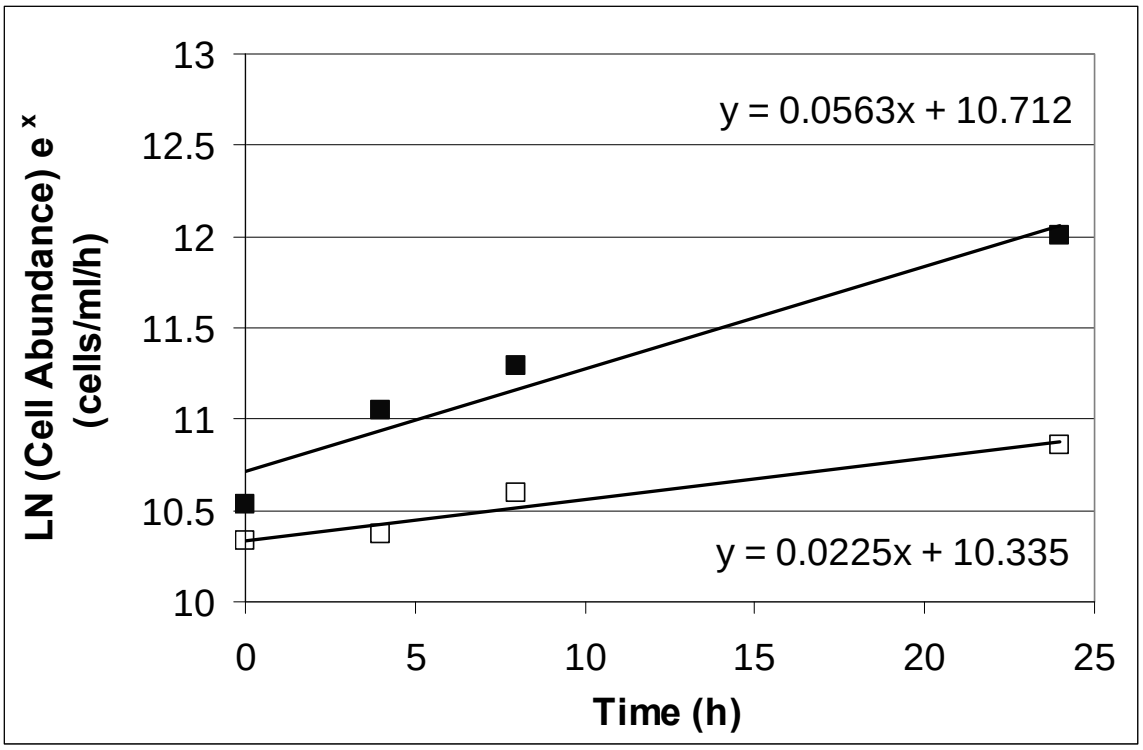


Figure 3.2 – Logarithmic plot of microbial abundance in filtered samples incubated over time in Peat Pond (■) and Shallow Wetland (□).

TABLE 3.2: Parameters used to calculate conversion factors in Peat Pond and Shallow Wetland.

Parameters	**Measured Values**	
	Peat Pond	**Shallow Wetland**
u	0.06	0.02
N(0)	37,664±11,863	30669±26,168
v(0)	0.02±0.00	0.03±0.03
CF	1.03×10^5	2.01×10^4

TABLE 3.3: Bacterial abundance and leucine incorporation rates in Peat Pond and Shallow Wetland during the conversion factor experiment.

Wetland	Time (h)	Abundance (cells/ml)	Leucine Incorporation Rate (pmol/ml/h)
PP	0	37,664±11,863	0.021±0.003
PP	4	62,773±33.554	0.042±0.014
PP	8	79,991±55,846	0.088±0.029
PP	24	163,210±99,162	0.156±0.173
SW	0	30,669±26,168	0.035±0.032
SW	4	31,835±33,190	0.092±0.101
SW	8	39,816±50,862	0.804±0.085
SW	24	52,012±33,410	0.651±0.090

Study 3: Bacterial Production

Bacterial production was compared among wetland treatments to evaluate the variation in biomass due to anthropogenic effects (OSPM present or absent), reclamation strategies (sediment amendment with organic materials or not) and age (younger vs. older), and to estimate the contribution of bacterial carbon potentially available to bacterivores. The rate of bacterial production measured in incubation experiments varied between wetland classes according to physico-chemical properties. Production ranged from 0.03±0.03 to 2.44±1.50 µg C/L/h or 0.68 to 58.67 µg C/L/d (Table 3.4).

There was no significant relationship between mean bacterial production and age, organic base or reclamation status (3-way factorial ANOVA; Table 3.5, Fig. 3.3). However, there was a trend whereby mean bacterial production was approximately five-fold higher in reference wetlands compared to OSPM wetlands (Fig. 3.4, Table 3.5, One-way ANOVA; $p=0.10$).

Table 3.4: Mean (±SD, n= 6) bacterial production and mean (±SD, n=3) biomass as determined by leucine incorporation and cell counts in the study wetlands.

Wetland	Leucine Incorporation		Cell Counts		Wetland Status	Age (y)	Organic Base
	Mean±SD Abundance (cells/mL/h)	Mean±SD Production (µg C/L/h)	Mean±SD Abundance (cells/mL)	Mean±SD Biomass (µg C/L)			
PP	$1.22x10^5±4.92x10^4$	2.44±1.50	$6.44x10^4±1.64x10^4$	1.29±0.33	Reference	Young	Rich
BP	5.48x104±2.60x104	1.09±0.47	$5.66x10^4±7.15x10^4$	1.13±1.43	Reference	Older	Rich
NW	$8.01x10^3±1.63x10^4$	0.16±0.30	$2.81x10^4±3.26x10^4$	5.61±4.73	Reference	Older	Rich
HS	$1.03x10^4±1.16x10^4$	0.21±0.23	$7.66x10^4±7.01x10^4$	1.53±1.40	Reference	Older	Rich
CNRL	$4.40x10^4±2.69x10^4$	0.88±0.48	$3.02x10^5±1.11x10^5$	6.04±2.22	Reference	Young	Poor
4mCT	$2.47x10^4±3.65x10^4$	0.49±0.70	$1.06x10^5±6.89x10^4$	2.12±1.38	OSPM	Young	Rich
SW	$2.05x10^4±2.23x10^4$	0.41±0.47	$1.19x10^5±7.85x10^5$	2.37±1.57	OSPM	Older	Poor
TP9	$5.99x10^3±3.20x10^3$	0.12±0.06	$2.50x10^5±2.06x10^5$	5.00±4.13	OSPM	Older	Poor
MP	$1.41x10^3±1.67x10^3$	0.03±0.03	$1.40x10^5±6.92x10^4$	2.79±1.38	OSPM	Young	Poor

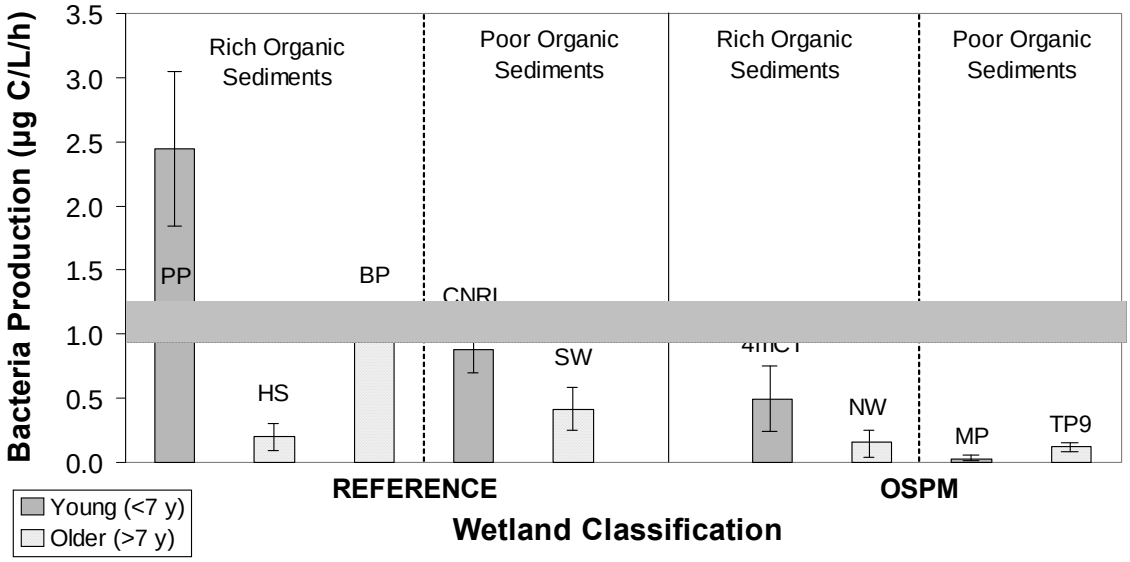


Figure 3.3 - Mean (±SE, n=9) bacterial production (µg C/L/day) in reference and OSPM wetlands of contrasting ages and sediment organic bases. The grey rectangle represents the range (± 1 SE) of bacterial production (µg C/L/day) in Beaver Pond (BP), the control wetland of natural origin in a relatively undisturbed landscape. Wetland abbreviations correspond to wetland names as follows: Peat Pond (PP), High Sulphate (HS), Canadian Natural Resources Ltd. Wetland (CNRL), Shallow Wetland (SW), 4m Consolidated Tailings Demonstration Pond (4-m CT), Natural Wetland (NW), Mike's Pond, (MP) and Test Pond 9 (TP9).

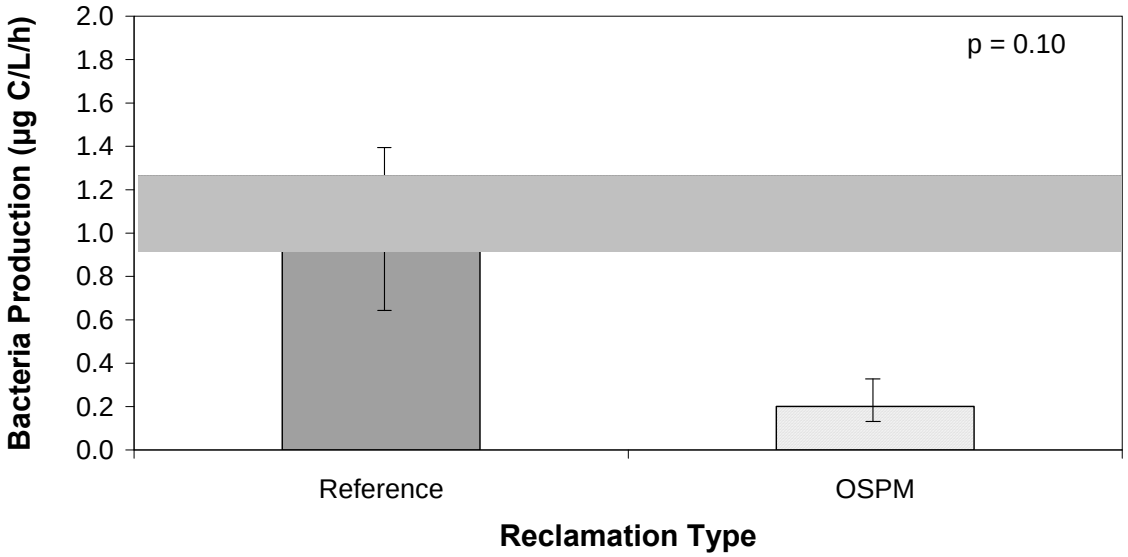


Figure 3.4 – Mean (±SE) bacterial production (µg C/L/day) in reference (n=5) and OSPM-affected (n=4) wetlands. The grey rectangle represents the range (± 1 SE) of bacterial production (µg C/L/day) in Beaver Pond (BP), the control wetland of natural origin in a relatively undisturbed landscape.

Table 3.5 - Three-way factorial ANOVA table examining the effects of wetland status (OSPM vs. reference), age and organic base on bacterial production (µg C/L/h).

Effect	**Degrees of freedom**	**Sum of Squares**	**Mean Square**	**F**	**p**
Reclamation Status (R)	1	7.22	7.22	8.11	0.10
Age (A)	1	0.58	0.58	0.65	>0.05
Organic Base (B)	1	2.49	2.49	2.80	>0.05
R*A	1	0.99	0.99	1.11	>0.05
R*B	1	0.52	0.52	0.59	>0.05
A*B	1	1.59	1.59	1.78	>0.05
Discrepance	2	1.78	0.89		

Principal Components Analysis (PCA) was used to combine environmental, abiotic and nutrient variables (pH, specific conductivity, salinity, temperature, DO, NAs, DOC, NH_4^+, Na^+, K^+, Mg^+, Ca^+, Cl^-, SO_4^-, HCO_3^-, B^+, Fe^+, Si^+, Sr^+) into indices of salts, abiotic factors, and DOC availability, which explained 34.8%, 24.8%, and 30.3% of the total variability, respectively. There were more variables than cases, so only 8 variables (pH, conductivity, salinity, temperature, DO, NAs, DOC and Fe) were used in the first PCA since they were deemed most likely to affect productivity. The other variables were included in additional PCA to see if they loaded onto factors determined in the primary PCA (salts, abiotic factors and DOC) (Fig. 3.5 to 3.7).

No relationship was found between bacterial production and abiotic factors or DOC availability (linear regression analyses, $p > 0.05$). However, production was significantly negatively correlated with salinity (simple linear regression; $R^2 = 0.47$, $p < 0.02$; Fig. 3.8). There was concern that one reference wetland data point may be mainly responsible for the significant correlation. Therefore, the correlation between bacteria production and salinity was examined with a non-parametric test, Spearman rank correlation. According to the non-parametric test, production was weakly negatively correlated with salinity but the relationship was not quite significant ($r_s = -0.63$, $p = 0.07$; Fig. 3.8).

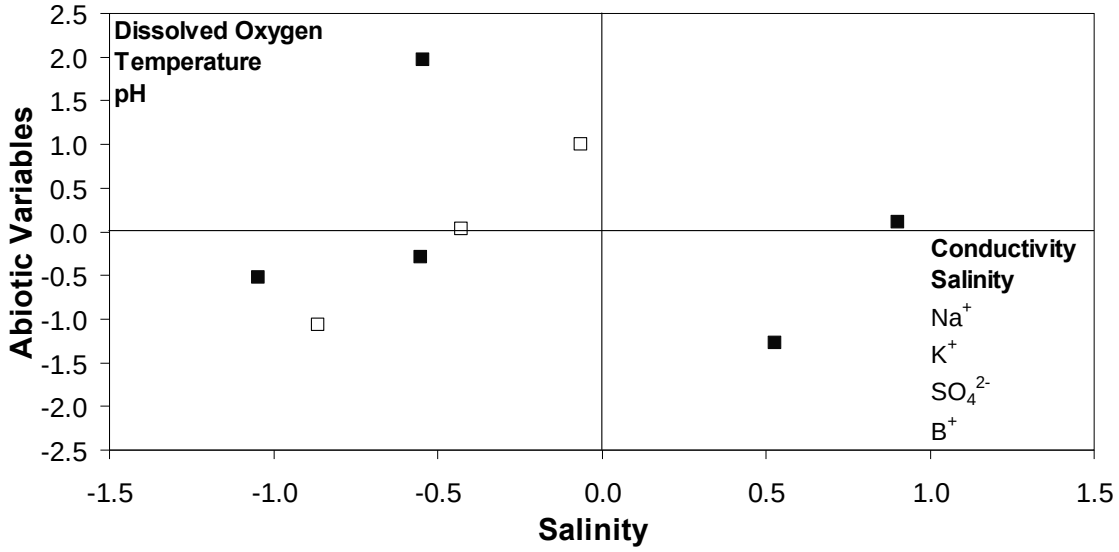


Figure 3.5 – PCA plot of salinity (factor 1) vs. abiotic variables (factor 2) for reference (□) and OSPM wetlands (■). **Bolded** variables represent variables used in the first PCA that loaded with a factor 1, 2 or 3. Non-bolded variables represent other variables whose values were subsequently found to be correlated with values of PC-1 (salts, abiotic factors and DOC).

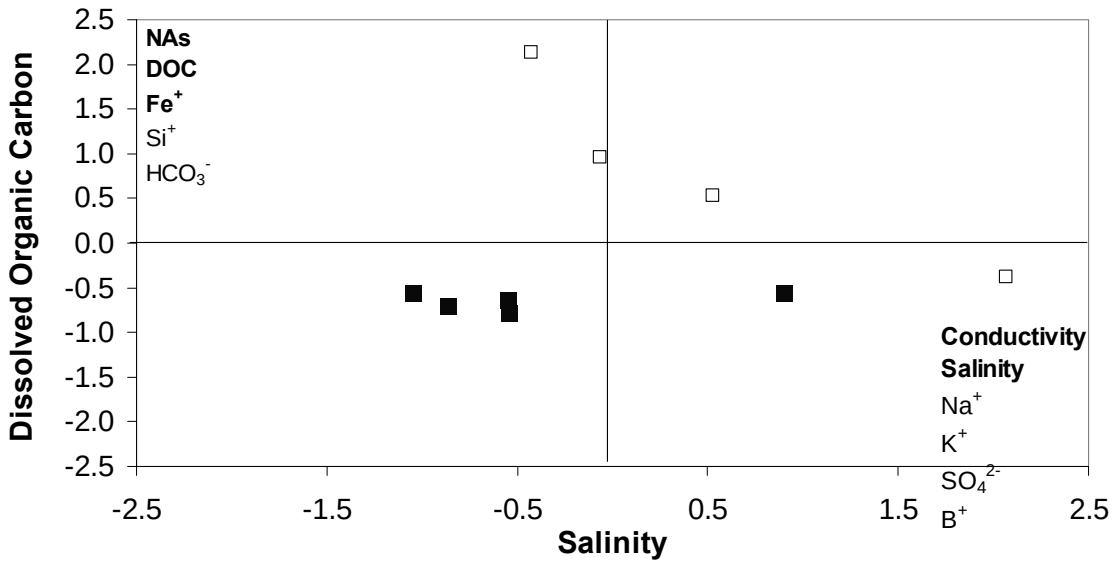


Figure 3.6 – PCA plot of salinity (factor 1) vs. dissolved organic carbon availability (factor 3) for reference (□) and OSPM wetlands (■). **Bolded** variables represent variables used in the first PCA that loaded with a factor 1, 2 or 3. Non-bolded variables represent other variables whose values were subsequently found to be correlated with values of PC-1 (salts, abiotic factors and DOC).

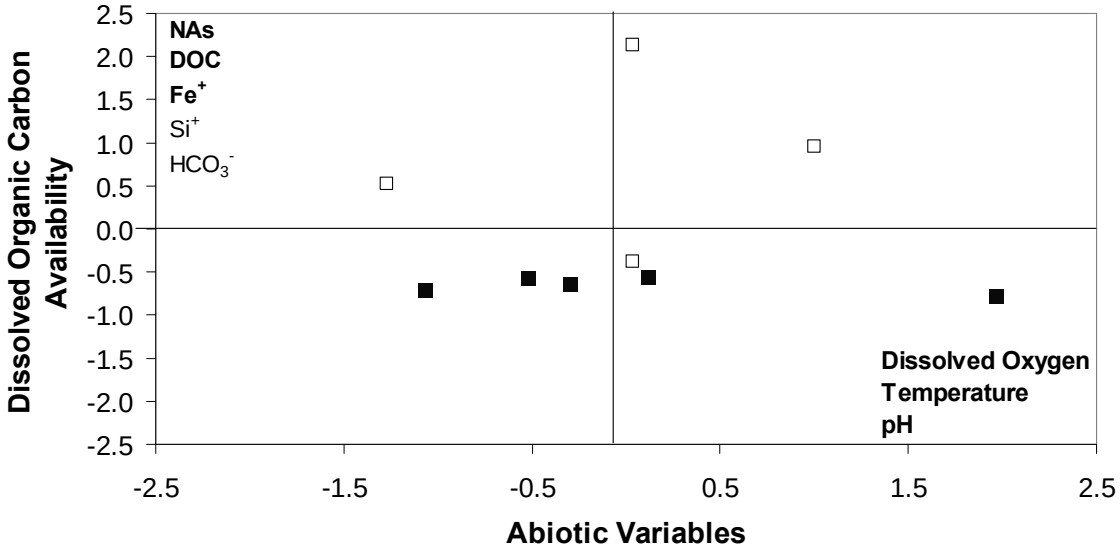


Figure 3.7 - PCA plot of abiotic variables (factor 2) vs. dissolved organic carbon availability (factor 3) for reference (□) and OSPM wetlands (■). **Bolded** variables represent variables used in the first PCA that loaded with a factor 1, 2 or 3. Non-bolded variables represent other variables whose values were subsequently found to be correlated with values of PC-1 (salts, abiotic factors and DOC).

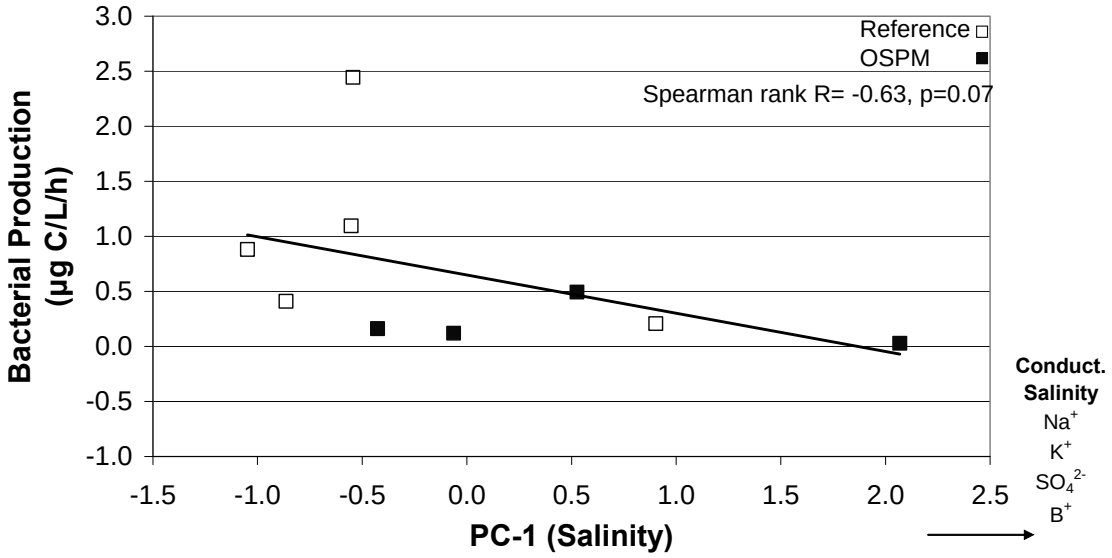


Figure 3.8 - Bacterial production (µg C/L/day) in reference and OSPM-wetlands with varying levels of salinity.

Study 4: Bacterial Biomass and Abundance

Bacterial biomass and abundance ranges were 1.13±1.43 to 6.04±2.22 μg C L^{-1} and 5.66 $x10^4$±7.15$x10^4$ to 3.02 $x10^5$±1.11$x10^5$ cells mL^{-1}, respectively (Table 3.4). There was no significant difference between bacterial biomass and age, status or organic base (Table 3.6; $p>0.05$). However, there was a weak significant interaction with bacterial biomass between age and wetland status (Fig. 3.10, 3-way factorial ANOVA; $p = 0.05$). Biomass in older OSPM wetlands was 2.2 times higher than in younger OSPM wetlands and 3.2 times higher than in older reference wetlands. In contrast, biomass in young reference wetlands was 2.2 times higher than older reference wetlands. There was also a weak significant interaction with bacterial biomass between reclamation type and organic base (Fig. 3.11, 3-way factorial ANOVA, $p<0.10$). Biomass in reference wetlands with rich organic bases was at least 2.9 times lower than other classes of wetlands including reference wetlands with poor organic bases and OSPM-affected wetlands with rich and poor organic bases.

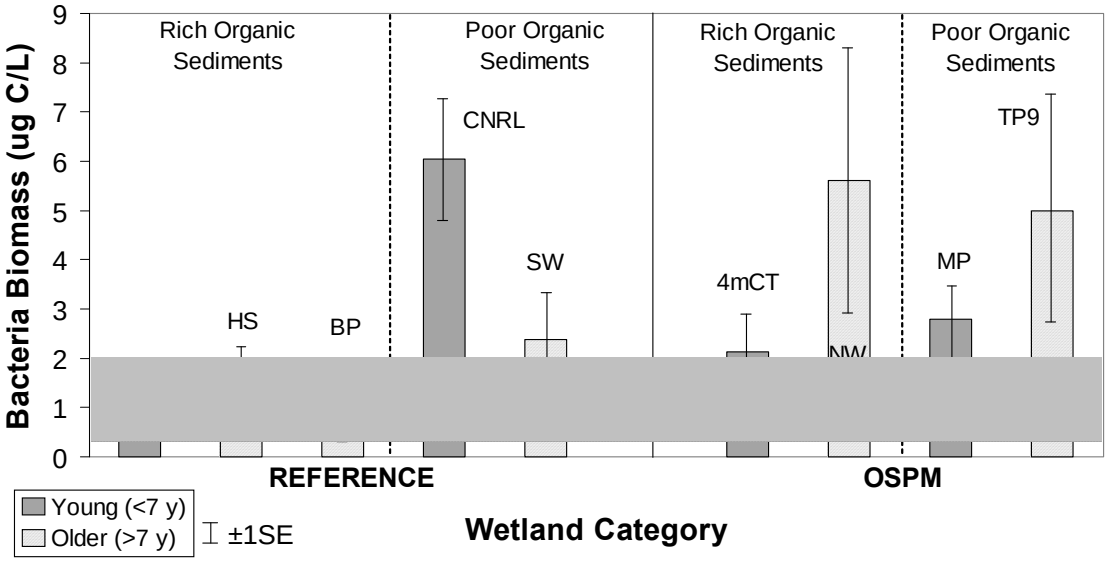


Figure 3.9 - Mean (±SE, n=9) bacterial biomass (µg C/L) in reference and OSPM wetlands of contrasting ages and sediment organic bases. The grey rectangle represents the range (± 1 SE) of bacteria biomass (µg C/L) in Beaver Pond (BP), the control wetland of natural origin in a relatively undisturbed landscape.

Figure 3.10 - Bacteria biomass (µg C/L) in reference and OSPM-affected wetlands of contrasting ages. The grey rectangle represents the range (± 1 SE) of bacteria biomass (µg C/L) in Beaver Pond (BP), the control wetland of natural origin in a relatively undisturbed landscape.

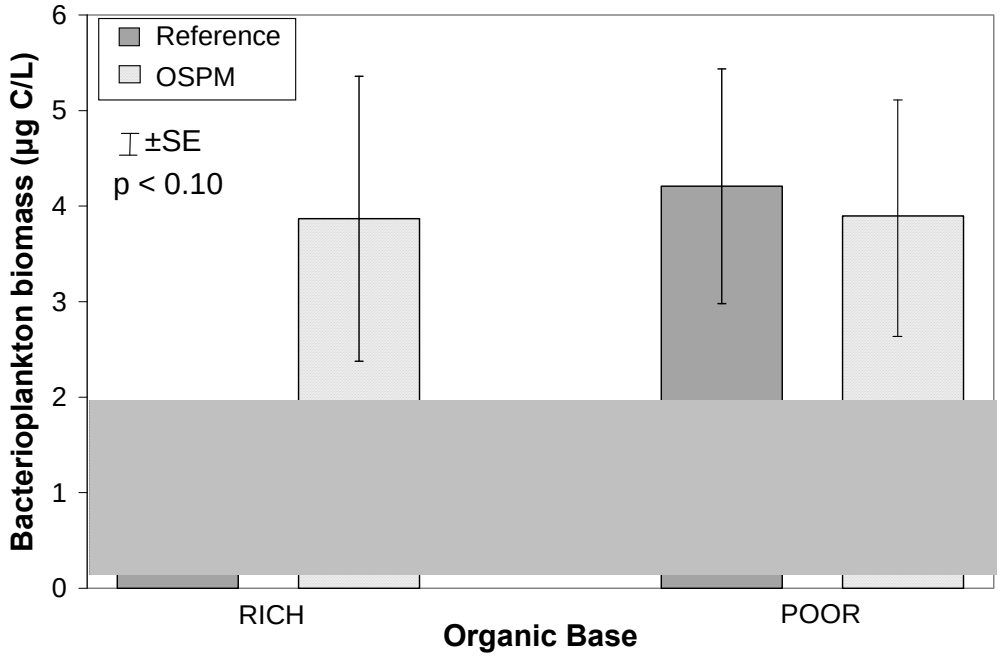


Figure 3.11 - Bacteria biomass (µg C/L) in reference and OSPM-affected wetlands with contrasting organic bases. The grey rectangle represents the range (± 1 SE) of bacteria biomass (µg C/L) in Beaver Pond (BP), the control wetland of natural origin in a relatively undisturbed landscape.

