Examining the Growth and Stable Isotopes of Phytoplankton and Periphyton Communities Exposed to Oil Sands Reclamation Strategies

by

Monique Boutsivongsakd

A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Master of Science
in
Biology

Waterloo, Ontario, Canada, 2013

Author's Declaration

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

I understand that my thesis may be made electronically available to the public.

Abstract

The impacts of oil sands processed materials (OSPM) on phytoplankton and periphyton community growth and stable carbon and nitrogen isotopes were examined. Estimates of plankton and periphyton community growth, measured as chl *a* and dry weight, were low and similar in reference and OSPM reclamation wetlands. The use of stable isotope analyses revealed higher $\delta^{15}N$ of plankton and periphyton in OSPM wetlands than reference wetlands, possibly due to increased TN concentrations in some OSPM wetlands.

In the laboratory, water-soluble fractions (WSF) of two types of OSPM (mature fine tailings, MFT and consolidated tailings, CT) and an amendment material (peat-mineral mixture), potential fill materials in wetland or end pit lake reclamation, were examined for phytoplankton community growth and stable carbon and nitrogen isotopes. All WSF treatments had higher chl *a* compared to reference water and maximum growth was observed at a 50:50 ratio of peat:CT or peat:MFT. In general, WSFs of peat had the highest concentration of total nitrogen (TN) whereas WSFs of MFT had the highest total phosphorus (TP; 3x higher). The results suggested that the addition of peat as an amendment to OSPM (particularly for MFT), contributing additional TN, could improve phytoplankton community growth in oil sands reclamation. At higher percentages of MFT WSF, there was increased turbidity due to fine clay particles that likely contributed to reduced phytoplankton growth. Turbidity could be an important factor limiting phytoplankton growth and thus reducing dietary resources and biological detritus (via sedimentation) in the initial development of an end pit lake. The WSFs also promoted the unfavourable growth of filamentous algae, highest at intermediate concentrations of peat and CT WSFs and inhibited in MFT WSFs due to light limitation. Stable N isotopes of plankton and filamentous algae suggests that ^{15}N enrichment of algae could be a useful indicator of nutrient inputs, including OSPM seepage into natural aquatic systems, for oil sands regional monitoring programs.

Acknowledgements

I would like to thank my supervisor Dr. George Dixon for first taking me on as an undergraduate research student, lab technician and seeing me through my graduate work with his unwavering support and guidance. Andrea Farwell without your guidance and help I would have been lost. Thanks to my committee member, Barbara Butler, for your insightful suggestions. Thanks to those who were a wonderful help in the field, Hao Chen and Allison Legg and various students from the University of Windsor. A special thanks to Jan Cibrowski, Carla Wytrykush and Richard Kavanagh for their help and guidance while in Fort McMurray. Thanks to the staff at Syncrude Canada Ltd and Suncor: Mike Mackinnon, Terry Van Meer, Clara Qualizza, Wayne Tedder, Jennifer Fudge and Lori Cyprien. Thanks to the staff at the University of Waterloo isotope lab: Lois Graham, Bill Mark, Phyllis Diebolt, and Stella Cuc. Thanks to Ralph Smith, Mark Servos, and Bruce Greenberg for the use of their laboratory equipment. Thanks to Ken Oakes for our long chats and words of encouragement. To all my labmates particularly Vince Nero, Patricia Videla and Rick Frank, who were a wealth of information and always willing to lend a hand. To all the volunteers, work study or co-op students that assisted me during my experiments or washed dishes, it was a big help.

Thanks to the loves of my life, my children who bring love and chaos into my life and to my significant other who shares the task of taking on the 2 princes and princess. I love you all.

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada, Syncrude Canada Ltd., Suncor Energy Ltd. Canadian Natural Resources Ltd., Albian Sands Energy (Shell Canada), Total E&P Canada, Ltd. and the Canadian Water Network to George Dixon.

Table of Contents

List of Figures

List of Tables

Chapter 1 Introduction

1.1 Thesis Overview

Oil sands mining activities generate a wide variety of waste products that could be used to reclaim wetlands, ponds and lakes in post-surface mining areas. There is a need to better understand the impacts of oil sands reclamation strategies on the various components that constitute a sustainable and healthy aquatic ecosystem. The focus of this study is on primary resources, plankton and periphyton, that contribute energy for growth of higher trophic levels.

1.2 Objectives

The goal of this study was to determine the impact of different types of oil sands reclamation on growth and stable carbon and nitrogen isotopes of plankton and periphyton. This goal was accomplished using two different approaches as outlined in Objectives 1 (Field Survey) and 2 (Laboratory Microcosms).

Objective 1 (Chapter 2):

The first objective was to estimate phytoplankton and periphyton community growth (using chlorophyll (chl) *a* and dry weight) in reference reclamation wetlands/ponds that contain no oil sands processed material (OSPM; e.g. tailings water or solids) and in OSPM reclamation wetlands/ponds. To assess carbon and nitrogen flow and cycling at the base of the aquatic food web, stable carbon and nitrogen isotopes of phytoplankton and periphyton were also examined for reference and OSPM reclamation sites.

Objective 2 (Chapter 3):

The second objective was to estimate phytoplankton community growth and stable carbon and nitrogen isotopes for two types of OSPM (mature fine tailings, MFT and consolidated tailings, CT) as well as a peat-mineral mixture used as a potential amendment material in oil sands reclamation. In this study, water-soluble fractions (WSFs) of construction materials (OSPM and muskeg overburden) were generated in order to assess the capacity of these WSFs to support phytoplankton production under controlled conditions without the influence of biotic factors such as grazing.

1.3 Oil Sands Mining Activities

The Athabasca oil sands in Alberta, Canada, contain one of the largest petroleum reserves in the world with an estimated 178.8 billion barrels of oil (US EIA, 2005). Bitumen is a natural, heavy crude oil composed of a complex mixture of petroleum hydrocarbons as well as other organic and inorganic compounds. Surface mining techniques are frequently used to first remove the overburden and then recover the oil sands ore for processing. The bitumen recovery process utilizes the Clark caustic hot water extraction method whereby the oil sand ore is mixed with warm water and NaOH to separate the bitumen from the sand. This process produces large amounts of waste water and slurry composed of silt, clay, and unrecovered bitumen. A substantial amount of water is required, therefore, creating slurry waste (tailings) (FTFC, 1995). The provincial government has set up a zero discharge policy, therefore, all waste materials resulting from the extraction of bitumen from sand are temporarily stored in tailings ponds prior to reclamation. In the tailings pond, the larger coarse tailings (sand) settle out first forming beaches and dykes (FTFC, 1995). The surface zone contains a layer of clear water where 70% is recycled back to the plant. Over time, fine tailings (FT) densify creating mature fine tailings (MFT) (Madill *et al*., 1999) which is composed of 85% water, 13% clay, and 2% bitumen (FTFC, 1995). In some cases, gypsum ($CaSO_4$) was added to MFT to aid in the precipitation of clay particles (Whelly, 1999). This type of reclamation is referred to as composite or consolidated tailings (CT).

1.4 Oil Sands Reclamation Strategies

Alberta has a zero discharge policy that forbids the release of process-affected material, therefore, the waste material is held in large settling basins on site. One reclamation method is the wet landscape approach which uses process-affected material to create wetlands, ponds or lakes. To date, various experimental wetlands and ponds have been constructed utilizing various reclamation substrates such as MFT, and CT and/or process-affected water (see examples in Farwell *et al*., 2009a). The reclaimed sites are classified as oil sands processed material (OSPM) sites if any type of processed material was used in the construction of the aquatic system, otherwise the reclaimed site is classified as a reference site. In some cases, peat-mineral mix that was stock-piled during the removal of overburden early in the mining process, is later used as an amendment in aquatic reclamation. Peat provides a source of organic matter and nutrients that may help the initial colonization of newly constructed wetlands or ponds.