Table 3.6 – Three-way factorial ANOVA table examining the effects of wetland age, organic base and status (OSPM vs. reference) bacterial biomass (μg C/L) on bacteria biomass (μC/L).

Effect	**DF**	**SS**	**MS**	**F**	**p**
Age (A)	1	0.57	0.57	1.29	>0.05
Organic Base (B)	1	4.57	4.57	10.37	<0.10
Reclamation Status (R)	1	2.69	2.69	6.11	>0.05
A*B	1	3.32	3.32	7.55	>0.05
A*R	1	11.58	11.58	26.32	<0.05*
B*R	1	4.38	4.38	9.95	<0.10
Discrepance	2	0.87	0.44		

No relationship was found between bacterial biomass and salinity or abiotic factors (Simple linear regression analyses, $p > 0.05$). However, biomass was weakly significantly positively correlated with DOC availability (Fig. 3.12; simple linear regression; $R^2 = 0.26$, $p = 0.09$). There was concern that one reference wetland (CNRL) data point may be mainly responsible for the significant correlation. Therefore, the correlation between bacteria biomass and DOC availability was examined with a non-parametric test, Spearman rank correlation. According to the non-parametric test, biomass was weakly positively correlated with DOC availability (Fig. 3.12; Spearman $R = 0.55$; $p = 0.12$). Generally, the highest biomass values were measured in the older OSPM wetlands, which also had the highest NAs concentrations.

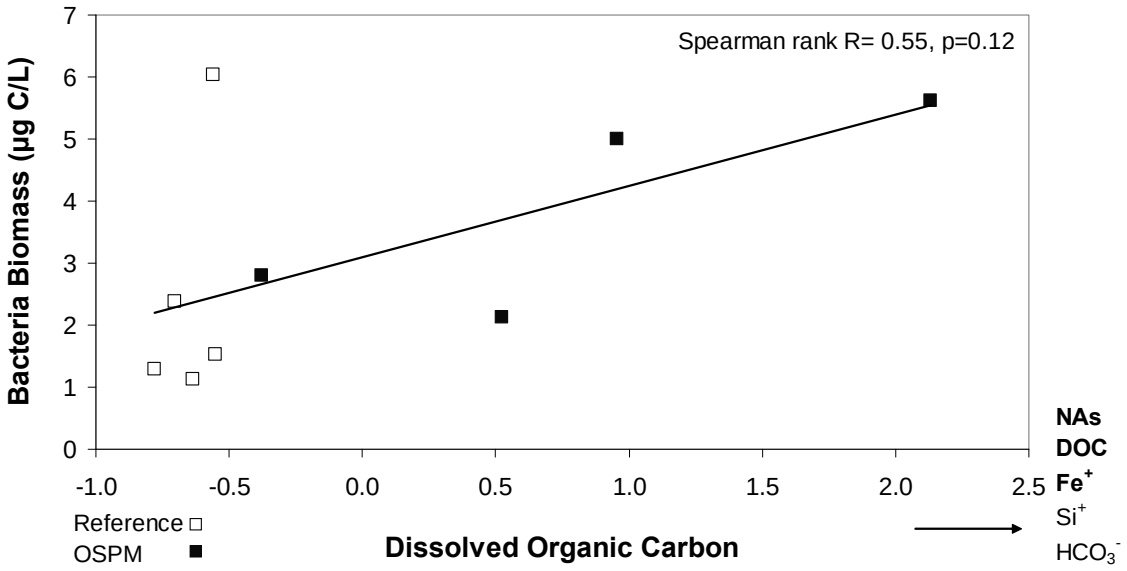


Fig. 3.12 – Planktonic bacteria community biomass (µg C/L) in reference (□) and OSPM-wetlands (■) with varying levels of dissolved organic carbon. CNRL is the reference wetland outlier with unexpectedly high biomass and low DOC.

Discussion

Study 1: ^{3}H Leucine Saturation

Isotope dilution is the contribution of unlabeled leucine derived from the water column or synthesized during incubation that increases the amount of leucine beyond what was added by the investigator (Cole and Pace 1995). Bacterial carbon production would be underestimated if intracellular isotope dilution was not accounted for (Jorgensensen 1992b). Study 1 showed that the ^{3}H-leucine uptake rates had reached an asymptote at leucine concentrations of 40 nM, and this was deemed the appropriate concentration to maximize extracellular incorporation during protein synthesis and to repress intracellular synthesis. An additional uncertainty in carbon production calculations relates to the possibility of radioisotope being incorporated into nonbacterial biota (Kirchman and Ducklow 1993). However, studies have shown that leucine incorporation is almost exclusively restricted to prokaryotes since they can outcompete eukaryotes at nanomolar concentrations (Kirchman 1985, Buesing and Gessner 2003). Most eukaryotes are large relative to bacteria. Consequently, the 1.0 μm filtration step was used to eliminate biota larger from the incubation study. However, if larger bacteria were also removed by the filtration step, my bacterial production estimates may be underestimated.

In general, the substrate saturation concentration measured in this study fell within the range of concentrations reported by other investigators in similar nutrient-rich habitats. Saturation of leucine incorporation in a littoral freshwater wetland in Switzerland was 120 nM (Buesing and Gessner 2003) while it was 50 nM in two eutrophic lakes in Denmark (Jorgensen 1992a). Cole and Pace (1994) reported that leucine saturation was reached at 17 nM in 3 lakes near the U.S. Wisconsin-Michigan border. Cole and Pace (1995) reported that leucine saturation varied between 12 and 34 nM in 9 lakes of varying trophic status. Jorgenson (1992b) recommended using 100nM of leucine for eutrophic waters. Bernard et al. (2000) added ^{3}H-leucine at a final concentration of 77 nM in samples from the Mediterranean coast of France including coastal lagoons, a bay, a harbour, and a river.

It is possible that uptake would saturate at higher levels in the study wetlands where leucine saturation experiments were not conducted, especially those supporting very high bacterial abundances i.e. Test Pond 9, CNRL Wetland, and Natural Wetland. The quantity of leucine added to maximize incorporation rates in the current study was sufficient for other nutrient-rich environments, so the rates reported here are assumed to be comparable among wetlands. The nearly identical isotope dilutions calculated in High Sulphate and Mike's Pond, wetlands of contrasting age, reclamation type and organic base, also support the use of this isotope dilution in the other study wetlands.

Study 2: Conversion Factors

Conversion factor estimates were required to translate measurements of leucine incorporation (pmol/mL/h) into bacterial cell production (μg C/L/h). The measured conversion factors in Mike's Pond and Shallow Wetland were 1.03×10^5 cells/pmol and 2.01×10^4 cells/pmol, respectively, with an average conversion factor of 6.14×10^4 cells/pmol used in the other study wetlands. On average, 6.14×10^4 bacteria cells were produced in 1 mL of wetland water per hour for every pmol of leucine that was incorporated into bacterial proteins.

These factors were similar to or somewhat smaller than conversion factors reported by other investigators from similar freshwater environments. Kirchman (1983) reported a factor of 4.5×10^6 cells/pmol measured in a small freshwater pond in Massachusetts using a 5 nM concentration of thymidine (Kirchman 1983). A conversion factor of 6.4×10^4 cells/pmol was measured in a eutrophic lake in Denmark when a 100 nM concentration of leucine was applied (Jorgensen 1992a).

The validity of using empirically-derived conversion factors was confirmed by demonstrating that the incorporation of exogenous leucine was a linear function of bacterial growth for up to 8 h (Table 3.3). ^{3}H-leu additions are therefore not expected to

stimulate protein synthesis beyond normal rates during the 1-h incubations that were used in studies 1 and 3.

Study 3: Bacterial Production

Bacterial biomass production in the study wetlands ranged from 0.03±0.03 to 2.44±1.50 μg C L^{-1} h^{-1} and was in the range with measurements reported for other freshwater systems. A review by Cole et al. (1988) indicated that planktonic bacterial production in fresh and saltwater ecosystems ranged from 0.02 to 6.25 μg C L^{-1} h^{-1}. Production in a small freshwater pond in Massachusetts was 0.1 μg C L^{-1} h^{-1} during August (Kirchman 1983). In Lake Constance, Central Europe, it was 0.75 μg C L^{-1} h^{-1} in July 1982 (Simon 1987). June production values in a French river was 41.75 μg C L^{-1} h^{-1}, while numbers in brackish lagoons in the same area ranged from 1.06 to 1.89 μg C L^{-1} h^{-1} (Bernard et al. 2000).

The rate of bacterial production measured in the leucine incorporation studies varied between wetlands classes. Mean bacterial production was 5 times higher in all reference wetlands examined as group compared to all OSPM wetlands, indicating that factors associated with oil sands process materials (see below) were inhibiting microbial activity (Fig.3.4).

Environmental Factors

Environmental stress factors that can that can influence bacterial metabolism include temperature, salinity, growth limiting nutrient concentrations, and availability of organic compounds (reviewed by Griffiths et al. 1984). The knowledge of how these factors affect microbial production and abundance is key to understanding the ability of the natural microbial community to adapt to oil sands mining impacts on wetland carbon dynamics.

Planktonic bacterial production in freshwaters can be limited by C, N and P (Caron et al. 1988; Coveney and Wetzel 1992; Miettinen et al. 1997). Felip et al. (1996) reported that C, N and P co-limited bacterioplankton production in a eutrophic lake in New York, but that additions of either C, P, or N and P alone provided only minimal stimulation suggesting that both nutrients and organic carbon limit growth. The degradation rate of hydrocarbons in oil sands constructed wetlands could be limited by nitrogen and phosphorus availability (Bergstein and Vestal 1978, Ward and Brock 1978). However, bacterial production and biomass does not appear to be limited by inorganic nutrients in the constructed wetlands. There was no correlation between bacterial production and ammonium, the preferred nitrogen source (Vallino et al. 1996). This leads us to believe that nitrogen is not a limiting factor in the study wetlands. We also know from a review by Quagraine et al. (2005) that the trace elements (S, K, Mg, Ca, Mn, Fe, Co, Cu, Mo, Ni, and Zn) required for bacterial growth are not limiting in tailings pond water, such as Syncrude's Mildred Lake Settling Basin and Suncor Pond 1. However, we cannot eliminate growth-limiting macronutrients as a potential cause for lower production estimates in some wetlands, especially the OSPM-affected wetlands, since phosphate was not measured and is known to stimulate production. Bacterioplankton and phytoplankton production are often correlated (Azam et al. 1983; Cole et al. 1988). No correlation existed between bacterioplankton production and primary production (Wytrykush 2007, pers. comm.) (linear regression, $p > 0.05$). Although, primary production was quantified at the end of August, 2005 and bacterioplankton production was estimated between July 28 and August 1, 2007. Future studies should quantify primary and secondary production simultaneously. Lai et al. (1996) examined factors that affect NA degradation in laboratory microcosms containing wastewater collected from Syncrude and Suncor's tailings pond water, Syncrude Pit 5, Mildred Lake and Loon Lake. They reported that bacterial activity increased in tailings pond water with phosphate additions (Lai et al. 1996). There was no correlation between bacterioplankton production and DOC indicating that bacteria are not limited by carbon (produced during CO_2 fixation to organic C by phytoplankton (Kirchman 1994)) or by the exhaustion of the labile organic C fraction in OSPM-affected waters. However, the concentration of DOC bioavailable to bacteria was not measured during this study. Further examination of DOC content is

required to determine how much carbon is labile and how much is refractory (i.e., humic substances and some naphthenic acids), which may play an important role in bacterioplankton production in oil sands constructed wetlands.

Lower bacterioplankton community production was correlated with water quality characteristics associated with oil sands mining process materials suggesting that stress may be induced by oil sands process materials. Production was significantly negatively correlated with salinity (Fig. 3.8). A similar trend was reported by Klammer et al. (2002) who observed that bacterial abundance decreased with increasing chloride concentrations in Austrian lakes impacted by salt and soda ash industries. Ward and Brock (1978) showed that rates of hydrocarbon metabolism in ponds decreased with increasing salinity, and attributed the results to a general reduction in microbial metabolic rates. Salinity stress on freshwater bacterial populations can reduce bacterial activity (Griffiths et al. 1984). Although, Rheimheimer (1997; cited in Klammer et al. (2002)) reported that freshwater bacteria have a critical salinity value around 10 ‰, which is much greater than the values measured in the current study wetlands (Appendix 2.2), suggesting that salinity alone may not be reducing bacterial activity.

Stress from high molecular weight NAs in process waters have been known to exert toxicity in aquatic organisms (Holowenko et al. 2002). Several studies have reported NA toxicity to different organisms, including fish (MacKinnon and Boerger 1986), algae (Dixon et al. 2003), invertebrates (MacKinnon and Boerger 1986), mammals (Rogers et al. 2002) and the Microtox toxicity test bacterium, *Photobacterium phosphoreum* (Lai et al. 1996, MacKinnon and Boerger 1986). Leung et al. (2003) examined the effects of naphthenic acids and major ions on phytoplankton communities from 10 water bodies near Fort McMurray and reported that naphthenate and major ion concentrations explained variation in phytoplankton taxonomic composition. However, phytoplankton community biomass was not related to naphthenic acid or major ion concentrations (Leung et al. 2003). Leung et al. (2003) reported that the ecological effect of major ions appeared to be at least as great as naphthenates. The salts in oils sands wastewaters have been observed to increase the toxicity of NAs in algae (Dixon et al. 2003). This seems to

be related to the surfactant nature of NAs, which may act to induce osmotic stress on algae (Quagraine et al. 2005). However, production was not correlated with the DOC index (i.e. naphthenic acids). Salinity in combination with some unknown variable appears to be inhibiting bacterioplankton community activity. The results from the current study do not suggest that certain populations of bacteria are incapable of survival and even bioremediation in OSPM wetlands, only that the natural bacterial community as a whole has reduced activity levels.

Study 4: Bacteria Biomass and Abundance

Bacterial cell numbers were compared between wetlands to determine the relative variation in biomass due to anthropogenic effects, reclamation strategies and age, and to estimate the contribution of bacterial carbon potentially available to bacterivores. Bacterial abundance and biomass varied between wetland classes according to physico-chemical properties.

Bacterial abundance in the Athabasca wetlands ranged from $5.66x10^4 \pm 7.15x10^4$ to $3.02x10^5 \pm 1.11x10^5$ cells mL^{-1}. Abundance was similar to or lower than measurements reported for other freshwater systems. Direct microscopic counting methods of freshwater systems usually report bacteria numbers near 10^6 cells mL^{-1} (Cole 1982). The number of bacteria in a small freshwater pond in Massachusetts reached a maximum of 1.8×10^6 cells/mL in August (Kirchman 1983). The mean number of bacteria in a saline lake in Saskatchewan was 7.98×10^6 cells ml^{-1} (Robarts et al. 1999). Bacterial abundance in a South Carolina estuary during July was 2.52×10^5 cells/ml (DeLorenzo et al. 2001). Abundance was also similar to or slightly lower than measurements from tailings pond water in the Athabasca region and a similar anthropogenically impacted lake. Forrester et al. (1983) measured high bacterial abundances, ranging from 10^6 to 10^8 cells/mL in Mildred Lake Settling Basin, a tailings pond at Syncrude Canada Ltd. Foght et al. (1983) measured aerobic and anaerobic counts of bacteria in a tailings pond to be approximately 10^6 and 10^3 cells/mL, respectively. Total bacterial abundance in an oligotrophic lake in Austria altered by salt and soda industries was 1.2×10^6 cells/ml (Klammer et al. 2002).

Bacterial biomass in the study wetlands ranged from 1.13±1.43 to 6.04±2.22 μg C L^{-1} and, like abundance, was lower than measurements reported from similar environments (Table 5). A review by del Giogio et al. (1999) indicated that planktonic biomass in 20 southern Québec lakes of varying trophic status ranged from 15.0 to 48.8 μg C L^{-1}. Bacterial biomass in Santa Rosa Sound, FL ranged from 26 to 101 μg C L^{-1} (Coffin and Connolly 1997).

The low biomass and abundance of bacteria in the Athabasca wetlands may reflect top-down regulation, viruses, the size fraction examined or low carbon bioavailability. Predation by protozoans may also be responsible for low microbial abundance values in the Athabasca wetlands. Sanders et al. (1992) demonstrated a consistent 1000:1 relationship between biomasses of bacterioplankton and heterotrophic nanoplanktonic protozoa in eutrophic fresh and marine systems indicating that predation is an important regulator of bacteria abundance. Viruses are also an important source of microbial mortality (Fuhrman and Noble 1995).

The most plausible explanation for lower bacterial numbers may also be attributed to the bacterial size fraction enumerated. Planktonic microbes in the 0.2-1.0 μm size fraction were the focus of this study. Bacteria in this size fraction are capable of supplying 60 to 80% of the total microbial production in freshwater environments (Simon 1987). However, the biomass contribution of a larger bacterial fraction may be considerable in nutrient enriched wetlands (Servais et al. 1999), such as the Athabasca wetlands. I am confident that pelagic bacterial biomass and production measurements from this study provide a detailed comparative view of bacterial carbon cycling, at least in those microbes that are < 1 μm, and describe how bacterial metabolism is influenced by wetlands with contrasting ages, reclamation types, and organic bases. Qureshi (2007) examined bacterioplankton community production in many of the same wetlands (Beaver Pond, Shallow Wetland, Test Pond 9, Natural Wetland and High Sulphate Wetland) in June 2006 and determined that mean (±SD) production in picoplankton (0.2μm – 2μm) and nanoplankton (2μm – 20 μm) was 0.94±1.03 and 1.13±1.37 μg C L^{-1} h^{-1}, respectively

(Qureshi 2007). Results suggest that my bacterioplankton community production may have underestimated total bacterioplankton production. Total bacterioplankton community production, which includes bacteria that range in size from 0.2 to 20 μm, in oil sands constructed wetlands of northern Alberta, may be as much as twice my production estimates for small bacteria (0.2-1.0μm). The mean(±SD) production in my study wetlands was 0.65±0.76 μg C L^{-1} h^{-1}, thus total bacterioplankton community production may have approximated 1.3 μg C L^{-1} h^{-1}. This estimate was in the range with measurements reported for other freshwater systems (Cole et al. 1988; Kirchman 1983; Simon 1987; Bernard et al. 2000). Currently bacterioplankton biomass estimates in Beaver Pond, Shallow Wetland, Test Pond 9, Natural Wetland and High Sulphate Wetland have not been measured (Qureshi 2007).

Biomass could also be limited by the amount of bioavailable DOC (Friedrich et al. 1999) produced by phytoplankton or in the organic fraction present in OSPM waters. Bacteria found in oil sands tailings ponds can mineralize NAs (Herman et al. 1994, Holowenko et al. 2002, Lai et al. 1996). Bacteria have even been detected in untreated tailings pond water in the Athabasca oil sands region where other taxa are incapable of survival (Fedorak et al. 2003, Herman et al. 1994, Lai et al. 1996). Similarly, oil sands mine process waters containing moderate naphthenate concentrations (8-21 mg/L) can support tolerant phytoplankton taxa (Quagraine et al. 2005; Hayes 2004). It is believed that DOC can support microbial growth in those process waters where NAs account for much of the DOC (Fedorak et al. 2003). Unique enzymes allow bacteria to metabolize these water soluble organic compounds (Jackson and Meyers 2002). Therefore, these organics can be used by bacteria for as direct carbon and energy sources (Videla 2006). Biomass in older OSPM wetlands was 2.2 times higher than in younger OSPM wetlands and 3.2 times higher than in older reference wetlands, indicating that OSPM is limiting to bacterial abundance in young wetlands, possibly due to naphthenic acid toxicity, but as wetlands age OSPM wetlands become more suitable for the bacterioplankton community. Some molecular toxicity studies, in which stress-inducible genes from *E.coli* were exposed to NAs, implicated the surfactant characteristic of NAs with membrane disruption and cytotoxicity from osmotic stress as mainly responsible the toxicity response (Holowenko

et al. 2002: Envirotest cited in Quagraine et al. (2005). However, studies have shown that natural aging of oil sands tailings reduces toxicity (Holowenko et al. 2002; Mackinnon et al. 1986) and that microbial activity in laboratory cultures also reduces toxicity of NAs (Herman et al. 1994). Bacterial biomass is much higher in old OSPW wetlands than in young ones, even though production is uniformly low. Perhaps NAs in young wetlands are acutely toxic, whereas NA in the older wetlands permits bacterial persistence (possibly tolerant species) albeit at lower levels of growth than occurs in reference wetlands of equivalent age.

Conversely biomass in young reference wetlands was over 2.2 times higher than older reference wetlands. Furthermore, elevated bacterial biomass was paralleled by higher bacterial production rates in young reference wetlands (Peat Pond and CRNL Wetland) compared to the other study wetlands suggesting that initial reference wetland conditions were more favourable for the natural bacterial community or perhaps that predation limitation plays a greater role in older reference wetlands. A phytoplankton bloom observed in at least one of the young reference wetlands (Peat Pond) at the time of the planktonic bacterial production measurements may have stimulated bacterial activity. Many studies have noted a positive correlation between bacterial production and phytoplankton biomass in freshwater environments suggesting that resources provided by algal production regulate bacteria (Cole 1982, Cole et al. 1988, Friedrich et al. 1999, Robarts et al. 1999, Simon 1987; White et al. 1990). Net primary production (NPP) was quantified in some of the study wetlands (Peat Pond, Beaver Pond, Test Pond 9, Natural Wetland and 4-m CT) at the end of August (C. Wytrykush 2007, pers. comm.). There was no correlation between bacterioplankton biomass and NPP (linear regression; $p > 0.05$). However, no quantitative NPP measurements were made during the time of this study (July 28 to August 1, 2005) to evaluate this hypothesis appropriately.

According to the data in this study, DOC availability was statistically a positive weak significant variable in determining bacterioplankton community biomass (Fig. 3.12). Bacteria are generally accepted to be the primary decomposers and mineralizers of organic matter in aquatic ecosystems (Friedrich et al. 1999). Bacterial production is

related to the supply of labile organic matter (Judd et al. 2006), which may originate from primary production or residual carbon in OSPM (i.e. petroleum, naphthenic acids). Studies have shown that bacterial abundance (Bird et al. 1984; Cole et al. 1988) and production (Cole et al. 1988; Pace and Cole 1996; Friedrich et al. 1999) covary with phytoplankton biomass and production. Phytoplankton appears to serve as a direct source of organic matter for heterotrophic bacterioplankton (Friedrich et al. 1999). Residual hydrocarbons can also increase abundance of hydrocarbon-degrading microbes (Atlas 1992). Results suggest that more DOC supports more bacterioplankton community biomass. Perhaps the relationship between DOC and bacterioplankton community biomass would be strengthened if the amount of bioavailable carbon was measured in wetlands and related to bacterioplankton community biomass.

Ecological suitability of reclaimed wetlands

Planktonic bacteria community production in reference wetlands was in the range measured in Beaver Pond, the control wetland that was of natural origin and in a relatively undisturbed landscape. In contrast, production was comparatively low in OSPM-affected wetlands in relation to Beaver Pond indicating that the richness (i.e. nutritive value) of available DOM was less than adequate for the bacteria and that the environment of OSPM-affected wetlands was less than suitable for the microbial loop (Billen et al. 1990; Ducklow 2000). The reduced metabolic activity of the bacterial population in OSPM wetlands was correlated with salinity index.

Biomass in reference wetlands with rich organic bases was lower than other classes of wetlands including reference wetlands with poor organic bases and OSPM-affected wetlands with rich and poor organic bases, although the relationship was not significant (Fig. 3.11). Contrary to my expectations, bacterioplankton biomass in wetlands with rich organic bases was not higher compared to wetlands with poor organic bases. I thought nutrients leached from the peat amendments may act to stimulate bacterioplankton production. Also, there was little variation in bacterioplankton production between wetlands with and without peat amendments, suggesting that the total number of cells

within wetlands was not markedly influenced by this reclamation strategy. Phytoplankton carbon production and residual carbon in oil sands process water (OSPW) (i.e. naphthenic acids) are probably more important carbon sources for bacterioplankton. This reclamation strategy does not appear to affect bacterioplankton carbon production and, potentially, carbon transfer in the aquatic food web in oil sands constructed wetlands in northern Alberta.

Future Studies

To further elucidate the trophic role of bacterioplankton in oil sands affected wetlands one must know the fate of bacterial production, including respiration losses (Chapter 4) and grazing rates. Future microbial production studies should assess protozoan activity and grazing potentials to further elucidate the role of microbes in at the base of the food web in constructed wetlands of the oil sands area of northern Alberta

Further examination of the relationship between bacterioplankton production and abundance with DOC (i.e. naphthenic acids) is required and should quantify how much carbon is labile and how much is refractory (i.e. humics and some naphthenic acids). Since there is often a correlation between primary production and bacteria production (Azam et al. 1983; Cole et al. 1988; White et al. 1990), future studies should also include chlorophyll *a* and primary production measurements. The bioavailability of DOC to the microbial loop likely plays an important role in the food webs of oil sands constructed wetlands.

Synopsis

1. The maximum rate of ^{3}H-leucine uptake into bacterial proteins was 40 nM, and was deemed the concentration that would maximize extracellular ^{3}H-leucine incorporation during protein synthesis and repress intracellular leucine synthesis. This measured concentration was in the range (12 to 120 nM) reported by other investigators in similar nutrient-rich habitats.
2. Conversion factors were required to translate measurements of leucine incorporation into bacterial cell production. The measured conversion factors in Mike's Pond and Shallow Wetland were 1.03 x 10^{5} cells/pmol and 2.01 x 10^{4} cells/pmol, respectively. The average of these two values (6.14 x 10^{4} cells/pmol) was used in the other study wetlands. These factors were similar or somewhat smaller than conversion factors reported by other investigators from similar freshwater environments.
3. Bacterial biomass production in the Athabasca wetlands ranged from 0.03±0.03 to 2.44±1.50 μg C L^{-1} h^{-1} and was in the range with measurements reported for other freshwater systems.
4. Bacterioplankton production may have been underestimated by a factor of 2 by considering only small bacteria (0.2 to 1.0 μm).
5. Bacterioplankton production in reference wetlands was similar to the unconstructed reference wetland, Beaver Pond, however, lower in OSPM-affected wetlands indicating that bacteria activity was inhibited by OSPM. Reduced bacterial activity in OSPM-affected wetlands is correlated with increasing salinity stress.
6. Bacterial abundance and biomass ranges in the Athabasca wetlands were $5.66x10^{4}$±$7.15x10^{4}$ to $3.02x10^{5}$±$1.11x10^{5}$ cells mL^{-1} and 1.13±1.43 to 6.04±2.22 μg C L^{-1}, respectively. These values were lower than measurements reported for other freshwater systems and may reflect top-down regulation, viruses, the size fraction examined or lower carbon bioavailability to bacterioplankton.
7. Elevated bacterial biomass and abundance in young reference wetlands compared to the other study wetlands suggests that initial reference wetland conditions were more favourable for the natural bacterial community, perhaps as a result of elevated algal production or predation limitation.

8. Biomass in older OSPM wetlands was 2.2 times higher than in younger OSPM wetlands and 3.2 times higher than in older reference wetlands, indicating that OSPM is limiting to bacterial abundance in young wetlands, possibly due to naphthenic acid toxicity, but as wetlands age OSPM wetlands become more suitable for the bacterioplankton community.
9. Amending wetlands sediments with peat, as a reclamation strategy, does not appear to affect bacterioplankton community production and biomass or potential carbon transfer to higher levels in the aquatic food webs.
10. Bacterioplankton biomass in all the study wetlands was higher than quantified in the unconstructed reference wetland, Beaver Pond, indicating that bacterial numbers are not atypical in the oil sands affected wetlands.