Naphthenic acids (NAs), polycyclic aromatic compounds (PACs) and salinity are known constituents of concern in oil sands reclamation. The concentration of these constituents is dependent on the type and quantity of OSPM used in the construction of the reclamation wetlands.

NAs are a complex group of cyclic or acyclic alkanoic carboxylic acids (Clemente and Fedorak, 2004). NAs are naturally found in surface waters at concentrations of 1-2 mg/L, however, greater than 35 mg/L have been found in OSPM reclamation (Leung *et al*., 2001; Leung *et al*., 2003). Laboratory studies have shown that indigenous microbial species were able to degrade oil sands NA extracts as well as a commercial NAs thereby reducing acute toxicity (Herman *et al*., 1994). Initial NA concentration in the process-affected water may decrease as a result of the biodegradation of NAs with smaller C numbers (<21) leaving higher molecular weight NAs (>22 C number) (Holowenko *et al.*, 2002). This suggests that although initial process materials are elevated in NAs, they can be degraded to some degree, adding a new carbon source to the reclaimed aquatic ecosystem.

Polycyclic aromatic compounds (PACs) are compounds composed of two or more fused benzene rings. The hydrophobic nature of these compounds causes an increased affinity for organic matter thus PACs are bound to the sediments (1300 µg/g, Colavecchia *et al*., 2004) at higher concentrations than those dissolved in the water column (<1 ug/L Madill *et al*., 1999). The Athabasca tributaries contain natural levels of PACs from the oil sands deposits of up to 34.7 µg/g; alkylated PACs are the predominate form of PACs found in the petroleum mixtures, particularly dibenzothiophenes (DBT) (Headley *et al*., 2001). Reference sites contain 0.03-2.4 µg/g of PAHs with the OSPM sediment containing the largest concentration of alkylated PAHs (Colavecchia *et al*., 2004).

Oil sands process-affected ponds can have elevated levels of salinity specifically, sulfate (43.6-98.6 mg/L), chloride (36.1-78.6 mg/L), and sodium (44.6-246 mg/L) (van den Heuvel *et al*., 1999). There are also elevated levels of other major ions including potassium, calcium and magnesium (van den Heuvel *et al*., 1999). Studies that examined the effects of salinity on phytoplankton suggest that there is a strong influence on phytoplankton species composition, which may be as strong as NAs. However, there does not appear to be a significant affect on total phytoplankton biomass; the suggested threshold whereby phytoplankton species composition changes is at greater than 1000 µS/cm (Hayes, 2005).

1.5 Oil Sands Primary Production Studies

Field surveys of OSPM reclamation and reference or natural systems have examined phytoplankton community composition and biomass. Leung *et al.* (2003) sampled various reference and OSPM ponds representing varied NA concentrations to examine the effects on phytoplankton. The highest biomass was found at the reference site (Mildred lake) however, the second highest was a site with a high NA concentration (>40 mg/L) which received seepage from

an active tailings pond, Mildred lake settling basin. Leung *et al.* (2003) suggested a threshold level of effect on phytoplankton species composition at 6-20 mg/L of NA. Cyanobacteria and Chlorophyta were the dominant groups of algae found at the ponds that were sampled. No one factor could explain the variability observed in phytoplankton biomass (TN, TP, NAs or conductivity). A field survey of 30 lakes and ponds revealed the taxa associated with the sites with higher NA concentrations consisted of *Navicula sp., Peridinium pisillum, Euglena spp., Carteria sp.,* and micro greens (Hayes, 2005). Hayes (2005) found that biomass was not correlated to the variables that were measured (NA, salinity, TP, TN) and suggested other variables such as biological (e.g. grazing) or physical (e.g. turbidity) factors may have played a role in the 30 lakes and ponds that were sampled. The survey found that the minimum concentration that had an effect on phytoplankton community composition was a NA concentration greater than 30 mg/L and conductivity greater than 1000 μS/cm.