CHAPTER IV:
PATTERNS OF CARBON DIOXIDE AND METHANE FLUX FROM OIL SANDS-AFFECTED WETLANDS OF NORTHEASTERN ALBERTA, CANADA

Abstract

Patterns and rates of generation of carbon dioxide (CO_2) and methane (CH_4) by aerobic and anaerobic soil respiration are a significant gap in knowledge of carbon dynamics in constructed wetlands affected by oil sands mining near Fort McMurray, Alberta. Partial pressures of CO_2 (pCO_2) were estimated by calculation from data on temperature, salinity, pH, and alkalinity. CO_2 and CH_4 flux from wetland sediments were measured in situ in 9 wetlands during July & August 2005 with a novel microcosm chamber. Good agreement between calculated pCO_2 and measured gas flux indicated that the microcosm chamber design provided credible gas measurements from the natural environment. Mean±SE rates of CO_2 and CH_4 accrual measured in situ ranged from -16.80±2.16 (n=10) to 15.60±4.80 (n=15) mg $m^{-2}d^{-1}$ and -0.48±0.46 (n=10) to 5.36±2.08 (n=14) mg $m^{-2}d^{-1}$, respectively. The pCO_2 estimates in wetlands ranged from 69 to 2,083 (mean±SE = 976.4±1124; n=4) μatm. Wetlands were 2.6 times supersaturated with respect to the atmosphere and currently appear to be small sources of C to the atmosphere. Averaged over all sampling dates and wetlands, total carbon emissions from the unvegetated sediments of study wetlands were estimated to be 15.59 mg m^2 d^{-1} (n_{CO2}=9, n_{CH4}=9). Methanogenic carbon accounted for 56% of the carbon emissions. No difference in C fluxes between oil sands process material-affected wetlands and reference wetlands indicated that anthropogenic effects did not appear to stimulate gaseous CO_2 and CH_4 fluxes from the unvegetated areas of OSPM-affected wetlands. A post-mining reclamation strategy of amending wetlands sediments with peat does not appear to stimulate microbial decomposition, thereby accelerating carbon loss over time. Methanogenesis, especially in OSPM-affected wetlands, was inhibited, by elevated sulphate concentrations. The study wetlands were minor net exporters of C from areas with unvegetated sediments and currently do not appear to be on a trajectory to becoming net sinks in these early stages of their development since gas emissions neither increased, nor declined as a function of age since construction.

Introduction

Northern peatlands mainly function as long-term sinks for atmospheric carbon dioxide (CO_2) and sources of atmospheric methane (CH_4) (Blodau 2002). Increased oxidation and microbial activity have the potential to release additional organic carbon to the atmosphere, thus contributing to global warming, by facilitating processes such as decomposition and respiration (Ali et al. 2006). According to one estimate (IPCC 2001) about 25% of anthropogenic emissions of CO_2 over the preceding 20 years were attributed to changes in land cover. For example, natural gas and oil pipelines crossing the Canadian boreal and subarctic regions may potentially lead to (1) loss of CO_2 uptake (through deforestation) and (2) a large increase in sediment respiration leading to loss of CO_2 from soils (Roulet 2000). Concerns about the negative effects of greenhouse gas emissions and toxic organic compounds and metals on environmental quality has indicated that there is a need for more research on the role of wetlands as sources and sink of these pollutants and factors affecting pollutant transformations (D'Angelo and Reddy 1999).

The Athabasca oil sands deposit covers about 42,000 km^2 surrounding Fort McMurray (57° 3.07` N, 111° 36.02` W) in northeast Alberta (Rogers et al. 2002) and contains over 200 billion cubic meters of petroleum, making it the world's largest single oil deposit (AOSTRA, 1990). Companies currently mining the oil sands hope to use wetlands constructed from oil sands process materials (water and fine tails) as part of their reclamation strategy required at mine closure. An understanding of gas exchange processes is essential to the development of appropriate reclamation plans for the Athabasca oil-sands constructed wetlands.

Constructed wetlands will be different from pre-development wetlands. They may evolve towards peatlands, which make up 20-40% of the premining landscape, but this will occur only over a long period (OSWWG 2000) since it took a few thousand years for northern peatlands to develop following the end of the last glaciation (Harden et al. 1992). The cool, moist climate of northern Alberta supports peatland formation

(OSWWG 2000). However, post-mining conditions may not be conductive to the formation of the vegetative communities typically associated with peatland ecosystems (OSWWG 2000). The presence of salts in oil-sands process waters (OSPW), used in wetland construction, will affect the ability of some vegetation to colonize these constructed wetlands. Some mosses are unable to establish viable populations in areas where saline conditions occur (Vitt et al. 1993; Wytrykush & Hornung, in prep). Based on these factors, which influence wetland formation and the predicted future characteristics of the reclaimed landscape, it is not feasible to reclaim peatlands in the short term (OSWWG 2000). However, the Environmental Protection and Enhancement Act (EPEA) require oil-sands operators to "reclaim disturbed land to an equivalent land capability" (OSWWG 2000). Thus, it is important that constructed wetlands function like northern peatlands by accumulating organic matter and ultimately acting as long-term CO_2 sinks. Currently, it is unknown how extensive the CO_2 and CH_4 losses are from recently constructed oil-sands affected wetlands and whether these systems are on a trajectory to becoming peat-forming wetlands. The purpose of this study was to examine rates and patterns of CO_2 and CH_4 production in unvegetated zones of wetlands of contrasting reclamation type (reference vs. OSPM-affected), age (young vs. older) and organic base (rich vs. poor) in the Athabasca oil sands region.

Partial Pressure of CO_2

The exchange of CO_2 across the air-water interface is an important process that affects the C budget of aquatic systems. The direction of CO_2 exchange depends on the CO_2 concentration gradient between the atmosphere and natural waters (Cole and Caraco 1998). Gaseous fluxes of CO_2 from aquatic sediments to the atmosphere are commonly measured with a static chamber (Pulliam 1993) or can be calculated from the partial pressure differences between air and water (Kling et al. 1991). Lakes and rivers are commonly supersaturated with CO_2 relative to the atmosphere (Kling et al. 1991; Kling et al. 1992; Cole et al. 1994; Cole and Caraco 1998; Striegl et al. 2001). When there is evolution of CO_2 to the atmosphere it must be supported by some input of CO_2 (Cole and Caraco 1998). Inputs come from biologically and geologically mediated processes.

First, the largest fraction of CO_2 found in natural waters comes from decomposition of organic matter by microorganisms (Girard 2005). Secondly, allochthonous carbon enters natural waters via hydrological inputs of DIC derived from dissolution of carbonates (Otsuki and Wetzel 1974). Alternatively, atmospheric C can diffuse into surface waters when aqueous CO_2 is depleted via: (1) primary production by algae and macrophytes, and (2) when C storage in biota and sediments are high (Kling et al. 1992; vanLoon and Duffy 2005).

The distribution of CO_2 between natural waters and the atmosphere is critically dependent upon the pH, temperature, alkalinity, and salinity of the surface waters (Broecker 1997; Hauser 2001; Raven and Falkowski 1999; Sarmiento and Le Quere 1996). Higher temperatures, salinity and alkalinity reduce CO_2 solubility (Sarmiento and Le Quere 1996).

Methane

Wetland CH_4 emissions play an important role in global CH_4 cycling. Methane, a greenhouse gas, absorbs infrared radiation emitted from the Earth's surface, contributing to global warming (Schlesinger 1997). Each molecule of methane in the atmosphere has the potential to contribute about 25X as much greenhouse warming as each molecule of CO_2 over the next century (Lashof and Ahuja 1990; Albritton et al. 1995). Since concentrations of methane in the atmosphere have increased at an average rate of about 1%/y, faster than the rate of CO_2 increase (Schlesinger 1997), increases in atmospheric methane may be relatively more important to global climate change during the next century (Dickinson and Cicerone 1986). Freshwater wetlands are considered to be one of the major sources for atmospheric CH_4 (Pulliam 1993). Cicerone and Oremland (1998) developed a global CH_4 budget which found that natural wetlands were the largest single global source of atmospheric CH_4, contributing >25% of all biogenic emissions.

Methanogenesis in reclaimed wetlands could pose a problem for remediation of fine tailings. First, methane percolating into the water cap overlying the fine tailings could

speed the transport of toxic compounds (e.g. naphthenic acids) from the sediment pore water into the water above (Gulley and MacKinnon 1993). Secondly, methanotrophic bacterial consumption of methane in wetland waters could also lead to anoxic conditions in the water column (Wang et al. 1996). Low dissolved oxygen concentrations in the water column could prevent the establishment of higher pelagic organisms within wetland food webs (Holowenko et al. 2000). Thirdly, methane is a greenhouse gas.

Methane production in fine tailings from Mildred Lake Settling Basin (MLSB), the primary tailings pond at Syncrude Canada Ltd., has increased significantly over the last 2 decades (Holowenko 2000; Holowenko et al. 2001). During the first 15 years, approximately, of operation no visible methane production, in the form of ebullition, occurred in the MLSB (Holowenko 2001). Fought et al. (1985) examined microbial content and activities in MLSB tailings and measured methane production in samples from the 15-m depth that was supplemented with acetate or glucose and incubated at 37^{o}C. No methane was detected in the supplemented cultures incubated at 15^{o}C, which was the temperature of tailings in situ. Fought et al. (1985) concluded that methane evolution was unlikely to disrupt the consolidation of tailings. During the early 90s bubbling activity became visible in MLSB (Holowenko et al. 2000). Analysis of the MLSB tailings collected in 1996 indicated methanogenic activity (Soboloewski 1997). In 1999, 40-60% of the 12-km^2 water surface area had an estimated daily flux of 12 g CH_4/m^2 (Holowenko et al. 2000). Methanogenesis in the MLSB appears to have commenced once sulphate concentrations declined, thereby minimizing microbial competition with sulphate-reducing bacteria (SRB) (Holowenko et al. 2000). In most cases, methane production was observed in consolidated tailings (CT) and mature fine tailings (MFT) from Shell Canada Ltd. amended with acetate and glucose when the sulphate concentration was below 17-20 mg/L (Fedorak et al. 2002).

The substrates in the tailings that support methanogenesis are unknown (Holowenko et al. 2001). Holowenko et al. (2001) showed that the anaerobic microbial community can adapt to NA concentrations that far exceed the in situ concentrations, and that NA

mixtures are not likely the direct source of methane in Syncrude Canada Ltd. tailings ponds.

Patterns and rates of generation of CO_2 and CH_4 by aerobic and anaerobic soil respiration remain a significant gap in knowledge of carbon dynamics in the Athabasca wetlands. Laboratory studies have examined CO_2 and CH_4 emissions from MFT and CT collected from tailings ponds (Holowenko et al. 2000; Holowenko et al. 2001; Fedorak et al. 2002; Fedorak et al. 2003). However, no studies have examined the rates of CO_2 and CH_4 generation from the Athabasca wetlands in situ. Furthermore, studies have not examined how the reclamation practice of amending sediments with peat affects gaseous C production. Additionally, to my knowledge CO_2 partial pressure has not been determined in wetlands, although pCO_2 has been extensively studied in lakes and marine systems. This paper presents measurements of CO_2 and CH_4 estimated from 9 constructed wetlands in northern Alberta and estimates of pCO_2 from 4 of those 9 wetlands. The objectives were to: (1) determine rates and patterns of CO_2 and CH_4 production in oil-sands affected wetlands; (2) assess if the presence of organic constituents (e.g., naphthenic acids and PAHs) in oil-sands process materials (OSPM) used in wetland construction potentially increase rates of gaseous C generation; (3) examine the success of a reclamation strategy from a carbon dynamics perspective; and (4) assess whether the flux estimates observed in these systems were consistent with a trajectory to becoming peat-forming wetlands.

It was hypothesized that CO_2 flux rates would be higher in wetlands amended with peat than in unamended wetlands and higher in older wetlands compared to young wetlands since older wetlands and peat-amended wetlands should contain more organic carbon, which can be respired. CO_2 production was expected to be higher in reference wetlands than in OSPM-affected wetlands since much of the organic carbon in OSPM-affected wetlands is petroleum residue, naphthenic acids and PAHs, which are largely refractory. Also, it was hypothesized that CH_4 production would be low in all wetlands since sulphate, which inhibits methanogenesis, occurs naturally, even in reference wetlands. However, the lowest CH_4 production rates were expected in OSPM-affected wetlands,

which were known to have very high sulphate concentrations (150 to 1220 mg/L). Finally, I expected to measure higher methane levels in Peat Pond, Natural Wetland, Beaver Pond and Shallow wetland since bubbling was observed in these wetlands prior to the commencement of the study.

Methods

Five sample plots were randomly selected near the shoreline in each of 9 study wetlands (Table 2.1). Static chambers have been extensively used to measure gas fluxes (Pulliam 1993). In this study I designed a novel microcosm chamber (0.65 L) to measure CO_2 & CH_4 effluxes from wetland sediment (Fig. 4.1a). The bottoms were removed from sterilized 2-L plastic soda bottles. Polypropylene tubing (90 cm length x 0.7 cm outer diameter; 0.5 cm inner diameter) was inserted into a hole created in the bottle cap. Silicon sealant and teflon tape were used around the cap and bottle to make an air tight seal in the microcosm's cap. A rubber septum was inserted into the end of the polypropylene tubing so gas samples could be collected using an air-tight syringe (150 cm^3). Teflon tape was wrapped around the rubber septum to reinforce an air tight seal.

At each plot 3 light and 3 dark (aluminum foil-wrapped microcosms were inserted 10 cm into wetland sediments away from wetland plants, to minimize plant respiration effects. The measurement of fluxes in light and dark microcosms allowed the comparison of autotrophic and heterotrophic microbial respiration. In this paper, only gas measurements from the dark microcosms are reported, and therefore reflected total heterotrophic flux rates from wetland sediments. In total, 270 microcosms representing 8 treatments (a 2x2x2 factorial design of contrasting ages, organic base, and reclamation strategy; Chapter 2) were used during the study.

Disturbance of sample plots was a significant concern, as each plot was visited many times during the study. Plot disturbance was minimized in several ways. Microcosms were inserted into wetland sediments at least 48 h prior to attaching the microcosm cap, to allow the restabilization of any disturbed sediment and eliminate unnatural gas elution

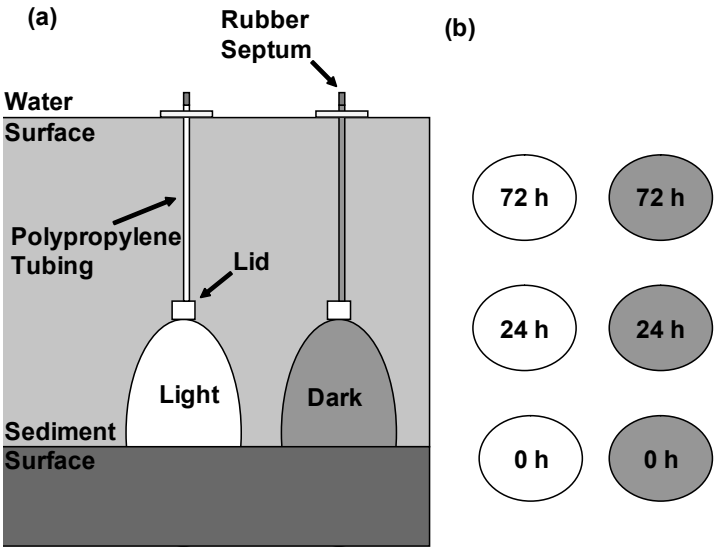


Figure 4.1 – Diagram of (a) the microcosm chamber design, and (b) time points after placement at which sampling occurred at each wetland site.

during the study. After this waiting period, caps were attached to the microcosms, thereby initiating a closed system. A sample could be collected while standing a good distance (50 cm or more) from the microcosm because of the long polypropylene tubing (90 cm).

Gas samples (containing CO_2 and CH_4) were collected from separate microcosms 0, 24, & 72 h after placing caps on both light and dark microcosms between July 26 and August 11, 2005 (Fig. 4.1b). The volume of produced gas was used to determine gas generation. Simultaneously with the gas sampling salinity, specific conductivity, pH, dissolved oxygen content (DO), and temperature were measured *in situ* with standard instruments (YSI). Additional abiotic and nutrient variables (concentrations of naphthenic acids (NAs), NH_4, SO_4, CO_3, HCO_3, $CaCO_3$, total cations, total anions, and the following ions: Na, K, Mg, Ca, F, Cl, Al, B, Ba, Fe, Mn, No, Ni, Se, Si, Sr, Ti, V, Zn, and Zr) were determined from 1-L water samples collected in July, 2005.

Gas flux measurements were made during the summer so as to ascertain what was expected to be the greatest efflux of gases during the year (Clair et al. 2002). Headspace-gas [CO_2] and [CH_4] were measured with a field-portable Agilent MicroGas™ Chromatograph (M200 Series) GC. Gas samples were collected from the microcosm tubing in 140-mL syringes and injected manually into the GC. A 4-m long 5 Å molecular sieve column and Ar carrier gas were used to separate O_2 and N_2 (column temperature of 70^{o}C, carrier gas pressure of 30 psi) and an 8 m Poraplot™ column and He carrier gas were used to separate CO_2 and CH_4 (column temperature of 65^{o}C, carrier gas pressure of 15 psi). Gas concentrations were measured with a thermal conductivity detector (TCD) at a precision and accuracy of ±1.3 % and ±1.3 % (Appendix 4.1). Both the gas analyzer and portable GC were calibrated with a reference gas mixture of 1.58 volumetric % CO_2, and 0.52 volumetric % CH_4.

Partial Pressure of CO_2

Expected CO_2 partial pressure (pCO_2) (atm) was calculated for each wetland from pH and alkalinity measurements using the following formula:

$pCO_2 = \alpha_{H+}\ \alpha_{HCO3-} / (K_1\ K_{CO2})$ (D. Fowle, University of Windsor, Pers. Commun),

where α, K_1, and K_{CO2} denote activity, the first dissociation constant, and Henry's law constant for CO_2. α_{H+} is given by 10^{-pH} (mol/L), while α_{HCO3-} is obtained by multiplying alkalinity (mol/L) by 16.38×10^{-6} (C. Weisener, University of Windsor, Pers. Commun.). Values of K_H (mol/L·atm) for CO_2 (Weiss 1974) and K_1 (mol/L·atm) for CO_2 (aq) (Harned and Bonner 1945) were determined for each wetland with appropriate corrections for salinity and temperature.

Statistical Analysis

The volume of headspace at 0 h in each microcosm was known (17.44 cm^3). However, the change in headspace over time was not recorded. The volume of CO_2 and CH_4 at 24 h and 72 h was calculated using the following formula:

$C_{final} \times (17.44\ cm^3 + X) = (C_{initial} \times 17.44\ cm^3) + (C_{mean} \times X)$

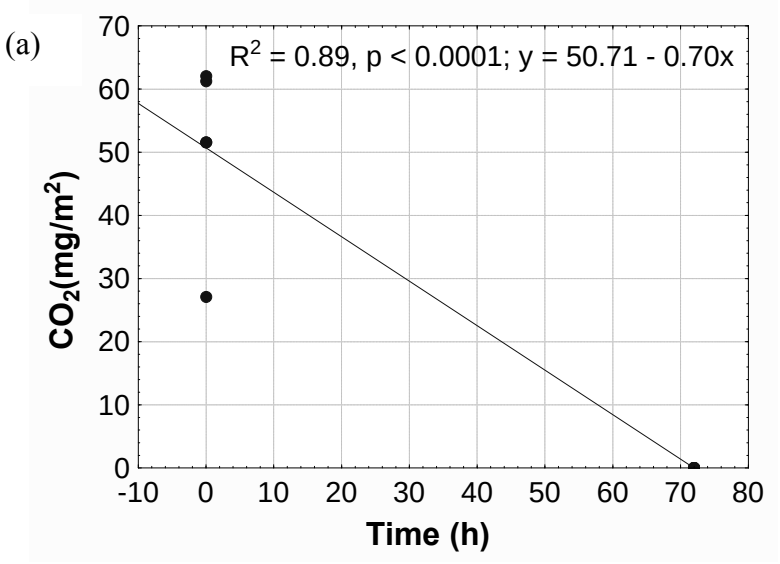
where C_{final} is the percent volume of gas (CO_2 or CH_4) in the headspace at 24 or 72 h, the $C_{initial}$ is the percent volume of gas (CO_2 or CH_4) in the headspace at 0 h, 17.44 cm^3 represents the known volume of headspace at 0 h, and C_{mean} represents the biogenic composition of gas, CO_2 or CH_4. Canton et al. (1989) examined CO_2 or CH_4 flux from vegetated and unvegetated sediments from 11 estuaries, marshes, creeks, and swamps in the U.S. and determined that 70% of the gas produced from unvegetated sediments was methane and 3% was carbon dioxide. Thus, the C_{mean} for CO_2 was 0.70 and the C_{mean} for CH_4 was 0.03.

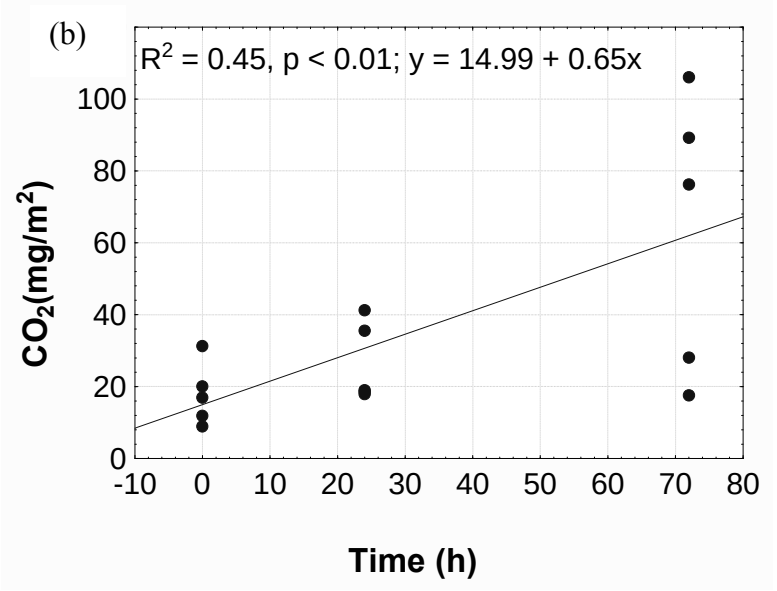
The number of moles of CO_2 and CH_4 gas present within the microcosms was calculated using the measured (0 h) and calculated (24 & 72 h) gas volumes and the ideal gas law. CO_2 and CH_4 masses were calculated by multiplying each gas' molar mass by the number of moles of that gas in the microcosms' headspace.

Gas fluxes, [CO_2] and [CH_4], were calculated by determining the slope of the least squares regression line of CO_2 and CH_4 concentrations in the microcosms vs. time for each observed wetland (Fig. 4.2, Appendices 4.2 and 4.3, respectively). Release of methane bubbles was sometimes indicated by extremely high CH_4 accumulations in the microcosms. Those observations were retained, even though they decreased statistical power. The slope of the CO_2 and CH_4 concentrations over time represents the emission or uptake rate. Slopes not significantly different from zero ($p > 0.05$) were reported as having an emission rate = 0.

Principal Components Analysis (PCA) (Statistica 6.0) was used to combine environmental, abiotic and nutrient variables (pH, specific conductivity, salinity, temperature, DO, NAs, DOC, NH_4^+, Na^+, K^+, Mg^+, Ca^+, Cl^-, SO_4^-, HCO_3^-, B^+, Fe^+, Si^+, Sr^+) into indices of salinity and sulphate, abiotic factors, and DOC availability, which explained 32.3%, 25.2%, and 32.03% of the total variability, respectively. Any environmental, abiotic and nutrient variables that did not conform to a normal distribution (Kolmogorov-Smironov test, $p > 0.05$) were log transformed to remove heteroscedasticity. Some parameters were not measured in CNRL (NAs, pH, NH_4^+, Na^+, K^+, Mg^+, Ca^+, Cl^-, SO_4^-, HCO_3^-, B^+, Fe^+, Si^+, Sr^+) and Shallow wetlands (NH_4^+, Na^+, K^+, Mg^+, Ca^+, Cl^-, SO_4^-, HCO_3^-, B^+, Fe^+, Si^+, Sr^+). The mean of these parameters in other reference wetlands was used in the PCA analysis for CNRL and Shallow wetlands. There were more variables than cases, so only 8 variables (pH, conductivity, salinity, temperature, DO, NAs, DOC and SO_4^-) were used in the first PCA since they were deemed most likely to affect C emissions. The other variables were included in additional PCA to see if they loaded onto factors determined in the primary PCA (salinity & sulphate, abiotic factors and DOC availability) (Fig. 4.3 to 4.5).

Fig. 4.2 – Graphical examples of least squares regressions of CO_2 concentrations vs. time in (a) 4-m CT and (b) BP. All graphs of least squares regressions of CO_2 & CH_4 vs. time are in appendix 4.2 & 4.3, respectively.





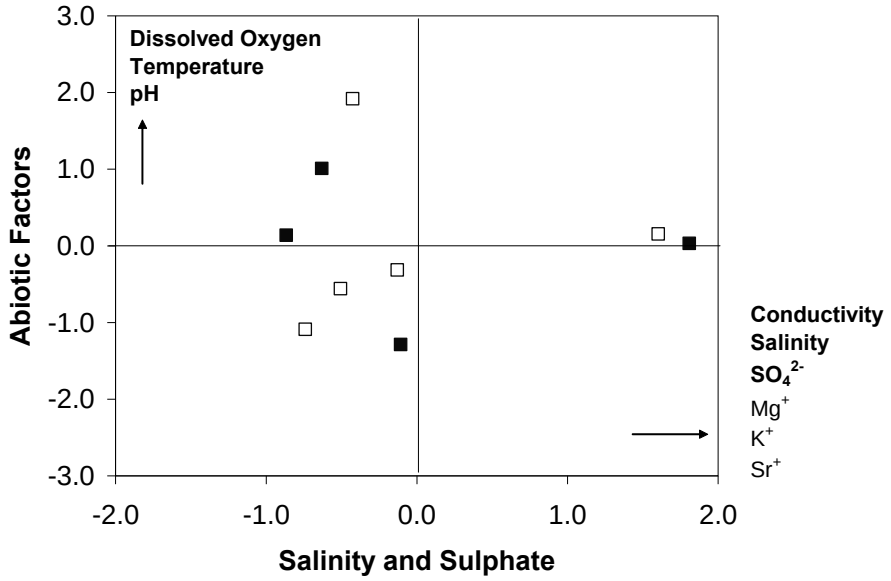


Fig. 4.3 - PCA plot of salinity and sulphate (factor 1) vs. abiotic factors (factor 2) for reference (□) and OSPM wetlands (■). **Bolded** variables represent variables used in the first PCA that loaded with a factor 1, 2 or 3. Non-bolded variables represent other variables included in additional PCA that loaded on the primary PCA factors (salinity and sulphate, abiotic factors and DOC).

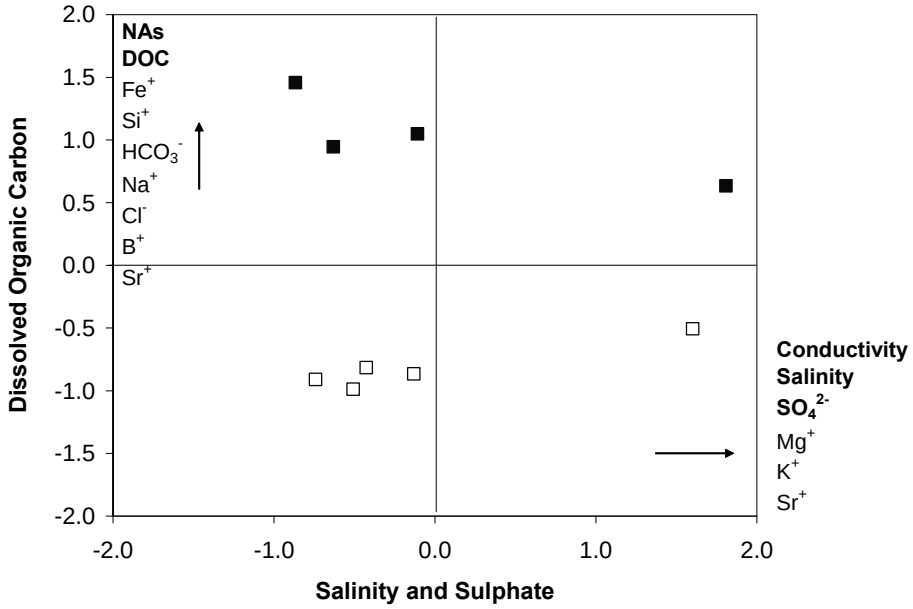


Fig. 4.4 - PCA plot of salinity and sulphate (factor 1) vs. dissolved organic carbon (factor 3) for reference (□) and OSPM wetlands (■). **Bolded** variables represent variables used in the first PCA that loaded with a factor 1, 2 or 3. Non-bolded variables represent other variables included in additional PCA that loaded on the primary PCA factors (salinity and sulphate, abiotic factors and DOC).

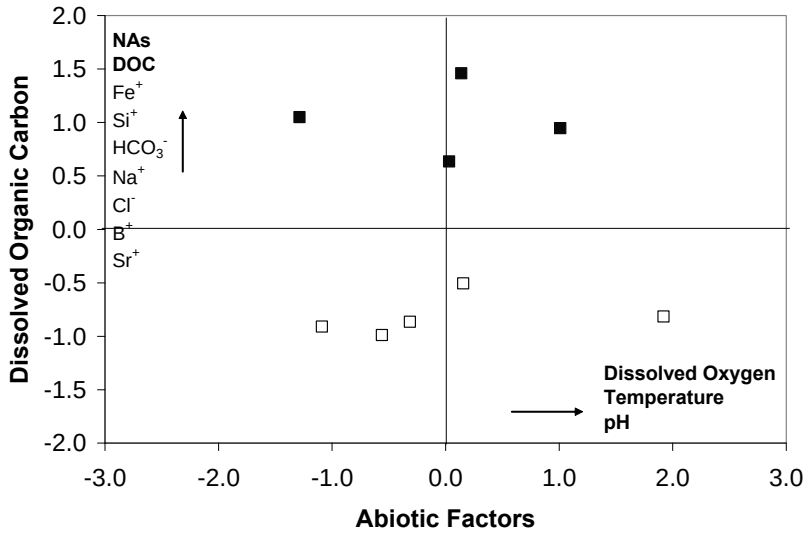


Fig. 4.5 - PCA plot of abiotic factors (factor 2) vs. dissolved organic carbon (factor 3) for reference (□) and OSPM wetlands (■). **Bolded** variables represent variables used in the first PCA that loaded with a factor 1, 2 or 3. Non-bolded variables represent other variables included in additional PCA that loaded on the primary PCA factors (salinity and sulphate, abiotic factors and DOC).

Parametric tests (Statistica 6.0, Statsoft Inc. 1998) were used to evaluate data since CO_2, CH_4 and pCO_2 values conformed to a normal distribution (Kolmogorov-Smironov test, $p > 0.05$). Three-way factorial analysis of variance (ANOVA) was used to test whether the CO_2 and CH_4 values were significantly related to age, organic base, and/or reclamation type. A main effect ANOVA was used to test whether pCO_2 was significantly related to age, organic base, and/or reclamation type. Regression analysis was used to test whether there was a significant relationship between gases and partial pressure with water chemistry and nutrient parameters. The null hypothesis was rejected at $\alpha = 0.05$.

Results

Rates of CO_2, CH_4 and pCO_2 flux were compared among wetlands to examine the variation in gas efflux due to anthropogenic effects, reclamation strategies and age, and to estimate the contribution of wetland carbon lost to the atmosphere. The rates of CO_2 and CH_4 efflux measured in microcosm chamber experiments varied among wetland classes according to physico-chemical properties. Mean pCO_2 ranged from 69 (n=1) to 2717±897 (n=2) µatm (Table 4.1). Mean CO_2 flux ranged from -16.80±2.16 (n=10) to 8.40±2.16 (n=15) mg $m^{-2}d^{-1}$ (Table 4.2). Mean CH_4 flux ranged from 0.48±0.46 (n=10) to 5.36±2.08 (n=14) mg $m^{-2}d^{-1}$ (Table 4.3). The CO_2 partial pressure was only calculated for 4 of the 9 study wetlands (Shallow Wetland, Peat Pond, High Sulphate and Natural Wetland) since alkalinity was only calculated for these 4 wetlands around the same time that CO_2 and CH_4 measurements from microcosms were made (Table 4.1).
Alkalinity, pH, salinity and temperature parameters used for the pCO_2 calculations were collected in June 27 and July 22, 2005 prior to CO_2 and CH_4 samples collections from microcosms between July 26 and August 11, 2005. Due to equipment malfunction during the middle of this study fewer than the expected 15 CO_2 and CH_4 flux samples per wetland (3 time points/location; 5 locations/wetland) were collected in Natural Wetland, High Sulphate, 4-m CT Wetland and Shallow Wetland (Table 4.2 & 4.3).

Table 4.1 – Parameters used to calculate CO_2 partial pressure (µatm).

Wetland	**Date**	**n**	**Alkalinity (mol/L)**	**pH**	**Salinity (mol/L)**	**Temperature (^oC)**	**α_{H+} (mol/L)**	**α_{HCO3-} (mol/L)**	**K_H (mol/L·atm)**	**Mean pCO_2 (µatm)**
SW	July 20 & 22, 2005	2	173	7.2	0.2	17.5	$5.83x10^{-8}$	0.001	0.0415	2717±897
PP	July 22, 2005	1	213	9.0	0.4	20.1	$1.00x10^{-9}$	0.001	0.0391	69
NW	June 27, 2005	3	391	9.0	0.6	15.5	$1.10x10^{-9}$	0.002	0.0441	109±9
HS	June 27, 2005	3	247	7.9	1.4	22.2	$1.21x10^{-8}$	0.001	0.0369	986±289

Table 4.2 – Statistics for CO_2 flux from wetland sediments.

Wetland	Sample No.	Slope(±SE) ($mg\ m^{-2}d^{-1}$)	R^2	p	Reclamation Type	Age	Organic Base
CNRL	15	3.12±0.72	0.59	<0.001	Reference	Young	Poor
4-m CT	10	-16.80±2.16	0.89	<0.0001	OSPM	Young	Rich
NW	10	-7.20±0.96	0.89	<0.0001	OSPM	Older	Rich
HS	15	3.84±0.96	0.54	<0.01	Reference	Older	Rich
PP	15	5.04±1.20	0.38	<0.001	Reference	Young	Rich
MP	15	2.40±0.24	0.90	<0.0001	OSPM	Young	Poor
SW	13	6.96±1.92	0.52	<0.01	Reference	Older	Poor
TP9	15	8.40±2.16	0.56	<0.01	OSPM	Older	Poor
BP	15	15.6±4.80	0.45	<0.01	Reference	Older	Rich

Table 4.3 - Statistics for CH_4 flux from wetland sediments.

Wetland	Sample No.	Slope(±SE) ($mg\ m^{-2}d^{-1}$)	R^2	p	Reclamation Type	Age	Organic Base
CNRL	15	0.11±0.11	0.20	0.09	Reference	Young	Poor
4-m CT	10	-0.48±0.46	0.12	0.32	OSPM	Young	Rich
NW	10	-0.21±0.38	0.04	0.60	OSPM	Older	Rich
HS	10	0.02±0.01	0.23	0.06	Reference	Older	Rich
PP	15	4.20±1.57	0.37	0.02	Reference	Young	Rich
MP	15	0.17±0.11	0.16	0.14	OSPM	Young	Poor
SW	14	5.36±2.08	0.36	0.02	Reference	Older	Poor
TP9	15	0.85±0.32	0.38	0.02	OSPM	Older	Poor
BP	15	1.71±0.78	0.29	0.05	Reference	Older	Rich

Principal Components Analysis (PCA) was used to combine environmental, abiotic and nutrient variables (pH, specific conductivity, salinity, temperature, DO, NAs, DOC, NH_4^+, Na^+, K^+, Mg^+, Ca^+, Cl^-, SO_4^-, HCO_3^-, B^+, Fe^+, Si^+, Sr^+) into indices of salinity & sulphate, abiotic factors, and DOC availability, which explained 32.3%, 25.2%, and 32.03% of the total variability, respectively. The other variables were included in additional PCA to see if they loaded onto factors determined in the primary PCA (salinity and sulphate, abiotic factors and DOC) (Fig. 4.3 to 4.5).

There was no relationship between pCO_2 and PCA factors: salinity and sulphate, abiotic factors and DOC (Regression; $p>0.05$). However, there was a significantly negative relationship between pCO_2 and pH (Fig. 4.6; $R^2 = 0.89$, $p < 0.05$). Figure 4.6 illustrates the dependence of pCO_2 on pH, because pH differed significantly between wetlands with rich and poor organic bases. The calculated partial pressures of CO_2 also differed. The pCO_2 was greater in wetlands with poor organic bases compared to those with rich organic bases (Fig. 4.7; Table 4.4; One-way ANOVA; $p=0.06$)). There was no relationship between pCO_2 and reclamation type (reference vs. OSPM) or age (Table 4.4; One-way ANOVA, $p > 0.05$).

There was no relationship between CO_2 elution and wetland age class, reclamation type or organic base (Table 4.5; Three-way factorial ANOVA; $p > 0.05$).

Carbon dioxide flux was greater from wetlands with higher dissolved organic carbon availability (Fig. 4.9; Regression analysis; $p = 0.07$). Carbon dioxide flux was unrelated to the PCA factors salinity & sulphate, and abiotic factors (regression analysis; $p > 0.05$).

The CO_2 flux estimates obtained from both methods suggested that High Sulphate and Shallow Wetland were carbon sinks at the time of sampling (Table 4.6). The pCO_2 values calculated for High Sulphate and Shallow Wetland indicated that both wetlands were supersaturated with respect to the atmospheric CO_2 concentration, which was 378 μatm in 2005 (http://www.cmdl.noaa.gov/ccgg/trends/). CO_2 gas evolution was recorded

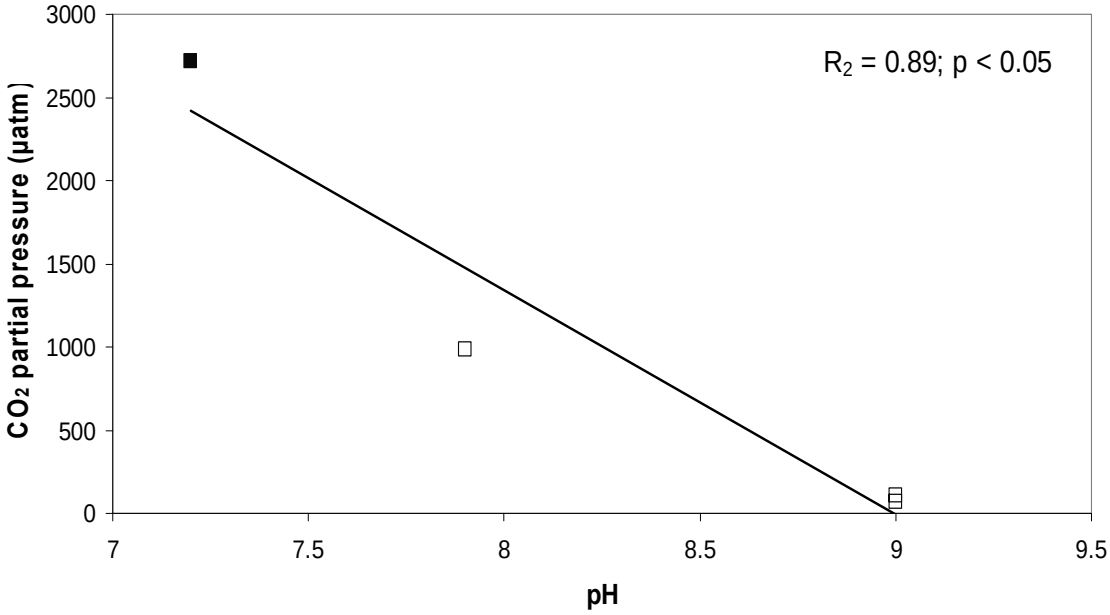


Fig. 4.6 – The relationship between pCO_2 (µatm) and pH (n=4) in wetlands with rich organic bases (□) and wetlands with poor organic bases (■).

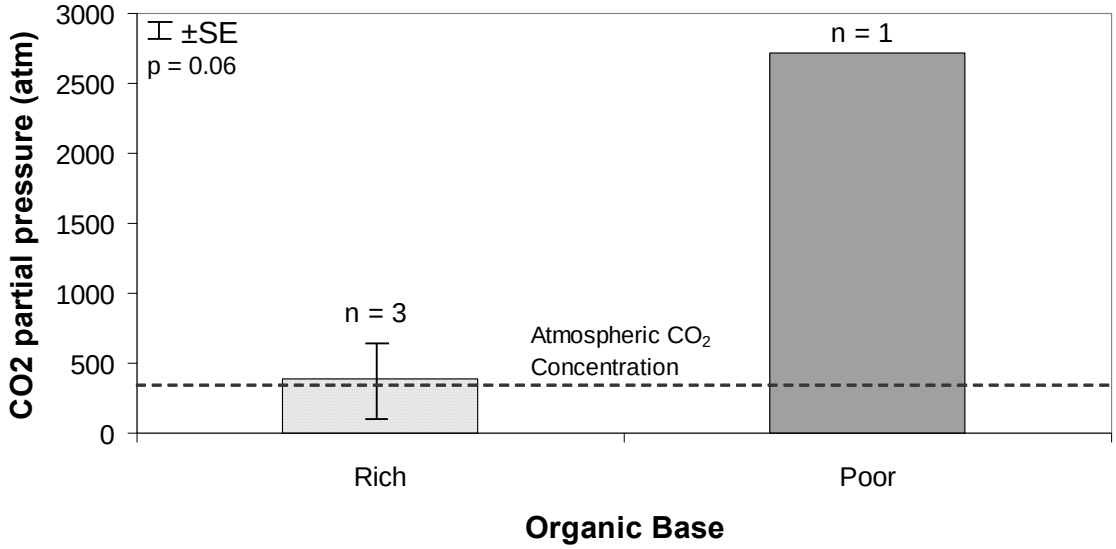


Fig. 4.7 – Estimated CO_2 partial pressure (μatm) in wetlands with rich or poor organic bases. The dashed line represents atmospheric pCO2.

Table 4.4 – One-way ANOVA examining the effects of pCO_2 on reclamation type, age and organic base.

Effect	**D.F.**	**Sum of Squares**	**Mean Square**	**F**	**p**
Reclamation Type	1	989,000	989,000	0.55	0.54
Age	1	1,083,002	1,083,002	0.62	0.52
Organic Base	1	4,068,181	4,068,181	15.15	0.06

Table 4.5 – Three-way ANOVA examining the effects of CO_2 flux from wetlands with different reclamation types, ages and organic bases.

Effect	**D.F.**	**Sum of Squares**	**Mean Square**	**F**	**p**
Age (A)	1	77.57	77.57	0.55	>0.05
Organic Base (B)	1	120.96	120.96	0.86	>0.05
Reclamation Type (R)	1	192.94	192.94	1.37	>0.05
A*B	1	2.63	2.63	0.02	>0.05
A*R	1	6.63	6.63	0.05	>0.05
O*R	1	207.82	207.82	1.48	>0.05
Discrepance	2	70.16	140.33		

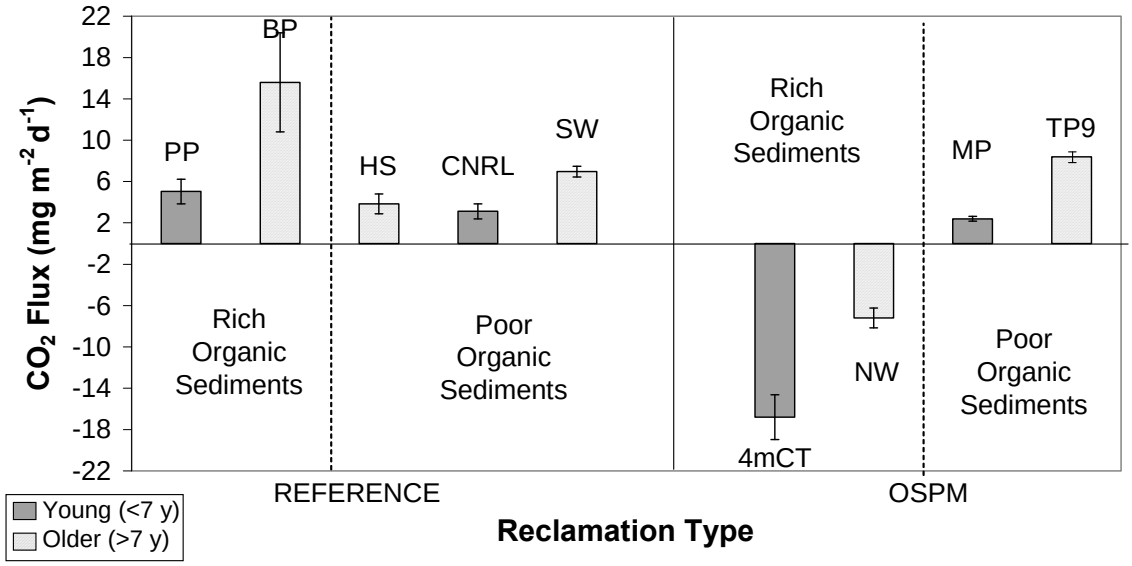


Fig 4.8 - Mean (±SE, n=9) CO_2 flux (mg m^{-2} d^{-1}) in reference and OSPM wetlands of contrasting ages and sediment organic bases.

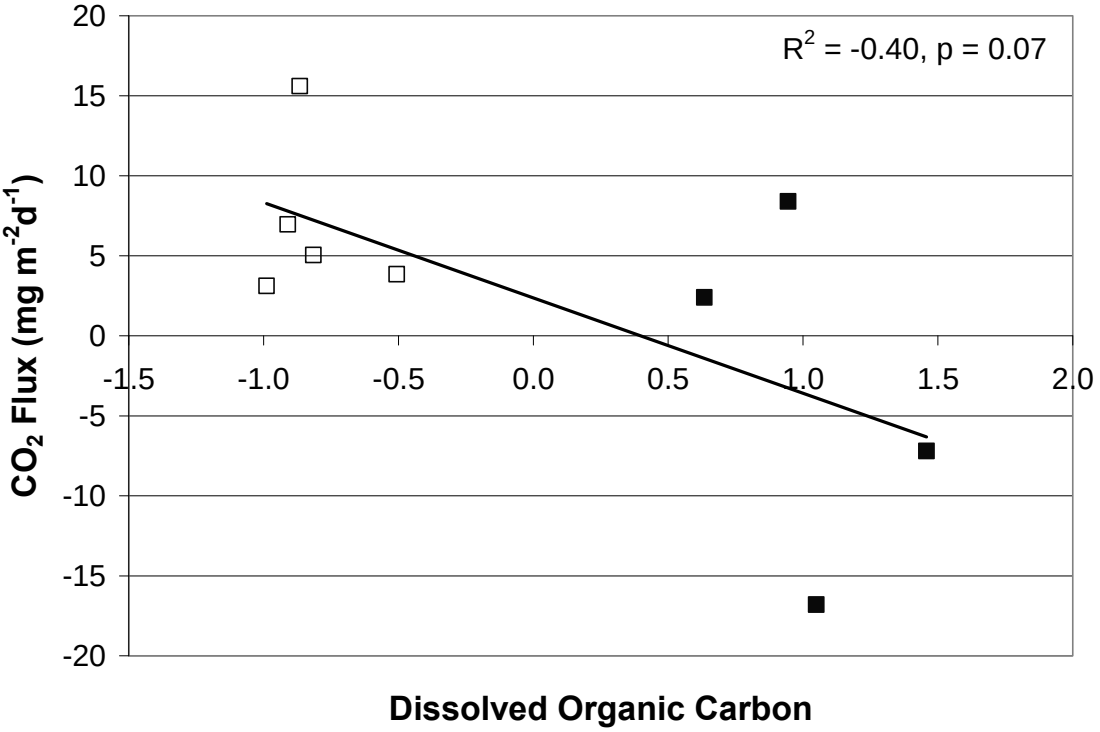


Fig. 4.9 - Carbon dioxide flux (mg $m^{-2}d^{-1}$) in reference (□) and OSPM-affected (■) wetlands with varying levels of dissolved organic carbon.

Table 4.6 – Comparison between calculated pCO_2 (µatm) values and in situ measurements of CO_2 flux (mg m^{-2} d^{-1}).

Wetland	**Mean pCO_2 (µatm)**	**Mean CO_2 Flux (mg m^{-2} d^{-1})**	**C Source or Sink**
Shallow Wetland	2717±897	0.29	Source
High Sulphate	986±289	0.16	Source
Natural Wetland	109±9	-0.30	Sink
Peat Pond	69	0.21	Sink or Source?

from microcosms within these wetlands. The pCO_2 value indicated that Natural Wetland was undersaturated, and gas consumption was measured the microcosms in this wetland (Table 4.6). However, calculated pCO_2 and measured CO_2 flux did not correlate in Peat Pond (Table 4.6).

There was no relationship between methane efflux and reclamation type, age or organic base (Table 4.7; Three-way factorial ANOVA, $p > 0.05$).

There was no significant relationship between CH_4 flux and abiotic factors, DOC or salinity & sulphate (for example, Fig. 4.10; Regression analysis, $p > 0.05$). The relationship between CH_4 flux and salinity and sulphate were examined independently of each other. There was a trend whereby methane flux was negatively correlated with SO_4^{2-} concentration (Fig. 4.11; $r_s = -0.77$, $p = 0.07$). The CH_4 emissions were greatest in Shallow Wetland, which also had the lowest sulphate concentration (Table 4.3). Similarly, the relationship between methane flux and salinity was examined independently of SO_4^{2-}. According to a non-parametric test, there was a no relationship between methane flux and salinity (Fig. 4.12; $r_s = - 0.48$, $p = 0.19$).

Table 4.7 – Three-way factorial ANOVA examining the effects of CH_4 flux from wetlands with different reclamation types, ages and organic bases.

Effect	**D.F.**	**Sum of Squares**	**Mean Square**	**F**	**p**
Reclamation Type (R)	1	13.89	13.89	0.67	>0.05
Age (A)	1	1.09	1.09	0.05	>0.05
Organic Base (B)	1	0.69	0.69	0.03	>0.05
R*A	1	0.12	0.12	0.01	>0.05
R*B	1	0.23	0.23	0.01	>0.05
A*B	1	10.79	10.79	0.52	>0.05
Discrepance	2	10.34	20.68		

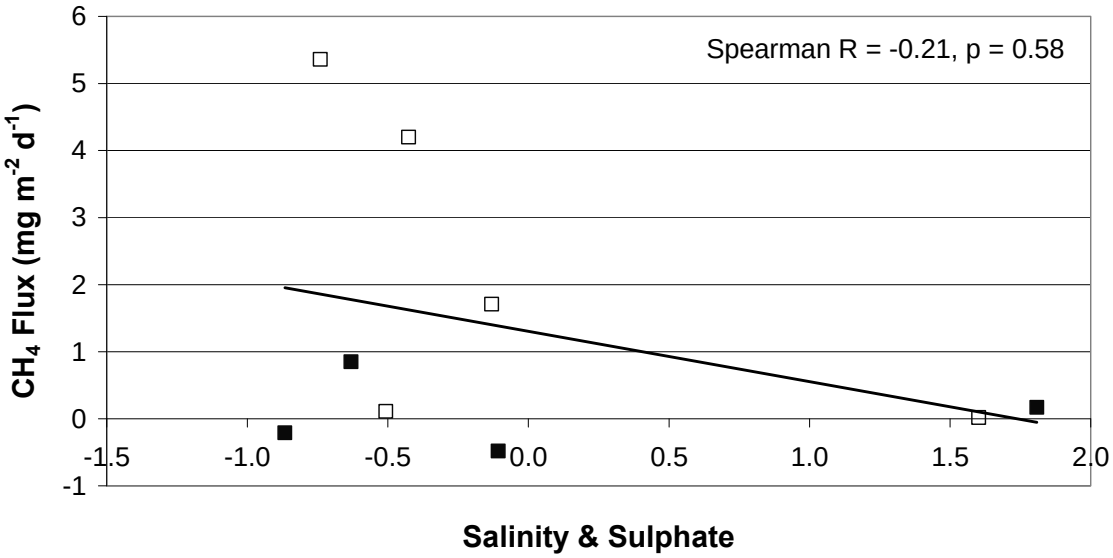


Fig 4.10 – Methane flux from reference (□) and OSPM (■) wetlands with varying levels of salinity & sulphate (factor 1) (n=9).

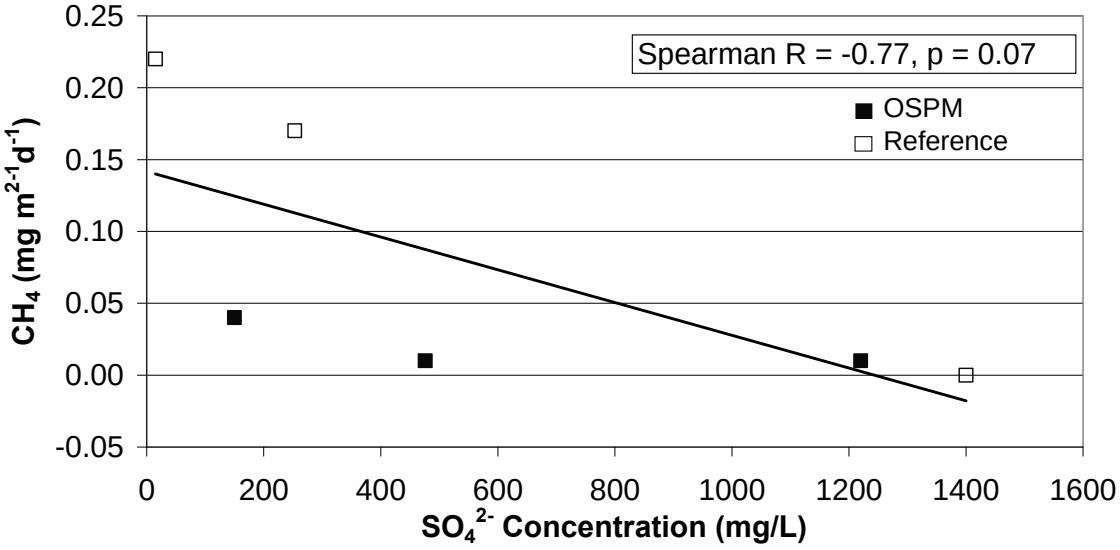


Fig 4.11 – The relationship between methane flux (mg m^{-2} d^{-1}) and sulphate ion concentration (mg/L) in wetlands (n=6).

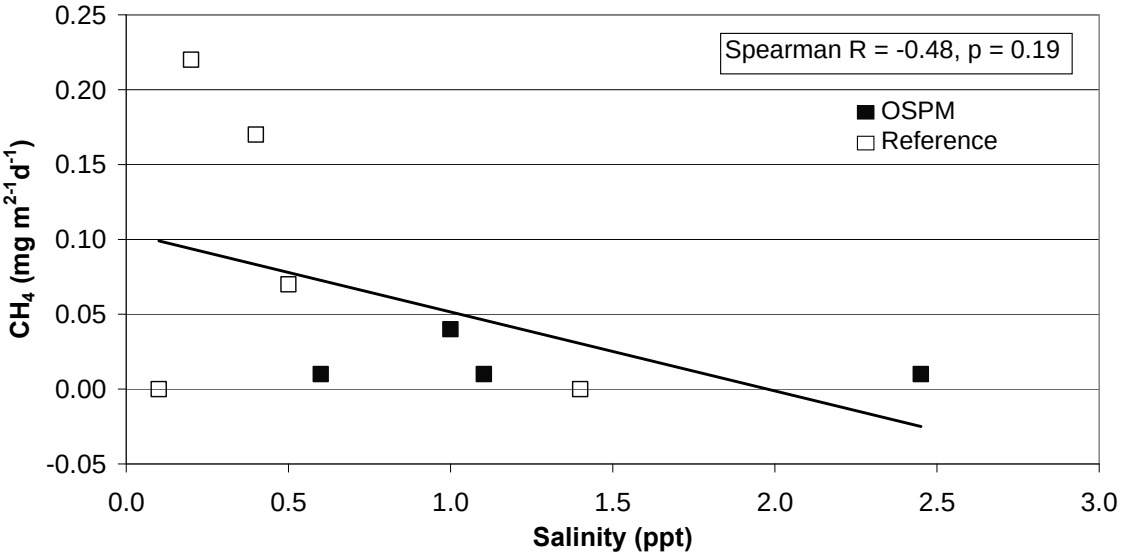


Fig 4.12 – The relationship between methane flux (mg m^{-2} d^{-1}) and salinity (mg/L) in wetlands (n=9).