Various microcosm studies have been conducted to examine the effects of NAs and salinity, measured as conductivity, on phytoplankton community composition and biomass. The phytoplankton community structure was affected at NA levels greater than 20 mg/L (Leung *et al*., 2001). Above these levels, a new phytoplankton community structure was composed mainly of *Navicula radiosa, Keratococcus sp., Gloeococcus schroeteri, Ochromonas spp., Chlorella spp.* and *Botryocuccus braunni.* shifting away from a community dominated by Cyanobacteria. Phytoplankton community biomass did not appear to be significantly affected by NA or major ion concentration (Leung *et al*., 2001). Since the treatments had both elevated salinity and NAs, Leung *et al*. (2001) was not able to separate the confounding factors of NA concentration and salinity and suggested salinity may have altered the effects of NA. Later, NA and salinity bioassays were conducted to determine the interactive effects of NAs and salinity on phytoplankton communities (Hayes, 2005). Low levels of NA were found to have stimulatory effects on phytoplankton biomass (chl *a*). Laboratory experiments were conducted to separate NA concentration and salinity effects; the phytoplankton composition became less similar to the reference community as NA and conductivity increased. Salinity and NA effects were found to be similar but uncorrelated (Hayes, 2005).

Growth of attached algae has also been examined in oil sands reclamation. Microcosm studies were used to evaluate the effects of various substrates, such as soil, sand, and/or CT, in the presence and absence of oil sands processed water, on biofilm growth (Frederick, 2011). In that study, mean chl *a* values were higher in treatments with processed water than treatments without processed water. Also, treatments with CT, as a substrate, had significantly higher chl *a* values than other treatments (Frederick, 2011). A nutrient enrichment study examined the effects

of peat and/or inorganic nutrient addition on plankton and periphyton growth in the presence of OSPM substrates, sand and sand-MFT mix (Chen, 2011). Peat amendments maintained increased nutrient levels of TN, TP, and DOC while nutrient additions were only a temporary relief as they were quickly utilized by the biota. Phytoplankton and periphyton growth were higher in all sand-MFT treatments supplemented with peat (Chen, 2011).

Differences in macrophyte communities and biomass decreased as the wetlands aged, however, submerged macrophyte biomass remained low at OSPM sites regardless of age (Kolavenko *et al*., 2013). A study that examined *Carex aquatilis*, a North American aquatic sedge, native to peatlands and marshes, found that plants in OSPM sites were morphologically different, with reduced culm height and leaf length compared to reference sites (Mollard et al., 2012). Physiologically, *C. aquatilis* had similar net photosynthesis and transpirations rates compared to reference sites. Macrophyte colonization is important to periphyton since they provide surface area for biofilm attachment as well as materials contributing to C assimilation and productivity (Mollard et al., 2012).

1.6 Stable Carbon and Nitrogen Isotopes

Overview

Stable carbon isotope analysis has been utilized to study carbon flow in food webs. Stable carbon isotopes provide information on energy flow and C sources since C isotopes are conserved between consumer and their dietary source (<1‰ per trophic transfer; Peterson and Fry, 1987). The use of stable C isotopes to trace sources utilized by bacteria in aquatic systems is based on the principle that the sources have distinct stable C isotopes. For example, the $\delta^{13}C$ of microbes was used to determine organic matter sources from either natural substrates such as plants and plant leachate or artificial substrates (glucose) based on the $\delta^{13}C$ differences between sources (Coffin *et al*., 1989).

The $\delta^{13}C$ signature of algae is determined by the source of C that is utilized by their cells. Dissolved inorganic carbon (DIC), derived from the atmosphere, rock weathering or microbial respiration in an aquatic system can exist as dissolved CO_2, bicarbonate, and carbonate depending on the pH (Hecky and Hesslein, 1995). Algae prefer to utilize dissolved CO_2 since this does not require energy. If algae must use HCO_3^-, this will result in cells becoming ^{13}C enriched. Differences in C species utilization by plankton (CO_2) versus benthic algae (HCO_3^-) has been used to study C flow in aquatic food webs. For example, in a turbid lake in the Mackenzie Delta, $\delta^{13}C$ signatures of primary consumers (^{13}C depleted) indicated that the dominant dietary source was plankton (^{13}C depleted) vs. benthic algae (^{13}C enriched) (Hecky and Hesslein, 1995).