Discussion

CO_2 Partial Pressure

Partial pressure was calculated in order to determine if wetlands were supersaturated with respect to the atmosphere and hence C sources (net exporters), or undersaturated with respect to the atmosphere and thus C sinks. Partial pressure was compared with field measurements of CO_2 flux (Table 4.6). There was good agreement between measurements of CO_2 flux and calculations of pCO_2, with the exception of Peat Pond (see below), since wetlands that produced CO_2 emissions from microcosms (Shallow Wetland and High Sulphate) were supersaturated in CO_2 with respect to the atmosphere according the pCO_2 calculations. Conversely, CO_2 was a sink in Natural Wetlands microcosms and was undersaturated with respect to atmospheric CO_2 according to pCO_2 calculations. Furthermore, partial pressure estimates were high for wetlands in which the highest mean CO_2 flux was observed (Table 4.6). Therefore, there was good consistency between measured gas flux and calculated pCO_2. Partial pressure could only be calculated for 4 of the 9 wetlands since alkalinity was not measured in all wetlands during the same period that the microcosm experiment took place. Future studies should calculate pCO_2 values for all wetlands studied.

According to the microcosm measurements, Peat Pond produced 2.83 mg CO_2 $m^{-2}d^{-1}$. The pCO_2 calculated from water chemistry measurements was 69 μatm and indicated that this wetland was undersaturated and should not have produced CO_2 emissions. Perhaps the microcosm was disturbed in Peat Pond (e.g. wind action) leading to overestimates of CO_2 flux. It is also possible that biological activity altered water chemistry between the time water chemistry measurements made on July 22, 2005 and gas flux measurements from microcosm were collected from August 2-5, 2005. Algal blooms were observed in Peat Pond during July. However, algal production declined through August as ambient temperatures fell. High rates of photosynthesis can reduce the pool of CO_2 (aq) in aquatic systems, resulting in less CO_2 evasion (Kling et al. 1992). Changes in photosynthetic activity in Peat Pond between the two sampling periods, spanning 2 weeks, may have caused differences between pCO_2 and CO_2 flux. In 2004, pH measured

in Peat Pond surface waters between June 18 and July 30, 2004 ranged between 7.7 and 8.5 (n=5) indicating that pH varies seasonally in this wetland. Future studies should collect gas flux measurements and the variables (pH, temperature, salinity and alkalinity) for calculating pCO_2 simultaneously.

The pCO_2 measured in the study wetlands ranged from 69 to 3,352 µatm and were within the range of measurements reported for similar freshwater environments (Appendix 4.4). Striegl et al. (2001) reported that pCO_2 values for north temperate and boreal lakes in US and Finnish lakes (n=34) ranged from 85 to 5,083 (mean 1,372) µatm and 770 to 42,339 (mean 4,994) µatm, respectively. Lakes from boreal Sweden had a mean±SE pCO_2 of 1,469±38 µatm and non-Arctic Lakes in North America, 1,087±23 µatm (Cole et al. 1994). Summer pCO_2 in 20 temperate lakes in Québec, Canada ranged from 139 to 1,020 µatm (Del Giorgio et al. 1999). Measurements of the pCO_2 in 25 lakes and 4 rivers across arctic Alaska, USA, showed values of 93 – 2,758±1215 (1,162±134) µatm and 369 – 845±182 (611±126) µatm, respectively (Kling et al. 1991).

Carbon Dioxide Evolution

The average rates of carbon dioxide flux in the study wetlands ranged from -16.80±2.16 to 15.6±4.80 mg $m^{-2}d^{-1}$ and were at the low end of the range of measurements reported for other freshwater systems. CO_2 flux from 25 Arctic lakes sampled during the summer from 1975-1989 ranged from -242.1 to 2,631.9 (mean 919.8) mg $m^{-2}d^{-1}$ (Kling et al. 1991). Mean CO_2 fluxes from a salt marsh in New Brunswick, Canada during the summer ranged from 300 to 3,700 mg C m^{-2} d^{-1} with an overall average of 2,500 mg C m^{-2} d^{-1} (Magenheimer et al. 1996). Total CO_2 emissions from a river floodplain in Georgia, USA averaged 2,518 mg m^{-2} d^{-1} (Pulliam 1993). CO_2 emissions from a harvested bog in Eastern Québec, Canada were 1,310 mg C m^{-2} d^{-1} in 2000 and 1,282 mg C m^{-2} d^{-1} in 2001 (Petrone et al. 2003). Measurements of CO_2 fluxes from salt, brackish and freshwater marshes in Louisiana, USA had accumulations of 1145, 493, 1693 mg C m^{-2} d^{-1}, respectively (Smith et al. 1983). CO_2 emissions measured from boreal peatlands ranged from 1872 to 6216 mg C m^{-2} d^{-1} (Silvola et al. 1996). A Nova Scotia wetland produced 456.14 to 1029.53 (mean 576.96) mg m^{-2} d^{-1} CO_2 (Clair et al. 2002). Daily rates of

carbon dioxide evolution from fens in Québec, Canada ranged from 822 to 1808 mg m^{-2} (Moore 1986).

Carbon dioxide production is the result of the mineralization of soil organic C and plant respiration (Blodau 2002). Numerous environmental factors have been reported to control carbon mineralization processes such as the availability of oxygen associated with the depth of the water table, microbial activity in the peat, soil temperature, type of vegetation, and chemical characteristics of the peat (Johnson and Damman 1991; Moore and Dalva 1993; Updegraff et al. 1996; Yavitt et al. 1997). No systematic pattern of CO_2 flux was observed in the study wetlands with respect to water chemistry parameters (salinity & sulphate, abiotic factors and DOC). There was no relationship between CO_2 production from wetland sediments and microbial biomass in the water column (chapter 3) (Table 3.4) (Regression analysis, $p > 0.05$). However, much less variation in exchange rates can be explained by environmental and ecological variables on smaller spatiotemporal scales, (Bellisario et al. 1999; Moore et al. 1994; Shannon and White 1994). It is possible that some physico-chemical variables controlled CO_2 flux in these wetlands since variables were measured in the water column and not in the sediment. Relationships between physico-chemical parameters in the soil may provide more insight into CO_2 production dynamics.

Patterns and Rates of Carbon Dioxide Flux

The mean pCO_2 for Peat Pond and Natural Wetland were undersaturated by factors of 0.2 and 0.3, respectively, with respect to the atmosphere. Mean pCO_2 in Shallow Wetland and High Sulphate were supersaturated by 7.2 and 2.6 times, respectively, with respect to the atmosphere. The mean±SE pCO_2 for the study wetlands as a group was 976.4±1124.6 µatm (n=9) and thus 2.6 times supersaturated with respect to the atmosphere. The control wetland, a natural beaver pond, displayed higher CO_2 production rates than all other study wetlands and had the second highest CH_4 production rate of all the wetlands. Overall, these wetlands currently appeared to be small sources of exported C, i.e, the amount of C extracted from the atmosphere during photosynthesis was slightly less than that respired and lost to the atmosphere (Bilings 1987).

Higher pH causes lower CO_2 solubility (Hausser 2001). Consequently, Shallow Wetland, which had the lowest pH (7.4), had the highest pCO_2 values compared to the other wetlands. In comparison Natural Wetland (pH 9.0), High Sulphate (pH 7.94) and Peat Pond (pH 9.0) had higher pH and lower pCO_2 values (Appendix 4.4).

There was no significant difference in pCO_2 or CO_2 production rate between OSPM-affected and reference wetlands. Also, CO_2 production in wetlands was negatively correlated with increasing DOC, where the highest concentrations of DOC were quantified in OSPM-affected wetlands. More DOC is present in OSPM affected wetlands because of elevated NA concentrations (Videla 2006). These results indicate that microbial decomposition rates in the sediments were not stimulated by OSPM such as petroleum, PAHs and naphthenic acids which are composed of refractory carbon.

Peat amendments provide benefits to the reclaimed wetlands, such as increasing nutrient availability in OSPM and providing structure for aiding in vegetation establishment (OSSWG 2000). Rapid revegetation in turn provides surface area that enhances the growth of microbes required to degrade chemicals and promote rapid detoxification of materials such as NAs (OSWWG 2000). There is a concern that peat amendments may be rapidly decomposed by microbes and that the benefits of this reclamation strategy may not be sustainable and thus are not cost-effective. According to data in this study there was no significant difference in CO_2 production between wetlands with rich and poor organic substrates. Although, there was a trend where organic base affected the pCO_2 in wetlands. The pCO_2 was greater in wetlands with poor organic sediments compared to richly organic sediments. Results suggest that rates of microbial decomposition of peat amendments were low, perhaps because the peat may already be highly decomposed and of low substrate quality. Additionally, there was no significant difference in pCO_2 or CO_2 production rate between young (< 7 years) and older (≥ 7 years) wetlands, indicating that microbial decomposition rates in the sediments do not increase with wetland age, contrary to my conjecture. If the goal of restoration is to accelerate the potential for succession while minimizing further losses of carbon to the atmosphere, amending

wetland sediments with peat during post-mining reclamation does not appear to accelerate carbon loss over time.

Patterns and Rates of Methane Evolution

Northern peatlands act as sinks by incorporating dissolved C into accumulating peat and organic matter. However, northern peatlands are also important emitters of C in the form of the greenhouse gas methane (Harris 1993). Methane is produced by methanogens, methane producing bacteria (Segers 1998). Methanogens produce CH_4 in anoxic sediments as an end product of decomposition of organic matter (Kankaala et al. 2003). Methane escapes from wetland sediments via diffusion, bubbling (Chanton et al. 1989; Schütz et al. 1989; Williams et al. 2007) and through plant mediated processes (Schütz et al. 1989). Methane emissions measured in this study incorporate diffusion and bubbling routes. However, methane production rates may be underestimated as CH_4 was not directly measured from vegetation. Not all of the wetlands were vegetated. For consistency in results, microcosms were installed in sediments where no vegetation was present on the sediment surface.

Methane emission rates for the study wetlands varied little between wetlands and were in the lower ranges reported for boreal wetlands and other similar aquatic environments. The average rates of methane flux in the study wetlands ranged from -0.48±0.46 to 5.36±2.08 mg $m^{-2}d^{-1}$. Total fluxes of CH_4 carbon from a forested floodplain in George, USA were 47 mg m^{-2} d^{-1} (Pulliam 1993). Mean CH_4 fluxes from a salt marsh in New Brunswick, Canada between mid July and September ranged from 0.2 to 11.0 mg C m^{-2} d^{-1} with an overall average of 1.6 mg C m^{-2} d^{-1} (Magenheimer et al. 1996). A Nova Scotia wetland produced 5.37 to 18.82 (mean 7.10) mg m^{-2} $d^{-1}CH_4$ between 1992 and 1998 (Clair et al. 2002). Methane production rates from low boreal wetlands in Canada ranged from -15.9 to 1,011.0 mg m^{-2} d^{-1} (Roulet et al. 1992). Methane production from wetlands in boreal Alberta ranged from 0-22,063.1 mg m^{-2} d^{-1} CH_4 (Vitt et al. 1990). Sebacher et al. (1986) measured methane emissions from boreal peatlands in Alaska. During the summer, 25–199 (mean 89) mg m^{-2} d^{-1} CH_4 were released from a coastal

tundra bog, 9–78 (mean 30) mg m^{-2} d^{-1} CH_4 a meadow tundra bog, and an average of 148 mg m^{-2} d^{-1} CH_4 from an alpine fen and marsh.

Spatial variability in CH_4 emissions was large within a wetland. High rates of release of methane carbon occurred irregularly even within a wetland. For example, 39% of the total CH_4 wetland emissions came from one of the five sites in Shallow Wetland (Appendix 4.5). Erratic escape of large bubbles or continuous release of tiny bubbles could account for some of the variance observed in the study wetland CH_4 emissions. The significance is that ebullition transports methane directly to atmosphere, bypassing the mediating effects of an oxygenated water column (Chanton and Martens 1988; Chanton et al. 1989). Copious amounts of gas were released from PP, BP, NW and SW sediments when individuals walked through parts of the wetland, prior to sample collection. However, there was no significant difference in CH_4 evolution between wetlands, even in wetlands where bubbling was observed. I had expected to measure higher methane concentrations in PP, BP, NW and SW because of the observed bubbling. Perhaps the microcosms were randomly installed on locations where bubbling did not occur due to the heterogeneity of wetland sediments, or gas pockets are trapped so deeply in the substrate that only severe compression permits bubbles to escape.

Methane emission measurements reported are of net methane release to the atmosphere with no attempt made to account for microbial oxidation of methane within the water column. Oxidation presumably increases with water column height and degree of stratification; thus, with all other factors being equal, one would expect more oxidation of methane in deeper wetlands, and consequently lower net flux (Rudd & Taylor 1980). The water depths in many of these wetlands are less than 1 m and thus considerable oxidation of methane is possible. In the Florida Everglades, more than 90% of methane production is consumed by methanotrophs before it diffuses to the atmosphere (King et al. 1990). (Sundh et al. 200) determined that the methanotroph biomass-specific oxidation rate ranged from 0.001 to 2.77 mg CH_4-C mg^{-1} C d^{-1} (Sundh et al. 2005). However, at high salinities (>9%) methane oxidation was completely inhibited, despite the presence of ample methane and oxygen (Conrad et al. 1995). Methane oxidation in

the OSPM-affected wetlands may be minimal or inhibited because their salinities range from 0.6 to 2.5 ppt Nevertheless, future studies should examine methane-oxidizing bacteria in constructed wetlands as they represent a potentially important pathway for reentry of carbon and energy into pelagic food webs that would otherwise be lost as evasion of CH_4 (Sundh et al. 2005).

Methanogenesis Inhibition by Sulphate

In this study, SO_4^{2-} was a weakly significant variable in determining CH_4 emission rates. Methane evolution was greatest in wetlands with the lowest SO_4^{-} concentrations, which were also the reference wetlands, except for High Sulphate wetland, which had the highest sulphate concentration. Methanogenesis was likely limited by the higher concentrations of sulphate present in OSPM-affected wetlands. Methanogenesis is limited in environments with high concentrations of sulphate ions (SO_4^{2-}) (Schlesinger 1997) since sulphate-reducing bacteria (SRB) are more effective competitors for the same compounds (Schönheit et al. 1982). Alternative electron acceptors, like NO^{3-}, Fe^{3+}, Mn^{4+}, and SO^{2-}_4 and possibly humic acids (Lovley et al. 1996), suppress methanogenesis because reduction of alternative electron acceptors supplies more energy than methanogenesis (Zehnder & Stumm 1988). SRB and methanogens both use acetate (Holowenko et al. 2000) and H_2 as a source of electrons. SRB are also more efficient in the uptake of H_2 than methanogens engaging in CO_2 reduction (Achtnich et al. 1995; Holowenko et al. 2000). Methanogenesis begins when sulphate is depleted (Abram and Nedwell 1978).

Fedorak et al. (2002) observed methane production in consolidated tailings (CT) and mature fine tailings (MFT) from Shell Canada Ltd. amended with acetate and glucose, In most cases, methane production was observed when the sulphate concentration was 17-20 mg/L (Fedorak et al. 2002). During the early and mid-1980's no methanogenesis was observed in Mildred Lake Settling Basin (MLSB) when sulphate concentrations were 60 – 120 mg/L in shallow (11-16 m thick) fine tailings and 35 – 70 mg/L in the deep (17-23 m thick) fine tailings areas (MacKinnon 1989 cited in Fedorak et al. 2002). In 1991, methane emissions were observed from MLSB (Holowenko 2000) when sulphate

concentrations were 30-40 mg/L in the shallow fine tailings (11-16 m) and <20 mg/L in the deep fine tailings (17-23 m) (MacKinnon and Sethi 1993 cited in Fedorak et al. 2002). In the current study, the greatest methane emissions were from Shallow Wetland, which also had the lowest sulphate concentrations (15 mg/L). These field observations are in reasonable agreement with lab observations of Fedorak et al. (2002). Somewhat higher methane emissions were measured in Peat Pond, Beaver Pond and Test Pond 9. However, the sulphate concentrations in the water column of Peat Pond and Test Pond 9 were 253 and 150 mg/L, respectively (Appendix 2.5). Sulphate concentrations were not measured in Beaver Pond. Sulphate concentrations are likely lower in sediment pore water, perhaps below 20 mg/L, thus accounting for the higher methane emissions.

The heterogeneity of wetland sediments could also possibly provide microhabitat for methanogens when sulphate concentrations are above 20 mg/L. Trace amounts of dissolved and entrapped methane were found in Syncrude and Suncor CT in laboratory mesocosm studies, even though the sulphate concentration in the Suncor CT mesocosm was 859 mg/L and was approximately 1,290 mg/L in Syncrude CT (Fedorak et al. 2003). Although methanogenesis usually occurs after most of the sulphate is depleted in an environment, simultaneous methane production and sulphate reduction have been observed (Mitterer et al. 2001; Purvaja and Ramesh 2001). Methanogens and SRB are able to co-habit and co-generate their respective gases when there is an abundant supply of electron donors, such as readily usable organic compounds or H_2 (Fedorak et al. 2003). Mitterer et al. (2001) examined Australian marine sediments to depths of 600 m for pore-water sulphate, H_2S and methane concentrations. They determined that sulphate concentrations ranged from approximately 1,400 to 4,800 mg/L, and H_2S and methane were detected in virtually all depths, suggesting the co-generation of H_2S and methane in the presence of elevated sulphate concentrations. Sansone and Martens (1981) postulated that methanogenesis can occur in "microzones" within sediments that are depleted of sulphate. Several compounds such as methanol, methylated amines, and dimethyl sulphide (termed non-competitive substrates) exist. Sulphate reducers do not have as strong an affinity for these compounds as they do with H_2 and acetate (Purvaja and Ramesh 2001). These noncompetitive substrates may be important CH_4 precursors in the

study wetlands. Thus, the study wetlands may have different sulphate concentrations within sediments, providing microhabitats where sulphate concentrations were depleted by SRB, yielding environments that are suitable for methanogens and methane evolution. Nonetheless, methane production was highest in wetlands with the lowest sulphate concentrations in the water column. Sulphate concentrations need to be measured in the pore-water of study wetlands.

Methane Evolution in relation to Salinity

Studies have indicated that CH_4 emissions are inversely related to salinity. Methane emissions measured in freshwater marsh, brackish marsh and salt marsh of the Gulf coastal area were found to increase with increasing distance from the coast and decreasing salinity (DeLaune et al. 1983). DeLaune et al. (1983) reported emissions from marsh sites having salinities of 0.4, 1.8 and 18 ppt (daily fluxes of 584, 266, and 16 mg CH_4 m^{-2}, respectively). Purvaja and Ramesh (2001) reported a clear negative correlation between salinity and CH_4 emission from a coastal wetland system in India. Daily methane emissions ranged from 74.4 – 517.44 mg m^{-2} d^{-1}, with the lowest emissions (approximately 0-144 CH_4 mg m^{-2} d^{-1}) occurring at sites with the highest salinities (>24 ‰) and the highest emissions (approximately 480 – 1128 mg CH_4 m^{-2} d^{-1}) occurring in sites with the lowest salinities (<15‰) (Purvaja and Ramesh 2001). Another study reported that CH_4 emissions from wetland rice fields on saline, low-sulphate soils were lower than CH_4 emissions from otherwise comparable non-saline rice fields indicating that methane production is inhibited by salinity (Denier van der Gon and Neue 1995). Contrary to other studies methane production from these wetlands was not related to salinity.

Studies have demonstrated a co-inhibition of methanogenesis by salinity and sulphate. For example, Ramesh et al. (1997) determined that sulphate and salinity inhibited methanogenesis in a polluted, tropical coastal wetland in Madras City, India. Similarly, in rice fields amended with gypsum, which increased pore water electrical conductivity to 2dS.m^{-1}, CH_4 emissions were reduced by 50-72% (Denier van der Gon and Neue 1994) possibly because SRB outcompete methanogens. Although the conductivity in the

gypsum-amended fields was a factor 2 lower, the reduction of CH_4 emission was much stronger than in the salt-amended fields (Denier van der Gon and Neue 1995). This indicated that salinity accompanied by high sulphate levels reduced methane emissions more strongly than salinity by non-sulphate containing salts (Denier van der Gon and Neue1995). The relationship between sulphate and salinity inhibition on methanogens is less clear. Sulphate constitutes a major component (24-28nM; Capone and Kiene 1988) of the salinity of seawater (~3.5-35 ppt) (http://en.wikipedia.org/wiki/Seawater). Consequently, methanogenesis can be inhibited by SO_4^{2-} rather than by associated salinity. There was a significantly positive correlation (Fig. 4.15) between sulphate concentrations and salinity. OSPM-affected wetlands had elevated SO_4^{2-} and salinity concentrations compared to reference wetlands. However, results from this study suggest that methanogenesis was inhibited by elevated SO_4^{2-} concentrations, characteristic of OSPM-affected wetlands, and not by associated salinity.

Total Carbon Emissions from Wetland Sediments

Averaged over all sampling dates and wetlands, mean total carbon emissions from the study wetland sediments were 15.59 mg m^2 d^{-1}. Methanogenic carbon accounted for 56% of the carbon emissions. Overall, wetland sediments were minor net sources of CO_2 and CH_4 during summer, the most productive season, albeit fluxes are low compared to other wetlands and lakes. CO_2 and CH_4 emissions in this study may be lower compared to other aquatic environments because these estimates pertain only to unvegetated sediments and not the wetland as a whole. Macrophytes can allow for conduit transport of CH_4 (Bubier 1995) and they also respire. Thus, macrophytes likely influenced CO_2 and CH_4 flux from the study wetlands and gas flux from the wetlands as a whole may be different from this study's estimates pertaining only to wetland sediments.

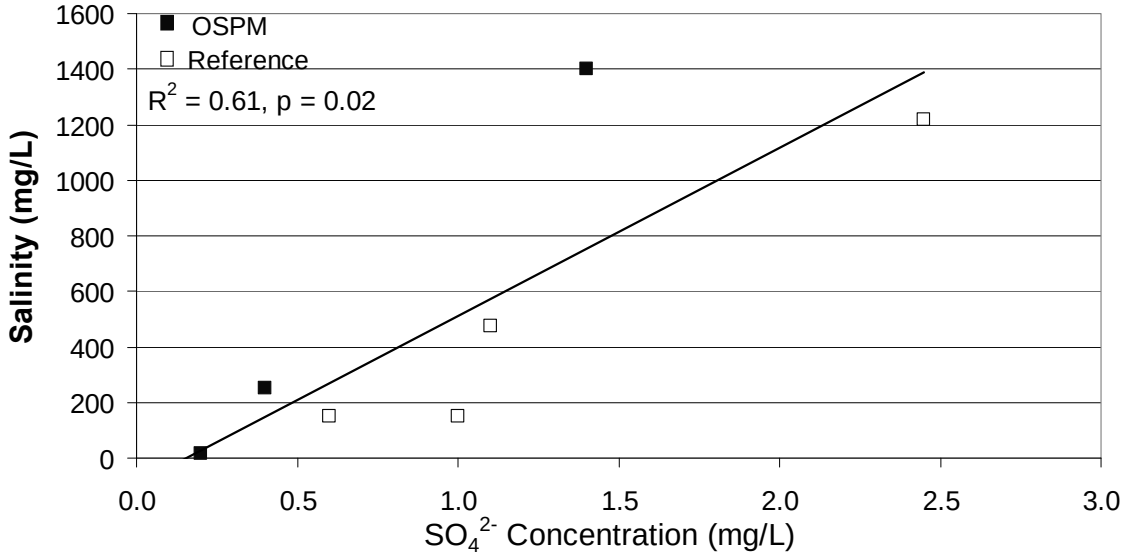


Fig. 4.13- The relationship between sulphate concentration (mg/L) and salinity (mg/L) in reference and OSPM-affected wetlands.

Technique Evaluation

Gaseous fluxes of CO_2 from aquatic sediments to the atmosphere are commonly measured with a static chamber (Pulliam 1993) or can be calculated from the partial pressure differences between air and water (Kling et al. 1991). This microcosm design was advantageous because it (1) allowed for intensive sampling at 5 sites within 9 wetlands over several days, (2) enabled gas measurements to be collected in the natural environment, as opposed to a laboratory study, and (3) was economical for large scale projects such as the current study, compared to more expensive static chambers composed of materials such as Plexiglas.

Future Studies

Further studies can improve upon the insight into CO_2 and CH_4 production dynamics presented in this thesis. Ongoing work should calculate pCO_2 values for all study wetlands since a larger sample size would increase the precision of estimates, and perhaps show a significant correlation between pCO_2 and CO_2 flux. Gas flux measurements and the water chemistry variables (pH, temperature, salinity and alkalinity) needed for calculating pCO_2 should be collected simultaneously. The relationships between physico-chemical parameters and CO_2 flux remain somewhat unclear as physico-chemical parameters were collected in the wetland waters and not sediment pore water.

To improve upon methanogenesis research in the Athabasca wetlands, other studies should examine methane-oxidizing bacteria in constructed wetlands as they represent a potentially important pathway for reentry of carbon and energy into pelagic food webs that would otherwise be lost as evasion of CH_4.

Gas flux rates are likely underestimated as microcosms were placed in sediments away from vegetation. Other studies should examine CO_2 and CH_4 emissions directly from

plants since they may allow for conduit transport of CH_4 (Bubier 1995) and they also respire. Furthermore, the sediments in which vegetation grows likely have different chemistry and microbial activity than unvegetated sediments.

Carbon emissions vary seasonally. This study represents what are potentially the largest CO_2 and CH_4 emissions rates during the year. McNeil and Waddington (2003) examined peatlands in Québec, Canada between May and August, 2000 and reported that gross ecosystem production (g CO_2 m^{-2} d^{-1}) was greatest in the month of August. Cooler and moister conditions beyond August resulted in lowered respiration rates in these peatlands (Petrone et al. 2001). Average emission rates of CO_2 from the Ogeechee River floodplain in Georgia, USA showed the highest rates in summer (Pulliam 1993). Although the largest CO_2 emissions from lakes, and possibly wetlands, commonly occur after ice melt when gases accumulated over winter are released (Michmerhuizen et al. 1996; Striegl and Michmerhuizen 1998). Quantitative results need to be obtained over the whole year to provide understanding of seasonal C dynamics.

Conclusion

Good agreement between measured gas flux and calculated pCO_2 indicated that the microcosm chamber design provided credible gas measurements from the natural environment.

The mean±SE pCO_2 for the study wetlands was 976.4±1124.6 µatm, 2.6 times supersaturated with respect to the atmosphere. Averaged over all sampling dates and wetlands total carbon emissions from unvegetated sediments and overlying water in the study wetlands was 15.59 mg m^2 d^{-1}. Methanogenic carbon accounted for 56% of the carbon emissions. Reference wetland unvegetated sediment areas were minor net sources of CO_2 and CH_4 during summer, the most productive season, albeit fluxes are low compared to other wetlands and lakes.

No difference in C fluxes between oil sands process material-affected wetlands and reference wetlands indicated that anthropogenic effects did not appear to stimulate gaseous CO_2 and CH_4 fluxes from the unvegetated areas of OSPM-affected wetlands.

CO_2 partial pressure values in wetlands with richly organic wetland sediments were low compared to wetlands with poor organic wetland sediments. Furthermore, there was no significant difference in CO_2 production between wetlands with poor and richly organic sediments indicating that there was little microbial decomposition of peat amendments, a reclamation strategy. If the goal of restoration is to minimize further losses of carbon to the atmosphere, amending wetland sediments with peat during post-mining reclamation does not appear to accelerate carbon loss over time.

Methane accounted for a greater percentage of C emissions from wetlands having lower sulphate concentrations. Methane emissions were highly variable both within and among wetlands. Since CH_4 emissions were relatively low, the ability of methanogenesis to increase transport of toxic compounds from OSPM pore waters to surface waters, establish anoxic conditions in wetland water and to potentially exasperate global warming is minimal in the sampled portions of constructed wetlands.

In these early stages of wetland creation, the unvegetated sediments and overlying water of constructed wetlands were minor net sources of C and currently do not appear to be on a trajectory to becoming net sinks, in that there was no difference in rates of gas emissions between age classes. By further understanding how mining affects wetland function, an improved understanding of wetland reclamation will be possible, which in turn will help achieve the goal of restoring the boreal landscape to a net carbon sink.

CHAPTER 5: CONCLUSIONS & RECOMMENDATIONS

A thorough understanding of the role of microbes in carbon cycling in relation to the food webs of oil sands constructed wetlands is important for determination of carbon and energy potentially available to higher trophic organisms, estimation of carbon losses via CO_2 and CH_4 emissions and developing guidelines for sustainable management of these systems. The carbon sources that supported the microbial community and the relative importance of bacteria biomass, production and microbial respiration were determined in 8 constructed wetlands and one naturally formed wetland of varying ages and condition in the boreal region of northern Alberta subject to oil sands mining.

Wetland Food Web Implications

My field research has confirmed previous laboratory observations (Coffin et al. 1997; Videla 2006) that petroleum can be incorporated into microbial biomass using stable isotope analysis. Little previous research has documented the assimilation of petroleum into the natural bacterial community in stable isotope field-based studies. According to the concentration-weighted linear stable isotope mixing model, the calculated proportions of carbon assimilated in microbial biofilm from OSPM-affected wetlands, Test Pond 9, 4-m CT Wetland and Natural Wetland were 68%, 62% and 97% bitumen, respectively. If there were no overlooked sources of carbon in these wetlands, these estimates confirm that oil sands mine-process derived carbon sources were incorporated into microbial biomass in OSPM-affected wetlands. Mean $\delta^{13}C$ measurements of DIC (-9.3±0.2 to -0.6±0.4‰) confirmed that a significant amount of DIC originated from microbial respiration and organic matter decomposition. Additionally, evidence was found for the transfer of carbon and nitrogen assimilated by microbes to higher trophic-level organisms (small chironomids and *Daphnia*). Therefore, microbial assimilation of petroleum indicates that oil sands mine-process derived carbon sources can fuel the microbial component of the food web in these constructed wetlands. This carbon in the bacterial biomass of OSPM-affected wetlands can potentially support higher trophic levels.

To my knowledge no prior research investigating microbial dynamics in tailings pond process waters or wetlands constructed with OSPM materials in the Athabasca region have examined how microbial community biomass changes over time. High molecular weight NAs in process waters have been known to exert toxicity in aquatic organisms (Holowenko et al. 2002), including the Microtox toxicity test bacterium, *Photobacterium phosphoreum* (Lai et al. 1996, MacKinnon and Boerger 1986). However, it is believed that DOC can support microbial growth in those process waters where NAs account for much of the DOC (Fedorak et al. 2003). This study reports a trend where DOC was positively correlated with bacterioplankton community biomass. The highest DOC values and some of the highest bacterioplankton biomass estimates were quantified in older OSPM-affected wetlands, while lower biomass values were estimated in young OSPM wetlands suggesting that NAs in young OSPM wetlands are acutely toxic, whereas NAs in older OSPM wetlands sustain more bacteria community biomass (possibly tolerant species). Less energy may be available for higher trophic organisms in young OSPM wetlands compared to older OSPM wetlands since carbon is known to pass through the "microbial loop" to higher trophic levels (Pomeroy 1974, Azam et al. 1983, Berman et al. 1987). However, overall bacterioplankton biomass in all the study wetlands was higher than quantified in the unconstructed reference wetland, Beaver Pond, indicating that bacterial numbers are not atypical in the oil sands affected wetlands and sufficient energy may be available to higher trophic levels, albeit at lower levels in young OSPM wetlands.

Oil Sands Process Material (OSPM) Effects on the Microbial Community

Stable isotope studies in some of the study wetlands have previously indicated an enrichment of $\delta^{15}N$ in various biota in OSPM-affected wetlands (Ganshorn 2002), although the reason for this enrichment was unclear. Results from this study were consistent with previous studies since microbial biofilm collected in OSPM-affected wetlands was enriched ($\delta^{15}N = 7.32‰$) compared to biofilm collected in reference wetlands ($-\delta^{15}N = 1.85‰$). The $\delta^{15}N$ enriched values quantified in biofilm and other wetland biota in OSPM-affected wetlands were characteristic of volatization and

denitrification. The $\delta^{15}N$ enrichment appears to be the result of nitrogenous compounds introduced via either the bitumen extraction process or wastewater effluent disposal into mine process waters that were used in wetland construction.

This research demonstrated that OSPM characteristics, such as salinity, naphthenic acids and sulphate affected the microbial community. To my knowledge no prior work has examined bacterioplankton activity levels in tailings pond or wetlands in the Alberta oil sands region. The bacterioplankton community had reduced metabolic activity in OSPM wetlands, compared to a reference wetland of natural origin, and was correlated with salinity index. Studies have reported that bacteria biomass can be limited by C availability (Friedrich et al. 1999). Similarly, a positive correlation between microbial biomass and DOC concentration (i.e. naphthenic acids, primary production) suggests that carbon availability may limit bacterioplankton community in these wetlands. Methanogenesis is known to be inhibited by high concentrations of sulphate (Schönheit et al. 1982; Schlesinger 1997) and salinity (DeLaune et al. 1983; Denier van der Gon and Neue 1995; Purvaja and Ramesh 2001). This relationship was observed in tailings ponds (Fedorak et al. 2002; MacKinnon and Sethi 1993 cited in Fedorak et al. 2002). Results confirm that methanogenesis is also inhibited in the study wetlands, especially in OSPM-affected wetlands, likely because of elevated sulphate concentrations and not due to salinity.

Assessment of a Reclamation Strategy

The addition of peat amendments to wetland sediments is a reclamation tool believed to speed up wetland succession (Turetsky et al. 2002) by providing structure for aiding in vegetation establishment, and moisture and nutrients required for macrophyte growth (OSSWG 2000). Although Baker (2007) reported that peat slowed macrophyte development in wetlands in constructed wetlands of the Alberta oil sands region, including Test Pond 9. Amending wetlands sediments with peat does not appear to affect bacterioplankton community production and biomass and/or the potential carbon transfer to higher levels in the aquatic food webs. Furthermore, peat amendments do not appear

to stimulate microbial decomposition in unvegetated parts of the wetland, thereby, accelerating carbon loss over time. Therefore, this reclamation strategy does not appear to have a direct effect on the microbial community in oil sands affected wetlands.

The nonvegetated sediments of the study wetlands were small net exporters of C (CO_2 and CH_4) and currently do not appear to be on a trajectory to becoming net sinks in these early stages of their wetland development since gas emissions neither increased, nor declined as a function of age since construction.

Limitations, Uncertainties, and Future Research Directions

Further stable isotope studies are required to examine carbon sources that support the microbial community in oil sands constructed wetlands. This study determined that the concentration-weighted linear stable isotope mixing model results from all the reference wetlands were problematic since microbial biofilm fell outside of the mixing triangle, a triangular space enclosed by lines connecting the 3 potential carbon sources, leading to nonsensical negative biomass estimates for primary production and sediment carbon sources. Mean microbial biofilm $\delta^{15}N$ values in all reference wetlands (-4.08±1.4 (mean±SD) to -0.10±0.7‰) were more negative than their potential sources (2.63±0.05 to 12.12±0.01‰), indicating that a nitrogen source common to reference wetlands may have been overlooked. Nitrification may play an important role in nitrogen dynamics in reference wetlands. Further isotope studies are required in reference wetlands and oil sands constructed wetlands where nitrogen transformation appear to obscure microbial $\delta^{15}N$ origins. A dual $\delta^{15}N$ and $\delta^{18}O$ approach offers the potential to identify changes to the N stable isotope via mineralization, nitrification, industrial effluent and atmospheric deposition. Also, further stable isotope studies examining the microbial food web in these study wetland using mixing model applications should collect enough microbial biomass to analyze for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$. By increasing the number of stable isotopes used, more potential sources can be examined.

Stable carbon isotope values of bacteria are commonly determined (Coffin et al. 1989, 1990; Kelly et al. 1998; Kritzberg et al. 2004) with the bacterial bioassay method which involves bacterial concentrates grown in 0.2-µm filtered water samples for short-term incubations (Coffin et al. 1989). I attempted to quantify microbial $\delta^{13}C$ and $\delta^{15}N$ with the bioassay method, but not enough microbial biomass could be collected. I would recommend my biofilm technique for those examining the microbial community in aquatic environments because you can collect abundant microbial biomass from the natural microbial community to quantify microbial $\delta^{13}C$ and $\delta^{15}N$ signatures. Plus, after the initial study setup (installing pipes and waiting for biofilm development) collecting microbial biomass is quick and efficient compared to the long filtering time inherent in the bioassay method.

Good agreement between measured gas flux and calculated pCO_2 indicated that the microcosm chamber design provided credible gas measurements from the natural environment. I would recommend this technique for large scale studies because this is a more economical technique compared to the static chambers commonly used to quantify gas flux.

The relationship between bacterial production and respiration is commonly examined in aquatic ecosystems (Cole 1999). In this study, bacterial production, biomass and carbon sources that supported the microbial community were examined in the water, whereas microbial respiration was quantified from unvegetated wetland sediments. Therefore, I was unable to quantify the amount of bacterial production that was lost from wetland systems via respiration. In the early stages of this study, I was only aware of a methodology for quantifying bacterial production in marine and lake water columns. I have since learned of a method for quantifying bacterial production in wetland sediments (Buesing and Guessner 2003). Future studies need to measure bacterial production and respiration in wetland sediments so that carbon loss from wetlands can be quantified.

Conclusion

Since this research assesses only one part of the food web within a larger multidisciplinary study of wetland function, information provided by other research in progress may help me to better understand the implications and relative importance of the findings I've reported here. A stable isotope study examining zooplankton and zoobenthos in the same study wetlands was carried out contemporaneously with this study (Wytrykush 2007, Ph.D., University of Windsor, in prep.) and may further support my hypothesis that microbial community biomass supplies energy to higher trophic organisms if microbial $\delta^{13}C$ and $\delta^{15}N$ ratios are retained in zooplankton and zoobenthos.

In summary, understanding of microbial dynamics in wetlands, especially those affected by oil sands mining, has improved as a result of this research. Functional assessments of microbial carbon sources and respiration in constructed wetlands of the Alberta, Canada oil sands mining area provided important insights into these unique systems that couldn't be ascertained by other approaches. The natural microbial community in OSPM-affected wetlands can assimilate petroleum into microbial biomass, and sufficient biomass appears to be available to support higher trophic levels even in OSPM-affected wetlands, albeit at lower levels in young OSPM wetlands. Thus, microbes have the potential to greatly influence energy received by higher trophic organisms in the study wetlands. Continued research on microbial dynamics is important for understanding carbon flow through the food web of the constructed wetlands of the Alberta, Canada oil sands region.

Appendix 2.1 - Wetland Formation

Natural Wetland

The area where Natural Wetland would form was filled in with sand in the mid-1980's and then leveled off. In 1984 the area was reclaimed with the intention of it becoming a forest ecosystem. Muskeg soil (15 cm) was amended on top of sand. Natural wetland formed in a depression when water levels increased because of dyke seepage of process waters and precipitation. In 1991, a berm was created on the east side with sediment scooper from the wetland itself. With the removal of the muck the depth went from 50 cm to 1 m. Biota evolved naturally. In 1994 and 1995 the land-bridges were extended to promote flow circulation through the wetland since flow was only occurring in the centre of the pond. Over the years (i.e. 1996 and 1997) CT process water, with high levels of gypsum, was added to the west end of Natural Wetland (S. Tuttle, July 22 2004, pers. comm.).

4 m Consolidated Tailings (4 m-CT) Demonstration Pond

The 4 m-CT Demonstration Pond was constructed in 1999 on an area formerly known as "Waste Area 11". The wetland was filled with a combination of sand and CT material. CT process waters have flowed into 4 m-CT from an adjacent wetland known as "control wetland". There was a large amount of bitumen visibly floating on the surface of the water in the beginning. The inflow of CT process water comes from Pond 5, an adjacent tailings settling pond. Peat amendments were placed on top of the sand-CT sediment in certain areas (S. Tuttle, July 22 2004, pers. commun.).

High Sulfate Wetland ("Crane Lake Duck Pond")

The area where High Sulphate Wetland would develop was composed of slightly sodic overburden. The area was reclaimed by adding 15 cm of muskeg soil. High Sulphate wetland developed naturally in a depression in this reclaimed area in 1987. Water levels rose in the depression and were fed by precipitation, runoff and possibly seepage from overburden (S. Tuttle, July 22 2004, pers. commun.).

Canadian Natural Resources Ltd. (CNRL) Wetland

CNRL Wetland was constructed in 2004. A road was built through the CNRL mining lease and materials used for the constructed were dug out leaving behind a depression that would become CNRL wetland. The substrate is sand. Water levels rose in the depression and were fed by precipitation and runoff from a forest ecosystem on the east side and a logged area on the west side of the wetland (June 19, 2005, pers. commun.).

Beaver Pond

Beaver Pond formed naturally in a stream channel that existed on the mining lease site prior to mining companies' establishment. It showed no evidence of being affected by oil sands development activity. Its actual age is unknown, but estimated to be at least a few decades old.

Test Pond 9

Test Pond 9 was constructed in 1992. It has a clay base that were capped with process waters.

Mike's Pond

Mike's Pond was filled in during the fall of 1997. It is composed of CT process waters taken from a tailings pond (J. Ciborowski, 2005, pers. comm.). The substrate is composed of clay that has a low organic content. This pond is halosaline (M. Mackinnon, 2005, pers.commun.).

Shallow Wetland

The area in which Shallow Wetland formed contains sodic overburden that was capped with a clay layer that is 1m thick. Shallow Wetland formed opportunistically in 1992 in a depression. Initially the wetland water was derived from West Interceptor Ditch. Currently wetland water is recharged with surface water (C. Wytrykush 2007, pers. comm.).

Peat Pond

Peat Pond was constructed on top of mine tailings that were capped with 80cm of a clay-loam mixture. At least 20 cm of a peat-mineral mixture was placed on top of the clay-loam mixture. The surface water comes from recharge water (C. Wytrykush 2007, pers. commun.).

Appendix 2.2 - Average physical and chemical measurements collected in the study wetlands, July 2005.

Wetland	pH	Conductivity (µS)	Salinity (ppt)	Temperature (^{o}C)	DO (mg/L)
PP	9.0	898	0.4	20.4	11.3
BP	8.0	661	0.5	17.7	7.1
NW	8.8	1330	0.6	19.3	6.8
HS	7.6	2910	1.4	18.9	9.3
CNRL		245	0.1	16.1	8.1
4mCT	8.1	2340	1.1	16.6	5.3
SW	7.3	337	0.2	16.6	6.5
TP9	9.1	1952	1.0	17.9	11.5
MP	8.5	4660	2.5	19.2	7.0

Blank = no measurements

Appendix 2.3: Stable carbon and nitrogen isotope ratios for pelagic and benthic microbial biofilm collected in the study wetlands.

Wetland	Code	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Elemental %C	Elemental %N	C:N
SW	Epibenthic A	-31.59	-1.85	12.69	1.15	11.06
SW	Epibenthic B	-30.99	0.29	12.85	1.21	10.60
SW	Subsurface C	-31.38	0.01	18.29	2.14	8.54
TP9	Subsurface A	-28.46	4.42	12.02	0.90	13.34
TP9	Epibenthic A	-26.33	4.50	9.34	0.70	13.43
TP9	Subsurface B	-26.21	5.33	11.76	1.16	10.17
TP9	Subsurface C	-28.53	4.82	15.99	1.15	13.88
TP9	Epibenthic C	-25.30	4.17	7.84	0.60	13.15
CNRL	Subsurface A	-24.30	0.86	13.84	1.16	11.92
CNRL	Epibenthic A	-27.19	-0.90	14.53	1.39	10.42
CNRL	Epibenthic B	-20.80	-0.10	12.88	1.07	12.09
CNRL	Subsurface C	-20.46	0.14	12.05	1.07	11.26
CNRL	Epibenthic C	-20.85	-0.51	12.52	1.13	11.05
PP	Subsurface B	-26.31	-1.46	22.13	3.36	6.59
PP	Subsurface C	-30.94	0.04	14.38	1.53	9.43
PP	Epibenthic C	-27.34	-2.82	18.53	2.09	8.86
4mCT	Subsurface A	-25.62	7.42	9.99	0.57	17.44
4mCT	Subsurface B	-28.33	15.84	17.57	1.88	9.34
MP	Subsurface A	-23.40	4.47	5.87	0.49	11.88
MP	Epibenthic A	-23.44	4.70	5.06	0.44	11.44
MP	Subsurface B	-24.30	1.88	5.71	0.50	11.41
MP	Epibenthic B	-24.19	1.88	5.80	0.49	11.81
NW	Subsurface A	-26.91	9.40	15.45	1.19	12.98
NW	Epibenthic A	-27.11	9.94	15.92	1.26	12.66
NW	Subsurface B	-26.66	9.32	13.03	0.88	14.77
NW	Epibenthic B	-26.84	9.77	13.53	0.98	13.75
NW	Subsurface C	-27.04	10.75	14.68	1.28	11.48
NW	Epibenthic C	-26.82	9.42	13.91	1.03	13.50
HS	Subsurface A	-26.08	-3.45	20.13	2.46	8.19
HS	Epibenthic A	-26.44	-3.76	18.06	2.00	9.01
HS	Epibenthic B	-26.21	-3.30	18.24	2.03	8.99
HS	Subsurface C	-26.92	-2.11	14.87	1.47	10.09
BP	Subsurface A	-32.12	-2.65	16.75	1.67	10.04
BP	Subsurface B	-32.10	-3.75	15.12	1.41	10.71
BP	Epibenthic B	-34.34	-6.37	12.44	1.32	9.42

BP	Subsurface C	-31.56	-3.66	17.29	1.52	11.41
BP	Epibenthic C	-29.68	-3.95	12.84	1.18	10.92

Appendix 2.4: Stable carbon and nitrogen isotope ratios for emergent, submergent and floating macrophytes in the study wetlands.

Wetland	Code	Macrophyte Name	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Classification
SW	Veg A	Scirpus validus	-28.16	5.62	Emergent
SW	Veg B	Carex spp.	-27.64	4.58	Emergent
SW	Veg C	Carex spp.	-27.72	2.57	Emergent
SW	Veg D	Hippuris vulgaris	-28.90	4.44	Emergent
SW	Veg E	Myriophyllum exalbescens	-18.57	1.51	Submergent
SW	Veg F	Potamogeton spp.	-21.59	2.23	Submergent
SW	Veg G	Typha latifolia	-27.62	2.40	Emergent
SW	Veg H	Certaophyllum demersum	-23.07	-0.24	Submergent
SW	Veg I	Utricularia spp.	-33.18	1.99	Submergent
TP9	Veg A	Typha latifolia	-28.14	11.29	Emergent
TP9	Veg B	Scirpus validus	-26.50	12.63	Emergent
TP9	Veg C	Potamogeton spp.	-20.07	6.88	Submergent
TP9	Veg D	Chara spp.	-25.88	5.19	Submergent
CNRL	Veg A	Scirpus validus	-30.21	4.28	Emergent
CNRL	Veg B	Potamogeton pucillus	-21.22	4.24	Submergent
CNRL	Veg C	Carex spp.	-27.22	4.89	Emergent
CNRL	Veg D	Triglochin spp.	-27.11	0.06	Emergent
CNRL	Veg E	Utricularia spp.	-27.82	3.63	Submergent
CNRL	Veg F	Carex spp.	-28.87	6.03	Emergent
CNRL	Veg G	Typha latifolia	-29.89	4.78	Emergent
CNRL	Veg H	Potamogeton spp.	-24.40	3.59	Submergent
CNRL	Veg I	Potamogeton richardsonii	-23.44	3.79	Submergent
CNRL	Veg J	Melilotus officinalis	-25.99	4.09	Emergent
PP	Veg A	Hippuris vulgaris	-31.12	4.22	Emergent
PP	Veg B	Certaophyllum demersum	-23.59	2.19	Submergent
PP	Veg C	Potamogeton spp.	-19.36	3.19	Submergent
PP	Veg D	Typha latifolia	-30.75	4.87	Emergent
PP	Veg E	unidentified submergent	-29.69	4.56	Submergent
4m-CT	Veg A	Carex spp.	-27.72	11.32	Emergent
4m-CT	Veg B	Carex spp.	-26.37	10.36	Emergent
4m-CT	Veg C	Scirpus validus	-27.86	18.02	Emergent
4m-CT	Veg D	Typha latifolia	-27.85	18.91	Emergent
4m-CT	Veg E	Chara spp.	-31.81	18.65	Submergent
4m-CT	Veg F	Potamogeton spp.	-25.83	23.08	Submergent
MP	Veg A	Chara spp.	-17.07	12.43	Submergent
MP	Veg B	Scirpus validus	-27.76	14.14	Emergent

MP	Veg C	Carex spp.	-29.41	20.69	Emergent
MP	Veg D	Typha latifolia	-28.80	9.51	Emergent
MP	Vef E	Potamogeton spp.	-12.74	12.56	Submergent
NW	Veg A	Potamogeton spp.	-15.93	17.14	Submergent
NW	Veg B	Carex spp.	-25.42	4.85	Emergent
NW	Veg C	Typha latifolia	-25.48	4.59	Emergent
HS	Veg A	Potamogeton spp.	-15.93	-1.49	Submergent
HS	Veg B	Chara spp.	-23.08	-1.96	Submergent
HS	Veg C	Typha latifolia	-28.81	1.26	Emergent
HS	Veg D	Carex spp.	-28.37	1.16	Emergent
HS	Veg E	Lemna minor	-30.68	1.01	Floating
BP	Veg A	Carex spp.	-26.95	6.13	Emergent
BP	Veg B	Typha latifolia	-28.62	5.74	Emergent
BP	Veg C	Certaophyllum demersum	-31.32	5.37	Submergent
BP	Veg D	Myriophyllum exalbescens	-31.88	3.64	Submergent
BP	Veg E	Polygonum amphibium	-28.70	3.38	Floating
BP	POM B	Lemna minor	-40.92	0.96	Floating

Appendix 2.5 – July 2005 Water quality in the study wetlands. (Data courtesy of Mike MacKinnon (Syncrude Canada Ltd.))

Wetland	Date	Naphthenic Acids (mg/L)	DOC	NH_4	Na	K	Mg	Ca	F (anion)	Cl (anion)	SO_4 (anion)	CO_3	HCO_3	$CaCO_3$
PP	Jul-05	1.2	33.5	0.3	115	0.10	26	60	BDL	25	253	0	260	
BP	Jul-05	1.3												
NW	Jul-05	30.9	69	1.7	312	9.36	11	12	2.4	34		0	645	
HS	Jul-05	14.5		1.4	411	16.00	100	150	BDL	7	1400	0	186	
CNRL	Jul-05	N/A												
4mCT	Jul-05	26.0	47	0.3	490	16.60	64	48	BDL	65	476	0	996	
SW	Jul-05	2.0	20.3	0.6	40	0.01	14	28	BDL	10	15	0	215	176
TP9	Jul-05	25.5	52.7	0.2	511	0.01	9	8	BDL	210	150	0	885	
MP	Jul-05	26.0		0.4	1060	15.60	33	42	BDL	650	1220	0	422	

Wetland	Total Cations	Total Anions	Cation/ Anions	Na/Cl	Al	B	Ba	Fe	Mn	No	Ni	Se	Si	Sr	Ti	V	Zn	Zr
PP	10	10	0.99	7	BDL	0.1	BDL	0.02	0.007	BDL	BDL	BDL	0.6	0.4	BDL	BDL	BDL	BDL
BP																		
NW	15	15	1.04	14	0.3	2.6	BDL	0.67	0.01	0.173	BDL	BDL	8.7	0.3	BDL	BDL	BDL	BDL
HS			1.05	86	BDL	0.8	BDL	0.05	0.054	BDL	BDL	BDL	0.2	1.7	BDL	BDL	BDL	BDL
CNRL																		
4mCT			1.05	12	BDL	2.3	0.05	0.12	0.026	0.087	BDL	BDL	1.4	0.7	BDL	BDL	BDL	BDL
SW	4	4	1.04		0.1	0.1	BDL	0.07	BDL	BDL	BDL	BDL	1.0	0.2	BDL	BDL	BDL	0.02
TP9	23	24	0.99		0.9	1.8	BDL	0.36	BDL	BDL	BDL	BDL	5.5	0.1	BDL	BDL	BDL	0.02
MP	51	51	1.01		BDL	3.4	BDL	0.00	BDL	0.095	BDL	BDL	1.9	0.8	BDL	BDL	BDL	BDL

Appendix 2.6: Stable carbon and nitrogen isotope ratios for sediment cores from the study wetlands

Wetland	ID	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Elemental %C	Elemental %N	C:N	Description
SW	Core A	-27.84	1.02	3.26	0.26	12.60	Grey clay with a macrophyte detritus component
SW	Core B	-27.90	1.21	2.65	0.21	12.67	Grey clay with a macrophyte detritus component
TP9	Core A	-26.35	1.11	6.15	0.22	28.21	Grey clay
TP9	Core B	-28.32	0.19	6.60	0.28	23.89	Grey clay
CNRL	Core A	-29.90	BDL	0.40	BDL	BDL	Tan-brown sand
CNRL	Core B	-26.87	BDL	0.52	BDL	BDL	Tan-brown sand
PP	Core A	-27.66	0.47	14.89	0.64	23.41	Peat
PP	Core B	-28.83	0.90	6.05	0.09	66.45	Dark organic sediment
4mCT	Core A	-27.26	1.77	9.49	0.27	35.00	Brown-grey clay with an organic fraction
4mCT	Core B	-29.46	6.08	6.50	0.11	61.35	Dark brown organic sediment with a peat component
MP	Core A	-27.69	BDL	1.00	BDL	BDL	Grey silty clay
MP	Core B	-28.10	BDL	0.70	BDL	BDL	Grey silty clay
NW	Core A	-27.74	BDL	0.52	0.03	17.29	Brown silt & sandy soil with large organic fraction
NW	Core B	-28.30	0.33	1.07	0.07	14.34	Brown silt & sandy soil with large organic fraction
HS	Core A	-90.21	3.34	29.27	0.62	47.51	Dark brown silty-sand with a large organic fraction
HS	Core B	-28.40	-1.84	2.85	0.07	42.55	Dark brown silty-sand with a large organic fraction
BP	Core A	-28.75	6.75	2.51	0.09	28.83	Light grey-brown clay with detrital component
BP	Core B	-28.39	5.73	1.49	0.06	25.21	Light grey-brown clay with detrital component

BDL = below detection limit

Appendix 2.7: Stable carbon and nitrogen isotope ratios for algae and seston in the study wetlands.