Stable N isotope analysis is utilized to determine trophic level as well as understanding N cycling processes at the base of the aquatic food web. In general, the trophic position of an animal is defined based on the principle that the $\delta^{15}N$ signature of a consumer increases (or is ^{15}N enriched) from one consumer level to another. On average, ^{15}N enrichment of 3.4 ± 1.1‰ has been documented as trophic level increases (Minigawa and Wada, 1984). C and N isotopes can provide information on both the source and trophic status of various organisms when used in combination. For example, in a subartic lake, a study was able to determine the trophic positions of 3 classes of biota. $\delta^{13}C$ values suggested the phytoplankton made up only a small portion (~15%) of particulate organic matter (POM). The $\delta^{15}N$ of Daphnia was influenced by atmospheric nitrogen derived from a N_2-fixing cyanobacteria bloom; the $\delta^{15}N$ values also revealed the highest trophic position of the Heterocope (*Copepod*) which relied on the POM for nutrients (Gu *et al*., 1994). A study that examined *Chlamydomonas acidophila* in an acidified lake reported that NH_4^+ concentrations were negatively correlated with $\delta^{15}N$ signatures, ^{15}N depleted values were observed during higher concentrations of NH_4^+ suggesting that nutrients are a factor of phytoplankton $\delta^{15}N$ signatures (Doi *et al.*, 2004).

Oil Sands Stable Isotope Studies

Various studies have utilized both $\delta^{13}C$ and $\delta^{15}N$ of primary or microbial producers (Daly, 2007; Videla *et al*., 2009), invertebrates and fish (Ganshorn, 2002; Murchie and Power, 2004; Farwell *et al*., 2009ab), or entire food webs (Elshayeb, 2006) to establish trends in carbon and nitrogen dynamics in oil sands reclamation. Initial isotope research reported trends of extreme $\delta^{15}N$ enrichment and to a lesser extent $\delta^{13}C$ depletion in benthic invertebrates associated with some OSPM sites (Farwell *et al*., 2009). The survey of various reference and OSPM sites found that invertebrates at CT sites had the highest level of ^{15}N enrichment followed by MFT sites (Farwell *et al*., 2009). Based on food web analyses, there were no obvious large shifts in food web structure and function at the OSPM sites, but there were changes in positions of organisms relative to the base of the food web (Elshayeb, 2006). ^{15}N enrichment of invertebrates was thought to be a function of elevated levels of nitrogen, mainly NH_4^+, in the OSPM systems; elevated levels of NH_4^+ in tailings, from oil sands production processes, may be slowly released from the MFT in aquatic reclamation (Farwell *et al.*, 2009).

Additional studies focused on lower trophic levels in an attempt to understand the biogeochemical processes associated with the isotope trends observed in invertebrates and fish inhabiting OSPM vs. reference sites. Microbial microcosm studies found that there was a significant relationship between the biofilm $\delta^{15}N$ and reclamation type (Daly, 2007). Microbial

biofilm from OSPM sites were more ^{15}N enriched ($\delta^{15}N$, 7.32‰) than reference sites ($\delta^{15}N$, -1.85‰). DOC concentrations varied little between both reference and OSPM sites (-28.7 to -26.7‰) while the microbial biomass became ^{13}C enriched in OSPM sites (-26.2 ±1.3‰) compared to reference sites (-29.5 ±0.8‰) (Daly, 2007). This suggests there must be another C source in OSPM sites. Laboratory studies examined the degradation of DOC from an oil sands NA extract using oil sands-derived bacterial cultures and found microbial biomass ^{15}N enrichment (3.8 to 8.4 ‰) under conditions of semi-continuous NA and mineral media renewal (Videla *et al.*, 2009).

For OSPM sites (NW, CT, TP9), isotope values were most consistent with the petroleum source which suggested that OSPM carbon sources were assimilated successfully by the microbial component of the food web (Daly, 2007). Periphyton $\delta^{13}C$ values from the low (0 to 4 mg/L) and medium (4 to 15 mg/L) NA concentration sites differed significantly from the periphyton $\delta^{13}C$ signatures in high (>15 mg/L) NA concentration sites (Elshayeb, 2006). There was a weak trend of decreasing $\delta^{13}C$ values of plankton and plants with increasing OSPM influences with the exception of periphyton (Elshayeb, 2006).

To date, studies focused on the base of the food web have found trends of higher $\delta^{13}C$ and $\delta^{15}N$ values of microbial biomass and periphyton from OSPM sites, characterized by higher NA concentrations, relative to reference sites (Elshayeb, 2006; Daly, 2007) yet our understanding of the environmental factors influencing the isotope trends are limited. Laboratories studies found ^{15}N enrichment of bacteria grown in oil sands NA extract when supplemented with nutrients (Videla *et al*., 2009) which indicates the importance of nutrients in ^{15}N enrichment of microbes. To further our understanding of isotope trends in reclamation systems, the current study examined the relationship between nutrients and growth of phytoplankton and/or periphyton to determine the potential effects on the stable carbon and nitrogen isotopes for phytoplankton and periphyton in reference and OSPM reclamation (Chapter 2) and on the stable carbon and nitrogen isotopes for phytoplankton grown in WSFs of OSPM and an amendment material, peat (Chapter 3).

Chapter 2 Phytoplankton and Periphyton Growth and Stable Isotopes in Oil Sands Aquatic Reclamation

2.1 Overview

In this study, phytoplankton and periphyton growth were examined to determine the impacts of oil sands processed material (OSPM) on primary production. Measures of community growth, chl a and dry weight, were determined for plankton and periphyton from water samples and deployed artificial substrates, respectively, in both reference reclamation sites and reclamation sites influenced by OSPM. In addition, carbon and nitrogen flow at the base of the aquatic food chain was assessed using stable carbon and nitrogen isotopes of plankton and periphyton. Estimates of plankton and periphyton community growth were low but similar in reference and OSPM reclamation wetlands. The use of stable isotope analyses revealed consistently higher $\delta^{15}N$ of plankton and periphyton in OSPM wetlands than reference wetlands, and $\delta^{15}N$ of plankton and periphyton were correlated to TN concentrations. Stable N isotopes of plankton and periphyton suggests that ^{15}N enrichment of biota could be a useful tracer of exposure to OSPM associated with seepage into natural waters as part of oil sands regional monitoring programs.

2.2 Introduction

An estimated 178.8 billion barrels of oil is located in the Athabasca oil sands in Alberta, Canada, one of the largest petroleum reserves in the world (US EIA, 2005). Bitumen, the heavy crude oil, is separated from the sand via a NaOH hot water extraction process. All waste materials are held in large tailings ponds that allow coarse tailings (sand) to quickly settle out forming sand beaches and dykes. The waste water or fine tailings consists of mainly water (85%), silt and clay particles (13%), and unrecovered bitumen hydrocarbons (2%; FTFC, 1995). As the fine tailings settle, a layer of clear water is recovered (70%) and recycled back to the plant (FTFC, 1995). Mature fine tailings (MFT) are formed after the fine tailings densify over a considerable period of time (Madill et al., 1999). Tailings water and MFT accumulate in large quantities in settling basins since there is no discharge into the environment as set by the provincial government (FTFC, 1995). Efforts have been made in the past to accelerate the rate of sedimentation of clay particles in MFT by adding gypsum (CaSO4) to create consolidated tailings (CT; Whelly, 1999). The large quantities of tailings water and solids (MFT and CT) will eventually be used to construct new wetlands, ponds and lakes (referred to as end pit lakes). There are a number of possible strategies for oil sands aquatic reclamation which consists of varying the quantities of tailings water or solids used to reclaim aquatic systems (Farwell et al., 2009a). To assess the long term viability of these reclamation options, numerous experimental aquatic systems have been developed to study measures of ecosystem structure and function (Kolavenko et al., 2013). In the current study, growth of phytoplankton and periphyton were determined for reclamation wetlands constructed using oil sands process material (OSPM; e.g. tailings water or solids) and those constructed with no OSPM (e.g. reference site).