Wetland	Name	Code	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Elemental %C	Elemental %N	C:N	Description
SW	Algae	POM A	-25.51	-0.41	32.48	2.17	15.0	Abundant in open water; Associated with macrophytes
SW	Algae	POM B	-24.58	0.83	40.86	2.05	19.9	Abundant in open water; Associated with macrophytes
SW	Seston	POM C	-28.53	1.40	1.76	0.22	15.0	
SW	Seston	POM D	-29.62	0.15	2.21	0.25	19.9	
TP9	Algae	POM A	-25.95	6.09	22.60	1.50	15.0	Abundant in open water; Associated with Typha latifolia
TP9	Seston	POM C	-30.07	5.33	6.76	0.33	20.7	
TP9	Seston	POM D	-30.16	4.29	7.60	0.32	23.5	
CNRL	Algae	POM A	-12.26	-0.73	10.59	0.64	16.6	Floating algae was collected from water column surface
CNRL	Algae	POM B	-12.81	-0.81	11.14	0.74	15.0	Floating algae was collected from water column surface
CNRL	Seston	POM C	-30.03	1.93	2.72	0.29	9.3	
CNRL	Seston	POM D	-28.93	3.87	3.25	0.33	9.9	
PP	Algae	POM A	-19.46	0.54	33.15	2.23	14.9	Floating algae was collected from water column surface
PP	Algae	POM B	-32.44	-0.45	42.61	8.11	5.3	Green rod-shaped algae (1-4mm); Abundant throughout water column
PP	Seston	POM C	-33.40	-0.71	7.54	1.35	5.6	Pelagic water was filtered on GF/D; Dominated by rod algae
PP	Seston	POM D	-28.87	0.69	3.63	0.53	6.8	Filtered through 180 μm sieve onto GF/D filter; Excludes rod algae
4m-CT	Algae	POM A	-30.74	15.47	27.12	1.78	15.2	Bright green, filamentous floating algae
4m-CT	Algae	POM B	-30.28	24.18	26.56	2.92	9.1	Bright green, filamentous floating algae
4m-CT	Seston	POM C	-29.20	20.50	3.15	0.36	8.7	

4m-CT	Seston	POM D	-29.05	23.65	4.70	0.67	7.0	
MP	Algae	POM A	N/A	N/A	N/A	N/A	N/A	no metaphyton was observed
MP	Algae	POM B	N/A	N/A	N/A	N/A	N/A	no metaphyton was observed
MP	Seston	POM C	-23.31	3.87	1.58	0.14	11.1	
MP	Seston	POM D	-23.69	5.10	1.57	0.12	12.8	
NW	Algae	POM A	-23.62	14.23	19.46	1.84	10.6	Floating algae was collected from water column surface
NW	Algae	POM B	-22.04	23.84	39.00	2.50	15.6	Floating algae was collected from water column surface
NW	Seston	POM C	-21.04	13.26	6.03	0.67	9.0	
NW	Seston	POM D	-20.11	13.75	5.92	0.68	8.8	
HS	Algae	POM A	-23.85	0.71	45.16	3.12	14.5	Floating algae was collected from water column surface
HS	Algae	POM B	-23.95	0.77	44.71	3.10	14.4	Floating algae was collected from water column surface
HS	Seston	POM C	-25.25	-0.84	4.27	0.37	11.4	
HS	Seston	POM D	-23.31	-0.24	3.66	0.28	13.2	
BP	Seston	POM C	-30.62	8.73	1.50	0.20	7.5	
BP	Seston	POM D	-30.40	8.71	1.96	0.24	8.1	

N/A = not collected or measured

Appendix 2.8 - The stable carbon isotope ratios for dissolved inorganic carbon in the study wetlands.

Wetland	Replicate	DIC δ13C (‰)
SW	1	-4.66
SW	2	-4.21
SW	3	-5.51
TP9	1	-4.17
TP9	2	-3.18
TP9	3	-4.03
CNRL	1	-8.14
CNRL	2	-7.87
CNRL	3	-7.99
PP	1	-5.27
PP	2	-4.98
PP	3	-5.28
4mCT	1	-9.37
4mCT	2	-9.52
4mCT	3	-8.04
MP	1	-0.63
MP	2	-1.02
MP	3	-0.23
NW	1	-5.29
NW	2	-5.84
NW	3	-7.4
HS	1	-5.81
HS	2	-9.65
HS	3	-7.81
BP	1	-9.52
BP	2	-9.14
BP	3	-9.25

Appendix 2.9 - Stable carbon isotope ratios and concentrations of dissolved inorganic carbon and dissolved organic carbon in August 2005 courtesy of Vidella (2006).

Wetland	Mean (±SD)DIC $\delta^{13}C$ (‰)	DIC (ppm)	DOC $\delta^{13}C$ (‰)	DOC (ppm)
SW	-4.8±0.7	39.76	-27.82	22.99
TP9	-3.8±0.5	131.14	-26.72	66.45
CNRL	-8.0±0.1	20.67	-27.54	22.98
PP	-5.2±0.2	34.49	-26.65	37.63
4m-CT	-9.0±0.8	133.76	-27.22	63.09
MP	-0.6±0.4	75.06	-27.37	57.52
NW	-6.2±1.1	115.58	-27.9	73.15
HS	-7.8±1.9	21.30	-28.7	60.99
BP	-9.3±0.2	55.16	-27.45	33.20

Appendix 2.10 - Mean values for microbial biofilm $\delta^{13}C$ and $\delta^{15}N$ and dissolved inorganic carbon $\delta^{13}C$.

Wetland	Biofilm $\delta^{13}C$ (‰)	Biofilm δ^{15N} (‰)	DIC $\delta^{13}C$ (‰)
SW	-31.32±0.3	-0.52±1.2	-4.8±0.7
TP9	-26.97±1.5	4.65±0.4	-3.8±0.5
CNRL	-22.72±2.9	-0.10±0.7	-8.0±0.1
PP	-28.20±2.4	-1.41±1.4	-5.2±0.2
4m-CT	-26.98±1.9	11.63±6.0	-9.0±0.8
MP	-23.83±0.5	3.23±1.6	-0.6±0.4
NW	-26.9±0.2	9.77±0.5	-6.2±1.1
HS	-26.41±0.4	-3.16±0.7	-7.8±1.9
BP	-31.96±1.7	-4.08±1.4	-9.3±0.2

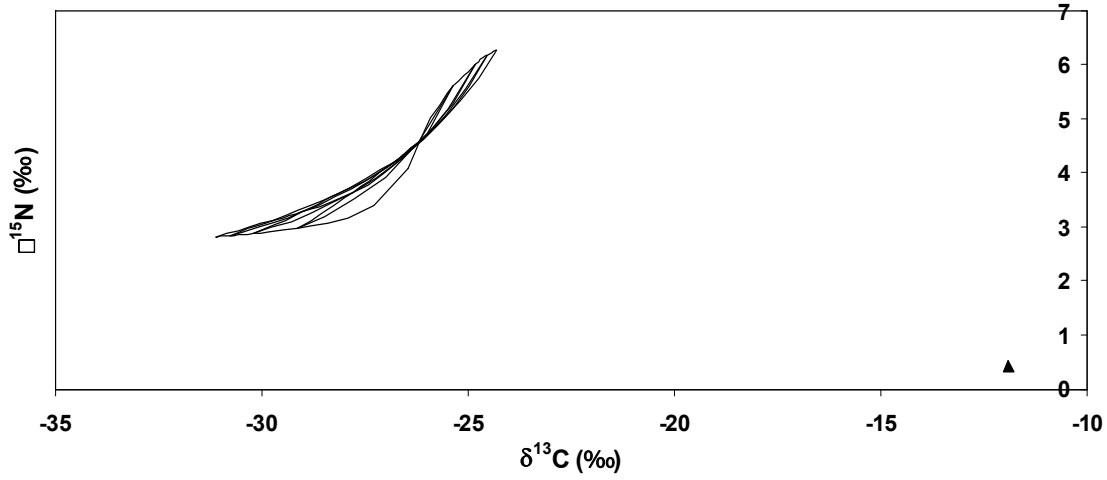
Appendix 2.11 - Stable carbon and nitrogen isotope ratios for bitumen.

$\delta^{13}C$ (‰)	**$\delta^{15}N$ (‰)**	**Elemental %C**	**Elemental %N**
-29.01	1.96	16.19	0.15
-29.09	1.94	12.57	0.11
BDL	1.78	BDL	0.11

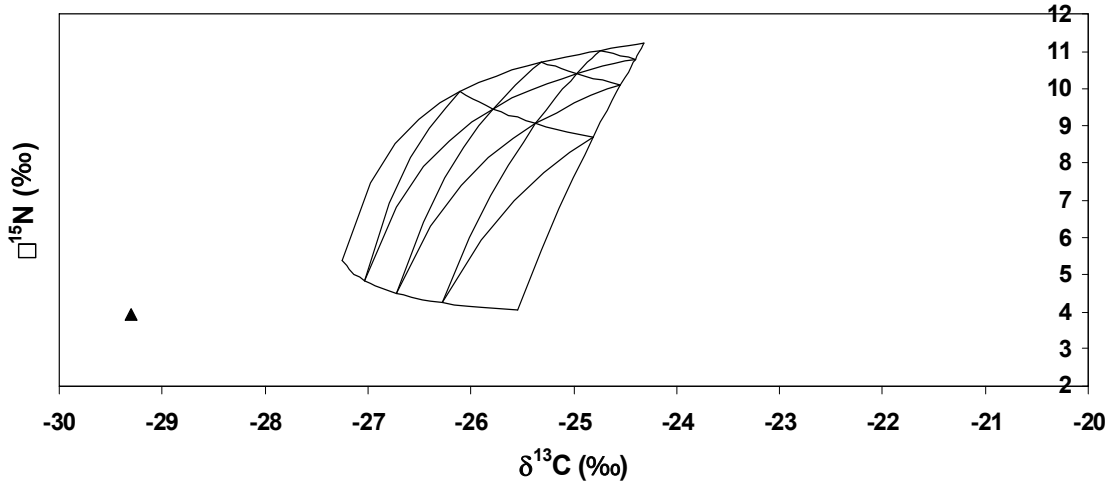
BDL = below detection limits

Appendix 2.12 – Dietary mixing triangles for mature microbial biofilm in reference wetland (a) Peat Pond and OSPM-affected wetland (b) Test Pond 9. Mean (±SD) microbial biofilm is represented by ▲. Lines within the triangles are "iso-diet" lines along which the proportion of one dietary component is invariant. Iso-diet lines increase from 0% on one side of the triangle opposing a vertex to 100% at the vertex (Phillips and Koch 2002). Note that scale of x and y-axes vary between graphs.

(a)



(b)



Appendix 3.1 - Leucine Incorporation measured during the conversion factor study.

Wetland	Incubation Time (h)	Replicate	^{3}H-Leu Incorporation (cpm/mL/h)	^{3}H-Leu Incorporation (dpm/mL/h)	^{3}H-Leu Incorporation (Ci/mL/h)	^{3}H-Leu Incorporation (pmol/mL/h)	Mean ^{3}H-Leu Incorporation (pmol/mL/h)
PP	0	1	298	2262.95	1.03E-06	1.86E-02	20.54
PP	0	2	359	2749.00	1.25E-06	2.25E-02	
PP	4	1	811	6350.60	2.89E-06	5.21E-02	42.16
PP	4	2	508	3936.25	1.79E-06	3.23E-02	
PP	8	1	1049	8247.01	3.75E-06	6.76E-02	87.82
PP	8	2	1668	13179.28	5.99E-06	1.08E-01	
PP	24	1	4264	33864.54	1.54E-05	2.78E-01	155.62
PP	24	2	529	4103.59	1.87E-06	3.36E-02	
SW	0	1	900	7059.76	3.21E-06	5.79E-02	34.91
SW	0	2	197	1458.17	6.63E-07	1.20E-02	
SW	4	1	325	2478.09	1.13E-06	2.03E-02	91.77
SW	4	2	2513	19912.35	9.05E-06	1.63E-01	
SW	8	1	13244	105418.33	4.79E-05	8.64E-01	804.16
SW	8	2	11407	90780.88	4.13E-05	7.44E-01	
SW	24	1	9010	71681.27	3.26E-05	5.88E-01	651.06
SW	24	2	10953	87163.35	3.96E-05	7.15E-01	

Appendix 3.2 - Cell abundance measured during the conversion factor study.

Wetland	Time (h)	Field of View (FOV)	Abundance (cells/FOV)	Abundance (cells/filter)	Abundance (cells/mL)	Mean Abundance (cells/mL)
PP	0H	1	43	3.32E+04	3.86E+04	3.77E+04
PP	0H	2	47	3.62E+04	4.21E+04	
PP	0H	3	61	4.70E+04	5.47E+04	
PP	0H	4	29	2.24E+04	2.60E+04	
PP	0H	5	30	2.31E+04	2.69E+04	
PP	4H	1	80	6.17E+04	7.17E+04	6.28E+04
PP	4H	2	130	1.00E+05	1.17E+05	
PP	4H	3	40	3.08E+04	3.59E+04	
PP	4H	4	60	4.63E+04	5.38E+04	
PP	4H	5	40	3.08E+04	3.59E+04	
PP	8H	1	191	1.47E+05	1.71E+05	8.00E+04
PP	8H	2	39	3.01E+04	3.50E+04	
PP	8H	3	91	7.02E+04	8.16E+04	
PP	8H	4	87	6.71E+04	7.80E+04	
PP	8H	5	38	2.93E+04	3.41E+04	
PP	24h	1	62	4.78E+04	5.56E+04	1.63E+05
PP	24h	2	195	1.50E+05	1.75E+05	
PP	24h	3	303	2.34E+05	2.72E+05	
PP	24h	4	274	2.11E+05	2.46E+05	
PP	24h	5	76	5.86E+04	6.82E+04	
SW	0h	1	62	4.78E+04	5.56E+04	3.07E+04
SW	0h	2	43	3.32E+04	3.86E+04	
SW	0h	3	67	5.17E+04	6.01E+04	
SW	0h	4	76	5.86E+04	6.82E+04	
SW	0h	5	55	4.24E+04	4.93E+04	
SW	0h	6	6	4.63E+03	5.38E+03	
SW	0h	7	8	6.17E+03	7.17E+03	
SW	0h	8	6	4.63E+03	5.38E+03	
SW	0h	9	4	3.08E+03	3.59E+03	
SW	0h	10	15	1.16E+04	1.35E+04	
SW	4h	1	8	6.17E+03	7.17E+03	3.18E+04
SW	4h	2	13	1.00E+04	1.17E+04	
SW	4h	3	4	3.08E+03	3.59E+03	
SW	4h	4	6	4.63E+03	5.38E+03	
SW	4h	5	4	3.08E+03	3.59E+03	
SW	4h	6	32	2.47E+04	2.87E+04	
SW	4h	7	75	5.78E+04	6.73E+04	
SW	4h	8	56	4.32E+04	5.02E+04	
SW	4h	9	114	8.79E+04	1.02E+05	
SW	4h	10	43	3.32E+04	3.86E+04	
SW	8H	1	162	1.25E+05	1.45E+05	3.98E+04
SW	8H	2	6	4.63E+03	5.38E+03	
SW	8H	3	77	5.94E+04	6.91E+04	
SW	8H	4	73	5.63E+04	6.55E+04	

SW	8H	5	105	8.10E+04	9.42E+04	
SW	8H	6	4	3.08E+03	3.59E+03	
SW	8H	7	5	3.86E+03	4.48E+03	
SW	8H	8	7	5.40E+03	6.28E+03	
SW	8H	9	2	1.54E+03	1.79E+03	
SW	8H	10	3	2.31E+03	2.69E+03	
SW	24h	1	120	9.25E+04	1.08E+05	5.20E+04
SW	24h	2	56	4.32E+04	5.02E+04	
SW	24h	3	20	1.54E+04	1.79E+04	
SW	24h	4	44	3.39E+04	3.95E+04	
SW	24h	5	50	3.86E+04	4.48E+04	

Appendix 3.3 - Bacterial Biomass Production in the study wetland.

Wetland	Location	Replicate	Bacterial biomass production (cells/mL/h)	Bacterial biomass production (ng C/mL/h)
PP	A	1	1.05E+05	2.09
PP	A	2	1.57E+05	3.14
PP	B	1	8.79E+04	1.76
PP	B	2	2.04E+05	4.09
PP	C	1	1.79E+05	3.59
PP	C	2	0.00E+00	0.00
HS	A	1	0.00E+00	0.00
HS	A	2	2.17E+04	0.43
HS	B	1	2.34E+04	0.47
HS	B	2	1.69E+04	0.34
HS	C	1	0.00E+00	0.00
HS	C	2	0.00E+00	0.00
4mCT	A	1	0.00E+00	0.00
4mCT	A	2	3.58E+04	0.72
4mCT	B	1	2.37E+04	0.47
4mCT	B	2	8.87E+04	1.77
4mCT	C	1	0.00E+00	0.00
4mCT	C	2	0.00E+00	0.00
NW	A	1	0.00E+00	0.00
NW	A	2	3.77E+04	0.75
NW	B	1	1.04E+04	0.21
NW	B	2	0.00E+00	0.00
NW	C	1	0.00E+00	0.00
NW	C	2	0.00E+00	0.00
BP	A	1	6.40E+04	1.28
BP	A	2	5.20E+04	1.04
BP	B	1	8.29E+04	1.66
BP	B	2	1.22E+04	0.24
BP	C	1	5.94E+04	1.19
BP	C	2	5.80E+04	1.16
CNRL	A	1	4.15E+04	0.83
CNRL	A	2	7.57E+04	1.51
CNRL	B	1	1.68E+03	0.03
CNRL	B	2	4.57E+04	0.91
CNRL	C	1	5.28E+04	1.06
CNRL	C	2	4.66E+04	0.93
TP9	A	1	9.45E+03	0.19
TP9	A	2	3.88E+03	0.08

TP9	B	1	8.94E+03	0.18
TP9	B	2	7.86E+03	0.16
TP9	C	1	2.30E+03	0.05
TP9	C	2	3.53E+03	0.07
SW	A	1	0.00E+00	0.00
SW	A	2	4.89E+04	0.98
SW	B	1	0.00E+00	0.00
SW	B	2	2.96E+04	0.59
SW	C	1	0.00E+00	0.00
SW	C	2	4.45E+04	0.89
MP	A	1	9.22E+01	0.00
MP	A	2	3.88E+03	0.08
MP	B	1	1.74E+03	0.03
MP	B	2	2.73E+03	0.05
MP	C	1	2.41E+01	0.00
MP	C	2	0.00E+00	0.00

Appendix 3.4 – Determining Quench Corrections

Introduction

Quenching is a reduction in counting efficiency due to anything that interferes with the conversion of radioactive decay energy emitted from a sample into photons of light. Counting efficiency may be influenced by reagents, scintillation cocktail, filters, and sample containers. Counting efficiency is calculated using the following formula:

$$\% \text{ Counting Efficiency} = \frac{\text{cpm}}{\text{dpm}} \times 100$$

where counts per minute (cpm) is the measured radioactivity and disintegrations per minute (dpm) is the theoretical maximum radioactivity. Counting efficiency for the bacterial biomass production samples collected from oil sands-affected wetlands in northern Alberta was determined by comparing the radioactivity of ^{3}H-leucine measured (cpm) by a liquid scintillation analyzer (Packard Tri-Carb 2900TR) to the calculated theoretical maximum radioactivity. The decay rate of tritium (^{3}H) (half-life: 12.26 y) was accounted in the theoretical maximum calculations.

Methodology

Triplicate 1-µL samples (A, B & C) of ^{3}H-leucine stock solution (1µCi/µL) were separately added to 999 µL of distilled water on May 4, 2006. Triplicate 50 µL subsamples from each 1:1000 dilution were micropipetted onto separate nitrocellulose filters (0.2µm, 25mm, Whatman) for a total of 9 subsamples. Filters were placed in separate 20 mL scintillation vials which were allowed to dry for 30 min at 70^{o}C. 0.5 mL of ethyl acetate was added to dissolve the filter and thus increase ^{3}H counting efficiency. Ten mL of Scintiverse® scintillation cocktail was added, the scintillation vial capped and the samples were radioassayed. Radioactivity was measured for 10 min per sample, using a liquid scintillation analyzer (Packard Tri-Carb 2900TR) and reported in cpm.

Results

Counts per minute ranged from 10,076 to 13, 456 and averaged 12,447±1379 (mean±SD) (Table 1).

Table 1 – Radioactivity measured (cpm) by the liquid scintillation counter.

Sample ID	**cpm**
A1	11841
A2	12250
A3	12292
B1	15262
B2	13456
B3	12138
C1	12584
C2	12128
C3	10076

Calculating Theoretical Maximum

Tritium decayed from its original concentration (1μCi/μL) during the 22 months between the purchase of new tritium and this study. According to a radioactive decay chart (Pers. Comm. Dr. Warner 2006) 90.15 % of tritium remained. Since $1\mu Ci = 2.2 \times 10^6$ dpm, therefore:

(a) Theoretical maximum= $C_{\text{stock solution}}$ x % remaining
= 2.2×10^6 dpm/μL stock x 0.9015
= 1,983,300 dpm/μL
= 1.98×10^3 dpm/μL (1:1000 dilution)
= 99, 165 dpm/50 μL

Calculating Counting Efficiency

$$\% \text{ Counting Efficiency} = \frac{12{,}447 \pm 1379 \text{ (Mean±SD) cpm}}{99{,}165 \text{ dpm}} \times 100$$
$$= 13\%$$

Results & Conclusion

Since the counting efficiency was 13%, therefore quench corrections accounted for an 87% reduction in counting efficiency that was likely influenced by reagents, scintillation cocktail, filters and sample containers.

Appendix 3.5 - Abundance (cells/mL) and biomass (µg C/L) in the Athabasca wetlands.

Wetland	Location	Abundance (cells/mL)	Biomass (µg C/L)
PP	A	69231	1.38
PP	B	78055	1.56
PP	C	46154	0.92
TP9	A	484167	9.68
TP9	B	94118	1.88
TP9	C	171947	3.44
NW	A	207785	4.16
NW	B	544620	10.89
NW	C	89141	1.78
HS	A	157467	3.15
HS	B	31222	0.62
HS	C	41177	0.82
BP	A	138553	2.77
BP	B	24073	0.48
BP	C	7240	0.14
SW	A	108327	2.17
SW	B	45883	0.92
SW	C	201812	4.04
MP	A	213305	4.27
MP	B	129684	2.59
MP	C	76019	1.52
CNRL	A	258554	5.17
CNRL	B	219278	4.39
CNRL	C	428511	8.57
4mCT	A	184617	3.69
4mCT	B	55295	1.11
4mCT	C	78824	1.58

Appendix 4.1 – Calculating the precision and accuracy of CO_2 & CH_4 gases measured with a thermal conductivity detector (TCD). (a) Measurements of CO_2 & CH_4. (b) Actual volumetric percent of CO_2 and CH_4 standards. (b) Precision and accuracy of CO_2 & CH_4 measurements.

(a)

Wetland	**Date**	**CO_2 Volumetric %**	**CH_4 Volumetric %**
4-m CT	7/26/05	14.82	0.92
4-m CT	7/29/05	1.38	0.43
CNRL	7/26/05	1.47	0.47
CNRL	7/27/05	1.53	0.55
CNRL	7/29/05	1.38	0.43
NW	7/26/05	14.82	0.92
NW	7/29/05	1.38	0.43
HS	7/28/05	1.53	0.55
HS	7/29/05	1.63	0.54
HS	7/30/05	1.63	0.54
PP	8/2/05	1.39	0.44
PP	8/3/05	1.40	0.44
PP	8/5/05	1.36	0.53
MP	7/2/05	1.39	0.44
MP	7/3/05	1.40	0.44
MP	7/5/05	1.36	0.53
SW	7/2/05	1.39	0.44
SW	7/3/05	1.40	0.44
SW	7/5/05	1.36	0.53
TP9	7/3/05	1.40	0.54
TP9	7/4/05	1.52	0.68
TP9	7/6/05	1.62	0.77
BP	8/8/05	1.79	0.61
BP	8/9/05	1.60	0.59
BP	8/11/05	1.63	0.57
	Mean	2.54	0.55
	Standard Error	3.70	0.14

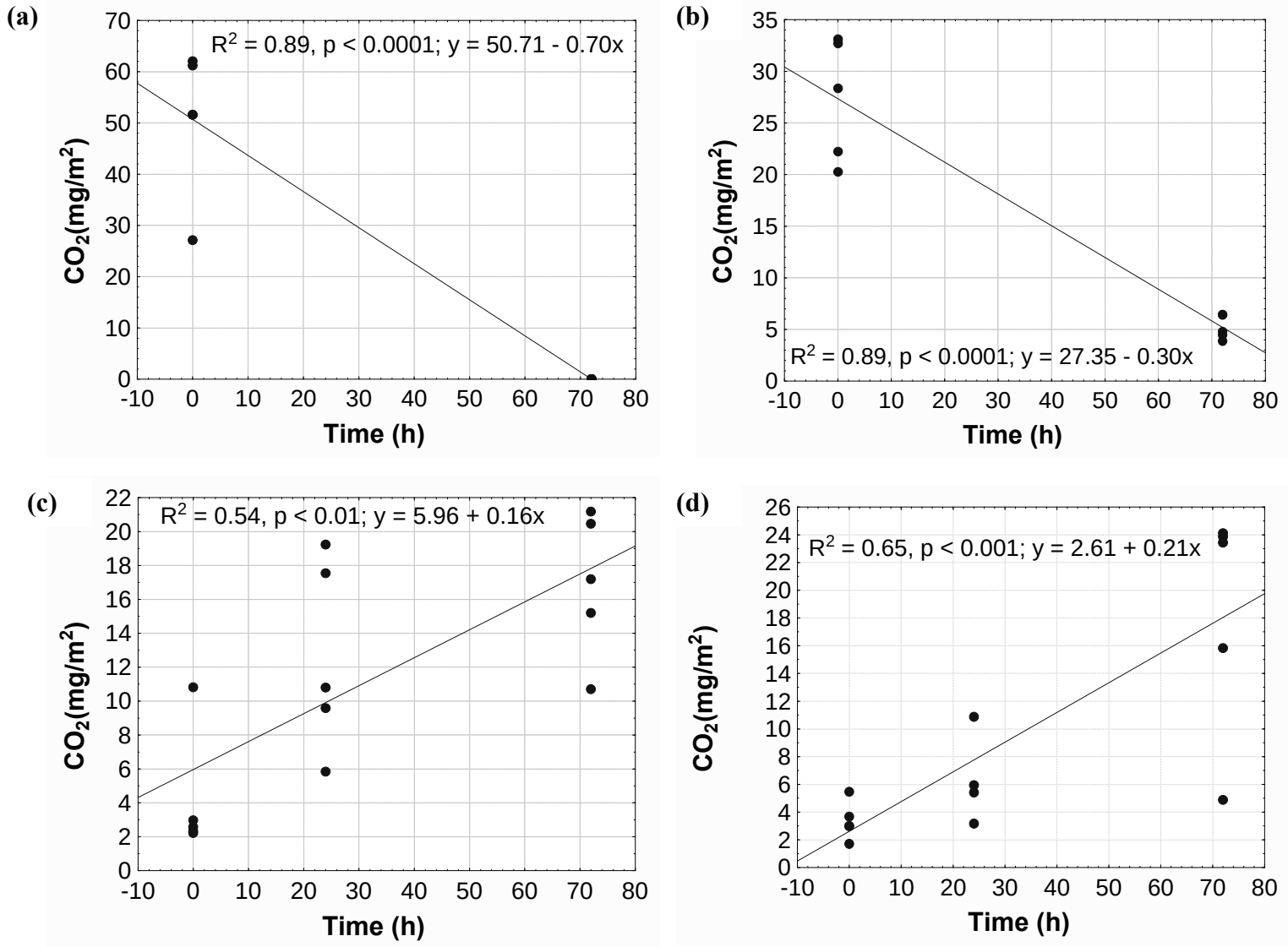
(b)