The ability to create healthy aquatic ecosystems from reclamation materials is a challenge due to the presence of a large variety of chemicals associated with natural oil sands, the addition of chemicals as part of the extraction (NaOH) or upgrading (NH_4^+) processes and MFT treatment strategies ($CaSO_4$) (Farwell et al., 2009a). Raw oil sands are composed of complex mixtures of hydrocarbons, polycyclic aromatic compounds, organics acids such as naphthenic acids (NAs), major ions and metals, all of which are present in tailings at varying concentrations. As a result, the tailings water, containing elevated levels of NAs and salinity cause acute and sub-acute toxicity to a wide range of animals and plants (Natural Resources Canada, CanmetENERGY, 2010). Ultimately, the reclamation strategies used to create wetlands and end pit lakes must be non-toxic and sustainable in the long term. In the current study, the focus is on the assessment of the resources at the base of the aquatic food chain (primary production), resources needed to sustain healthy ecosystems.

Oil sands phytoplankton communities have been studied in microcosms (Leung et al., 2001; Hayes, 2005) and in the field (Leung et al., 2003; Hayes, 2005) to determine the effects of NAs and salinity (conductivity). Species composition of the phytoplankton community was affected at NA levels greater than 20 mg/L where there is a shift to a community consisting of more NA tolerant species such as Navicula radiosa, Keratococcus sp., Gloeococcus schroeteri, Ochromonas spp., Chlorella spp. and Botryocuccus braunni (Leung et al., 2001). However the effects of co-factors, NA and salinity, could not be isolated in these microcosm or field studies (Leung et al., 2001 and 2003; Hayes, 2005). In laboratory studies, the richness of phytoplankton communities decreased with increasing NA concentration and the communities were dominated by species from Chlorophyta and/or Pyrrophyta divisions (Hayes, 2005). In the field, Chlorophyta were also predominant in the wetlands that had intermediate to high NA concentration and intermediate salinity (Hayes, 2005).

Although there were changes in species composition due to OSPM, phytoplankton community biomass was not significantly affected by either NAs or major ion concentrations. In a field survey by Leung et al. (2003), the highest biomass was observed in Mildred lake (a reference site) with the next highest biomass at an OSPM site that had greater than 40 mg/L of NAs. Hayes (2005) survey of 30 wetlands and lakes determined that biomass was not correlated to the variables that were measured (NAs, salinity, TP, TN) and suggested other biological or physical factors such as grazing or turbidity may be an issue.

Numerous studies have also examined the effects of OSPM on aquatic macrophytes, periphyton/biofilm or benthic invertebrates in oil sands reclamation. Carex aquatilis in OSPM sites had carbon assimilation rates that were similar to reference wetlands but their growth remained restricted in terms of height and leaf length relative to reference sites (Mollard et al., 2012). Reduced macrophyte growth has implications in terms of reduced surface area available for periphyton colonization and thus reduced energy resources for secondary production. Recent field studies have also found lower microbial biomass in OSPM reclamation sites (Daly, 2007). Biofilm, representing heterotrophic and autotrophic growth, differed depending on the type of OSPM present (Frederick, 2011) while peat amendments to OSPM increased periphyton chlorophyll (chl) a (Chen, 2011). Studies also have found low zoobenthic abundance and richness in new OSPM reclamation, but after several years (5-7) zoobenthic abundance and richness in OSPM wetlands become more similar to reference sites (Whelley, 1999; Leonhardt, 2003).