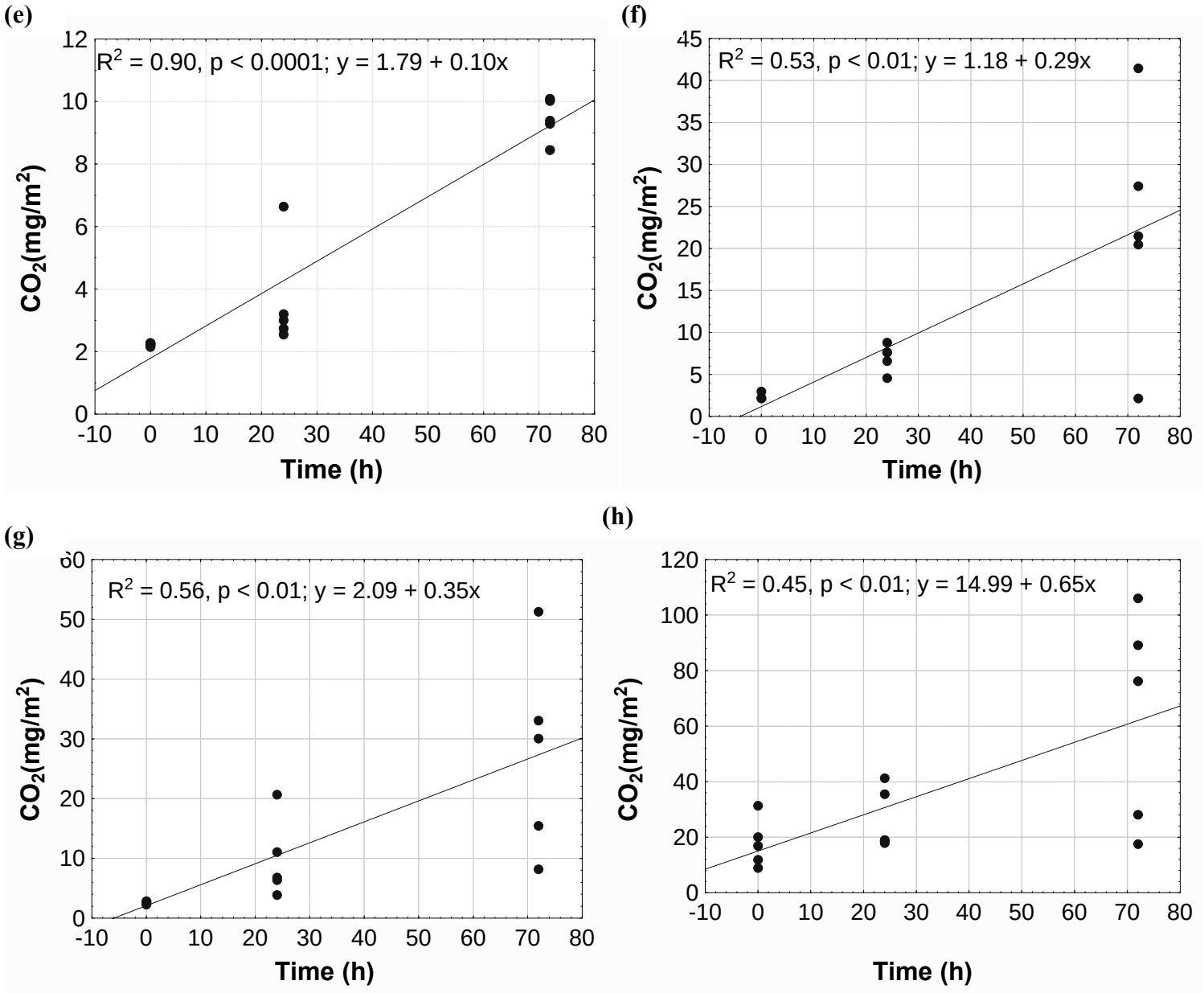
Gas	**Volumetric %**
CO_2	1.58
CH_4	0.52

(c)

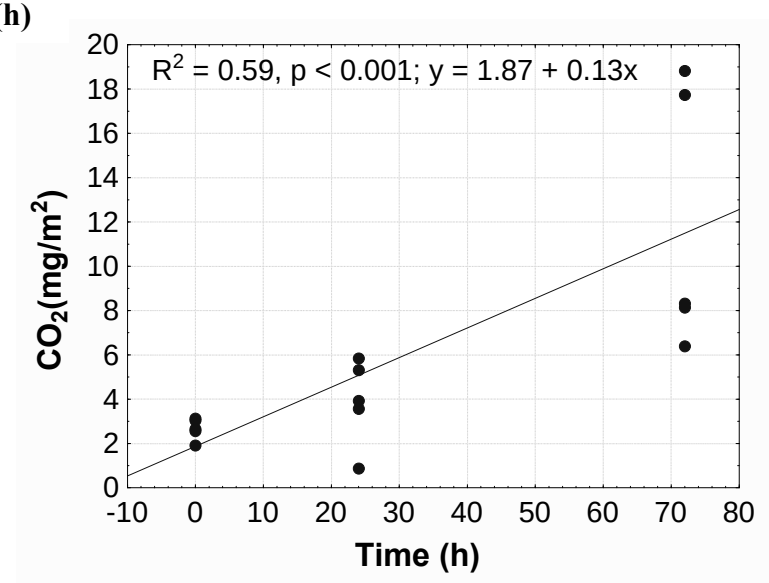
Gas	**Precision**	**Accuracy**
CO_2	2.34	1.61
CH_4	0.27	1.05
Mean	1.31	1.33

Appendix 4.2 - Least squares regressions of CO_2 concentrations vs. time in (a) 4m-CT, (b) NW, (c) HS, and (d) PP, (e) MP, (f) SW, (g) TP9, (h) BP, and (i) CNRL.

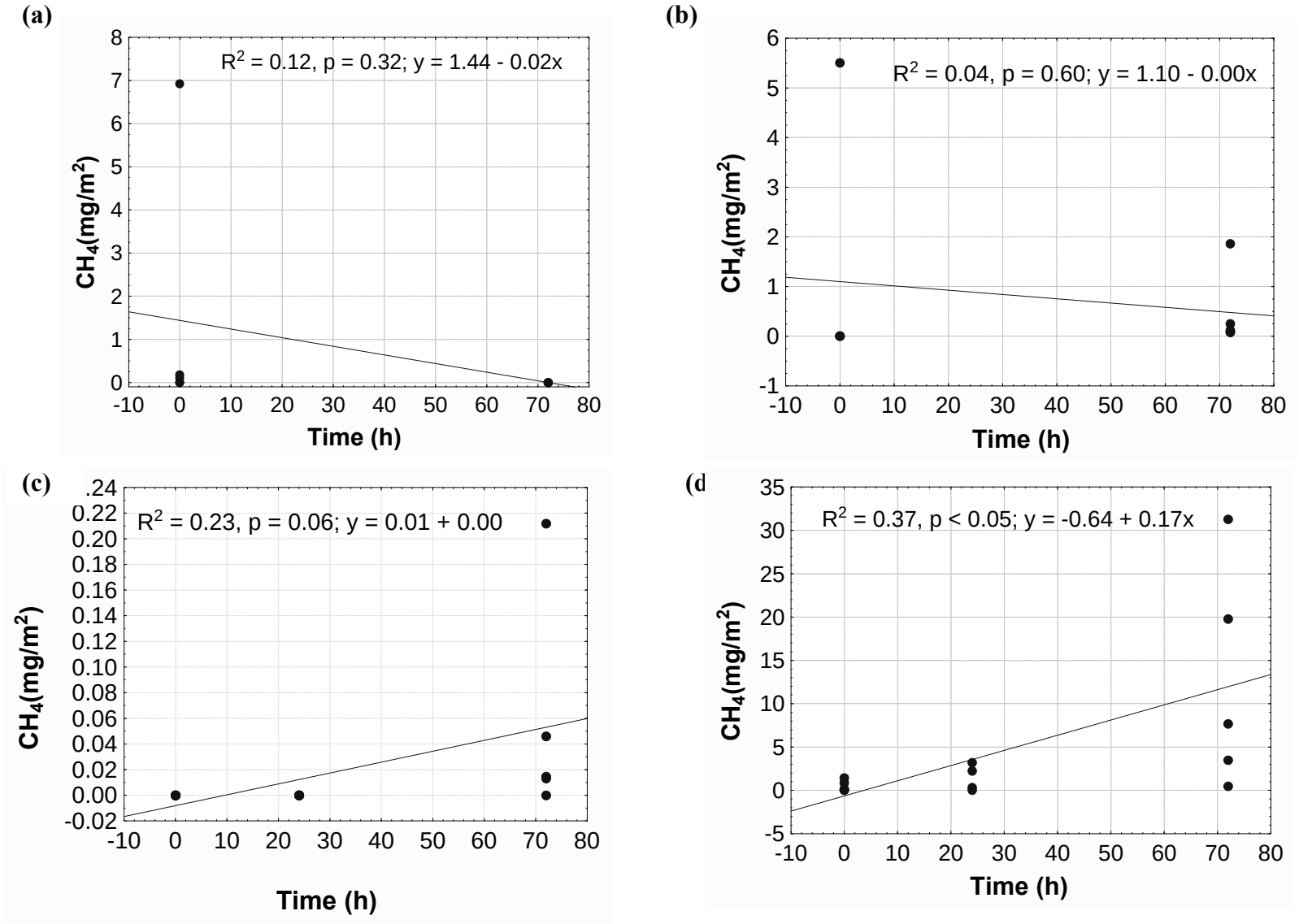



(e)
R² = 0.90, p < 0.0001; y = 1.79 + 0.10x
CO2(mg/m2)
Time (h)
(f)
R² = 0.53, p < 0.01; y = 1.18 + 0.29x
CO2(mg/m2)
Time (h)
(g)
R² = 0.56, p < 0.01; y = 2.09 + 0.35x
CO2(mg/m2)
Time (h)
(h)
R² = 0.45, p < 0.01; y = 14.99 + 0.65x
CO2(mg/m2)
Time (h)

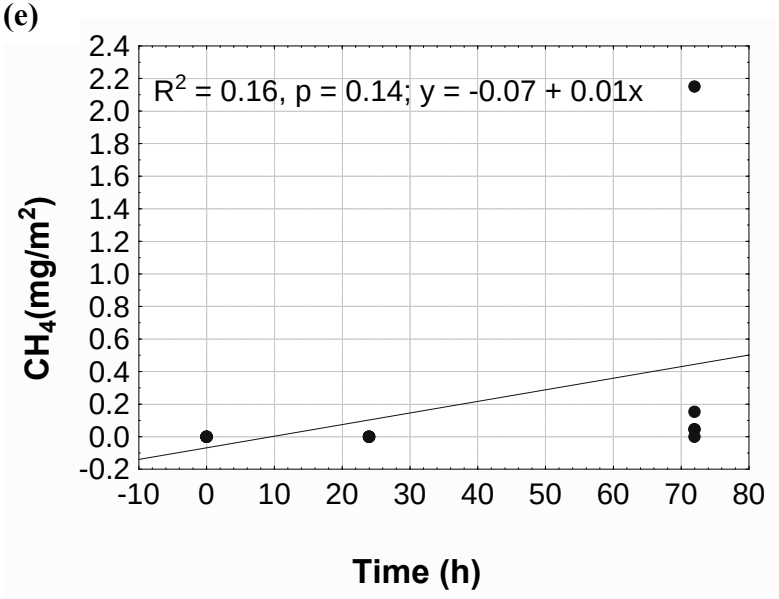
(h)


R2 = 0.59, p < 0.001; y = 1.87 + 0.13x
CO2(mg/m2)
Time (h)
20
18
16
14
12
10
8
6
4
2
0
-10
0
10
20
30
40
50
60
70
80

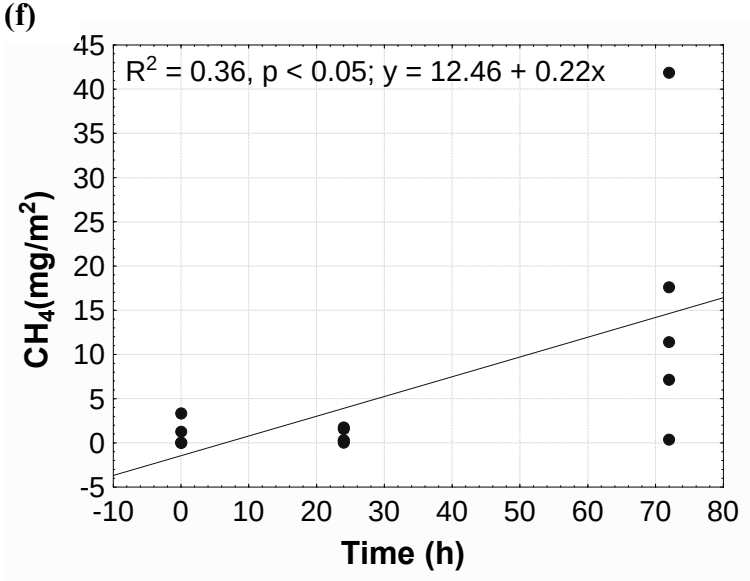
Appendix 4.3 - Least squares regressions of CH_4 concentrations vs. time in (a) 4m-CT, (b) NW, (c) HS, and (d) PP, (e) MP, (f) SW, (g) TP9, (h) BP, and (i) CNRL.

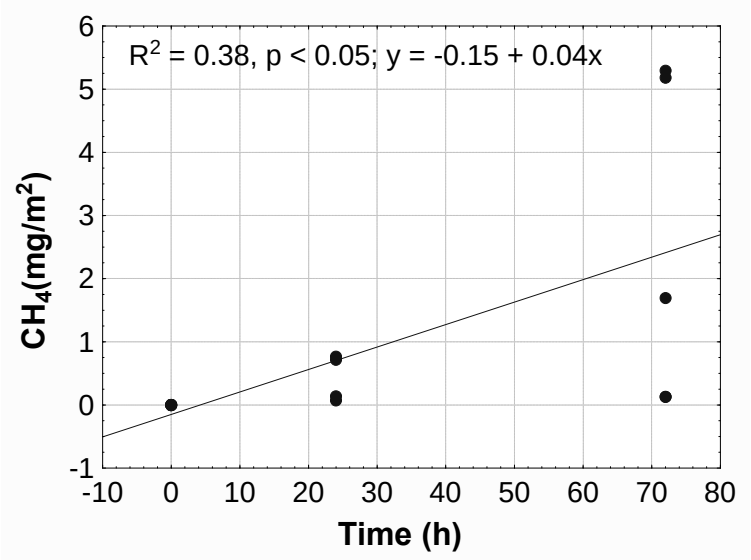


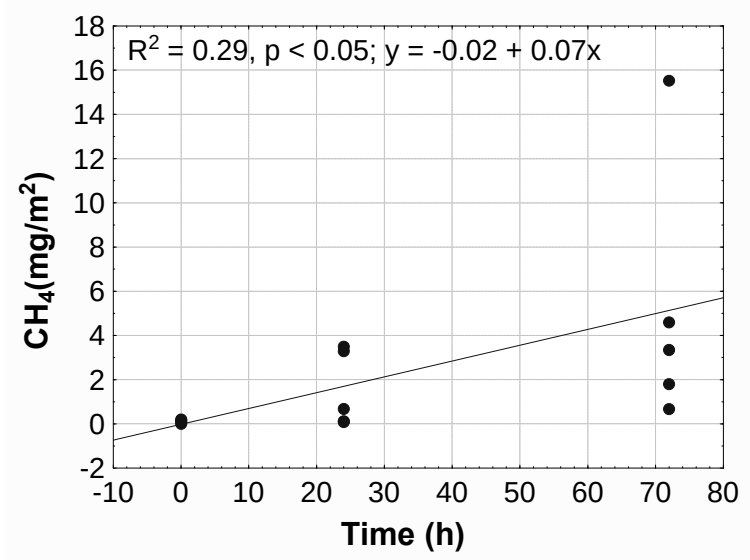
(e)


R2 = 0.16, p = 0.14; y = -0.07 + 0.01x
CH4(mg/m2)
Time (h)

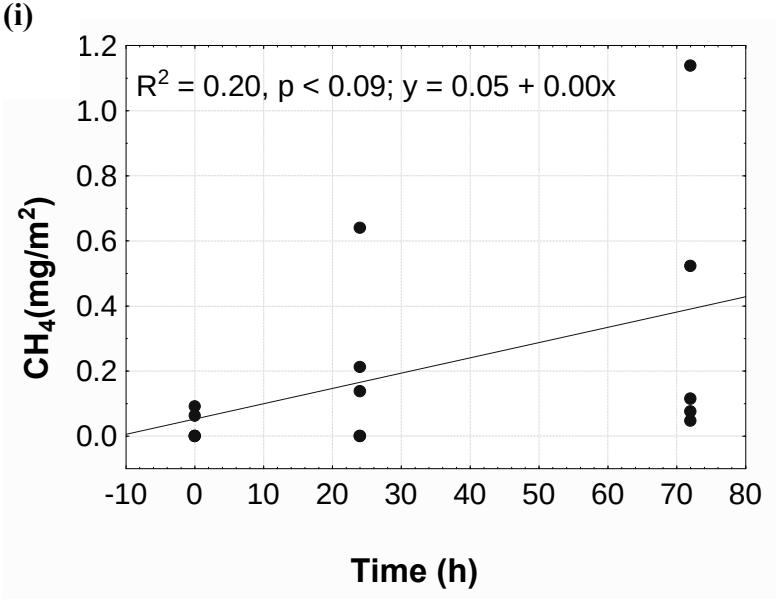
(f)


R2 = 0.36, p < 0.05; y = 12.46 + 0.22x
CH4(mg/m2)
Time (h)


R2 = 0.38, p < 0.05; y = -0.15 + 0.04x
CH4(mg/m2)
Time (h)


R2 = 0.29, p < 0.05; y = -0.02 + 0.07x
CH4(mg/m2)
Time (h)

(i)


R2 = 0.20, p < 0.09; y = 0.05 + 0.00x
CH4(mg/m2)
Time (h)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-10
0
10
20
30
40
50
60
70
80

Appendix 4.4 – Variables used to calculate pCO_2 and pCO_2 values measured in the study wetlands.

Wetland	Date	Alkalinity (expressed in $CaCO_3$) (mol/L)	pH	Salinity (mol/L)	Temperature (^oC)	α_{H+} (=10^-pH) (mol/L)	α_{HCO3-} (=alkalinity * $16.38x10^{-6}$) (mol/L)	K_{CO2} (mol/L*atm)	pCO_2 (uatm)
SW	20-Jul-05	169.67	7.3	0.2	17.5	4.57E-08	8.45E-04	0.042	2083
SW	22-Jul-05	176.23	7.2	0.2	17.5	7.08E-08	8.78E-04	0.042	3352
PP	22-Jul-05	213.11	9.0	0.4	20.1	1.00E-09	1.20E-03	0.039	69
NW	27-Jun-05	390.16	8.9	0.6	15.5	1.15E-09	1.94E-03	0.044	113
NW	27-Jun-05	386.07	8.9	0.6	15.5	1.17E-09	1.92E-03	0.044	115
NW	27-Jun-05	397.54	9.0	0.6	15.5	9.77E-10	1.98E-03	0.044	98
HS	27-Jun-05	279.51	8.2	1.4	22.2	6.92E-09	1.57E-03	0.037	659
HS	27-Jun-05	228.69	7.8	1.4	22.2	1.55E-08	1.28E-03	0.037	1207
HS	27-Jun-05	231.97	7.9	1.4	22.2	1.38E-08	1.30E-03	0.037	1091

Figure 4.5 – Carbon dioxide and methane fluxes from dark microcosms in wetland sediments.

Wetland	Site	Time (h)	CO_2 (mg m^{-2})	CH_4 (mg m^{-2})
CNRL	A	0	2.64	0.06
CNRL	B	0	1.91	0.00
CNRL	C	0	2.56	0.00
CNRL	D	0	3.12	0.09
CNRL	E	0	3.03	0.00
CNRL	A	24	0.87	0.64
CNRL	B	24	3.93	0.14
CNRL	C	24	5.83	0.00
CNRL	D	24	3.57	0.00
CNRL	E	24	5.32	0.21
CNRL	A	72	17.74	1.14
CNRL	B	72	8.13	0.52
CNRL	C	72	6.39	0.12
CNRL	D	72	18.82	0.08
CNRL	E	72	8.32	0.05
4mCT	A	0	51.58	0.10
4mCT	B	0	51.58	6.92
4mCT	C	0	62.06	0.18
4mCT	D	0	61.22	0.00
4mCT	E	0	27.09	0.00
4mCT	A	72	0.00	0.00
4mCT	B	72	0.00	0.00
4mCT	C	72	0.00	0.00
4mCT	D	72	0.00	0.00
4mCT	E	72	0.00	0.00
NW	A	0	33.13	0.00
NW	B	0	20.27	0.00
NW	C	0	28.36	0.00
NW	D	0	32.72	5.50
NW	E	0	22.24	0.00
NW	A	72	4.80	0.12
NW	B	72	3.89	0.25
NW	C	72	4.48	0.08
NW	D	72	6.46	1.86
NW	E	72	6.42	0.09
HS	A	0	2.97	0.00
HS	B	0	2.58	0.00

HS	C	0	2.22	0.00
HS	D	0	2.34	0.00
HS	E	0	10.80	0.00
HS	A	24	17.53	0.00
HS	B	24	10.79	0.00
HS	C	24	5.84	0.00
HS	D	24	9.58	0.00
HS	E	24	19.23	0.00
HS	A	72	17.18	0.21
HS	B	72	15.20	0.05
HS	C	72	10.70	0.01
HS	D	72	20.45	0.01
HS	E	72	21.17	0.00
PP	A	0	2.99	1.43
PP	B	0	1.71	0.03
PP	C	0	3.67	0.84
PP	D	0	2.99	0.02
PP	E	0	5.47	0.14
PP	A	24	55.31	67.57
PP	B	24	5.92	3.19
PP	C	24	10.87	2.25
PP	D	24	5.42	0.32
PP	E	24	3.17	0.02
PP	A	72	15.82	31.26
PP	B	72	4.87	7.67
PP	C	72	23.86	3.48
PP	D	72	24.11	0.47
PP	E	72	23.43	19.76
MP	A	0	2.26	0.00
MP	B	0	2.23	0.00
MP	C	0	2.15	0.00
MP	D	0	2.27	0.00
MP	E	0	2.20	0.00
MP	A	24	3.19	0.00
MP	B	24	3.00	0.00
MP	C	24	2.73	0.00
MP	D	24	2.54	0.00
MP	E	24	6.64	0.00
MP	A	72	9.29	2.15
MP	B	72	8.44	0.15
MP	C	72	10.02	0.00

MP	D	72	10.09	0.04
MP	E	72	9.39	0.05
SW	A	0	2.23	0.01
SW	B	0	N/A	N/A
SW	C	0	3.00	1.26
SW	D	0	2.14	0.02
SW	E	0	N/A	3.34
SW	A	24	6.58	0.20
SW	B	24	4.58	0.30
SW	C	24	7.62	0.05
SW	D	24	8.81	1.56
SW	E	24	7.62	1.74
SW	A	72	41.47	41.87
SW	B	72	2.16	11.41
SW	C	72	20.46	0.37
SW	D	72	21.49	17.60
SW	E	72	27.43	7.15
TP9	A	0	2.87	0.00
TP9	B	0	2.77	0.00
TP9	C	0	2.38	0.00
TP9	D	0	2.29	0.00
TP9	E	0	2.58	0.00
TP9	A	24	6.80	0.13
TP9	B	24	11.10	39.03
TP9	C	24	3.91	0.72
TP9	D	24	6.40	0.07
TP9	E	24	20.65	0.76
TP9	A	72	30.04	5.29
TP9	B	72	33.07	5.18
TP9	C	72	51.27	1.69
TP9	D	72	15.45	0.13
TP9	E	72	8.17	0.13
BP	A	0	11.86	0.20
BP	B	0	8.90	0.00
BP	C	0	20.00	0.16
BP	D	0	16.89	0.08
BP	E	0	31.29	39.69
BP	A	24	35.53	3.29
BP	B	24	19.02	0.69
BP	C	24	18.54	0.13
BP	D	24	41.28	0.10

BP	E	24	17.91	3.50
BP	A	72	28.06	0.66
BP	B	72	89.22	3.35
BP	C	72	106.02	1.80
BP	D	72	76.21	4.59
BP	E	72	17.57	15.53

N/A – Measurement not taken

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VITA AUCTORIS

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Year of birth: 1980

EDUCATION

St. Anne High School,
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1994-1999

University of Windsor
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1999-2003 B.Sc. in Honours Environmental Science with Thesis
Thesis: *Abundance and Isotopic Composition of Carbon and Nitrogen in Soil Organic Matter from Point Pelee Marsh*

University of Windsor
Windsor, Ontario
2003-2007 M.Sc. in Biological Sciences
Thesis: *Carbon sources, microbial community production, and respiration in constructed wetlands of the Alberta, Canada oil sands.*

PROFESSIONAL EXPERIENCE

University of Windsor, Windsor Ontario
2002
Laboratory Assistant

- Performed diagenesis and low-temperature geochemistry research.
- Conducted samples preparation and assisted in stable isotope analysis.
- Developed knowledge in the use of a Thermo-Finnigan mass spectrometer and elemental analyzer.

University of Windsor, Windsor, Ontario
2003
Field Assistant

- Participated in the Great Lakes Environmental Indicators (GLEI) research project which examined the condition of coastal wetlands surrounding all 5 Great Lakes.
- Identified fishes and aquatic plants and surveyed their diversity & abundance.

University of Windsor, Windsor, Ontario
2003-2005
Graduate Assistant

- Demonstrated principles and techniques in biology labs, including "Introduction to Microbiology with Techniques" and "Medical Microbiology."
- Proctored quizzes, midterms and final exams.

Manaaki Whenua Landcare Research, Nelson, New Zealand
2006-2007
Research Assistant

- Co-authored and published an environmental impact assessment report for Biosecurity New Zealand which identified potential environmental impacts of toxic bait formulations used for invasive ant control.
- Tested the effectiveness of toxic baits in controlling invasive ants and wasps in New Zealand.
- Surveyed the geomorphology of rivers with Trimble Real Time Kinematic (RTK) and Global Positioning System (GPS) and Electronic Distance Measurement (EDM) tools.
- Analyzed river cross-sections and determined riverbed degradation in the Motueka Catchment during the 20th and 21st centuries.
- Surveyed New Zealand Regional Councils on their views and uses of "integrated catchment management" and produced a summary of study results.
- Conducted fish studies using an eletrofishing technique and a New Zealand Wood Pigeon census.

WORKSHOPS

Canadian Water Network Southwestern Ontario Student Communications Workshop (December 2005). St. Jacobs, Ontario.

PRESENTATIONS

Daly, C. and J.J.H. Ciborowski, 2004. Microbial carbon sources in boreal wetlands. Poster presented in the proceedings of the 31st annual Aquatic Toxicity Workshop, Charlottetown, Prince Edward Island.

Daly, C. and J.J.H. Ciborowski, 2005. Microbial carbon sources in wetlands affected by oil-sands mining in northern Alberta. . Paper presented in the proceedings of the 32nd annual Aquatic Toxicity Workshop, Waterloo, Ontario.

Daly, C. and J.J.H. Ciborowski, 2005. Microbial carbon sources in wetlands affected by oil-sands mining in northern Alberta. Paper presented at the 1st annual Canadian Water Network Oil-sands Research Meeting.

Daly, C. and J.J.H. Ciborowski, 2006. Planktonic bacterial community production in oil-

sands affected wetlands. Poster presented in the proceedings of the 33rd annual Aquatic Toxicity Workshop, Jasper, Alberta.

Daly, C. and J.J.H. Ciborowski, 2006. Planktonic microbial productivity and its implications for the carbon cycle in oil-sands affected wetlands. Paper presented at the CONRAD Reclamation and Environmental Research Symposium, Edmonton, Alberta.

Daly, C. and J.J.H. Ciborowski, 2006. Planktonic microbial productivity and its implications for the carbon cycle in oil-sands affected wetlands. Paper presented at the 2nd annual Canadian Water Network Oil-sands Research Meeting.

IN PREP

Daly, C. and J.J.H. Ciborowski, 2007. Planktonic bacterial community production in oil-sands affected wetlands of northeastern Alberta, Canada

Daly, C., and J.J.H. Ciborowski, 2007. Determining energy flow and trophic structure in the microbial community of oil sands-affected wetlands: A stable isotope approach.

OTHER PUBLICATIONS

Forgie, S.A., O'Halloran, K, Ward, D.F., Stanley, M.C., Rees, J.S., and C. Daly. Environmental impact assessment for baits used during RIFA (red imported fire ant, *Solenopsis invicta*) incursions. Landcare Research Contract Report: LC0607/046, 1-34, 30-11-2006. Tamaki, Auckland, New Zealand, Manaaki Whenua Landcare Research.

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