In order to further the understanding of aquatic food webs in oil sands reclamation, tools such as stable carbon and nitrogen isotopes have also been utilized. Typically, stable C isotopes are conserved from source to consumer (<1‰ per trophic transfer, Peterson and Fry, 1987) and

thus can be used to infer differences in carbon source utilization if the carbon sources are isotopically distinct. In contrast, stable N isotope are used to determine the trophic position of an animal as there is 15N enrichment of 3.4 ± 1.1‰ as trophic level increases (Minigawa and Wada, 1984). However previous surveys of various oil sand reclamation wetlands or ponds found highly 15N enriched invertebrates, uncharacteristic of typical trophic position in OSPM reclamation (Farwell et al., 2009). Further study indicated that there were no large shifts in food web structure and function at the OSPM sites, but there were changes in trophic positions of organisms related to the base of the food web (Elshayeb, 2006). Studies focused on the base of the food web have found trends of higher δ13C and δ15N values of microbial biomass, phytoplankton or periphyton from OSPM sites, characterized by higher NA concentrations, relative to reference sites (Elshayeb, 2006; Daly, 2007) yet our understanding of the environmental factors influencing these isotope trends is fairly limited. Laboratories studies were able to culture highly 15N enriched bacteria on an oil sands NA extract but only when bacteria were intermittently supplemented with a nutrient media (Videla et al., 2009) which suggests the importance of nutrients in 15N enrichment of microbial biomass. To further our understanding of isotope trends in oil sands reclamation, the current study examines phytoplankton and periphyton growth in reference and OSPM reclamation that differ in nutrient levels to determine the potential effects of nutrient levels on the stable carbon and nitrogen isotopes for phytoplankton and periphyton.

The goal of this study is to determine the growth of both phytoplankton and periphyton communities in OSPM and reference reclamation that vary in nutrient levels to assess the impacts of reclamation involving OSPM on growth and carbon and nitrogen flow at the base of the aquatic food web. Earlier studies of fish populations and invertebrate communities suggested energy resource availability as a limiting factor for production in OSPM reclamation (van den Heuvel et al., 1999; Gould, 2000). Measures of community growth (using chl a and dry weight) of phytoplankton and periphyton communities from artificial substrates will be used to assess differences in resources in aged reference vs. OSPM systems.

2.3 Materials and Methods

2.3.1 Study sites

The study sites are located in Fort McMurray, Alberta (57°05'102" N, 111°41'623" W) on either the Syncrude or Suncor mining lease. Nine wetlands were chosen for the field survey; five reference and four OSPM sites (Table 2.1). All reference sites are constructed sites that have not been amended with processed material with the exception of South West Sands Beaver Pond (SSBP), which is a naturally formed wetland. OSPM sites contain either processed water and/or MFT or CT used as sediment. NA concentrations were lower for reference sites (range, 1.2 – 14.5 mg/L) than OSPM sites (range, 25.5 – 55 mg/L) (Table 2.1).

Table 2. 1 Wetland descriptions for reference and OSPM study sites

	Year Constructed	Wetlands	Description	NA Concentration (mg/L)
Reference				
	1996	Bill's Lake (BL)	20-50 cm saline sodic overburden[d]	nd
	1993	Shallow Wetland (SW)	Storage area for unprocessed WID water (muskeg drainage water) [b]	1.4 ±0.8[b]
	2001	Peat Pond (PP)	80cm of a clay-loam mixture + 20 cm peat-mineral mix, surface water[a]	1.2[a]
	1985	High Sulphate (HS)	Lean oil sands mixed with overburden material (peat) [b]	14.5[a]
	n/a	South West Sands Beaver Pond (SSBP)	natural, formed in stream channel prior to mining [a]	1.3[a]
OSPM				
	1997	Syncrude Consolidated Tailings (Mike's Pond) (SCT)	CT release water from 1997/1998 CT pilot test [b]	55 ±11[b]
	1992	Test Pond 9 (TP9)	MFT and water from MLSB [d]	25.5[a]
	1998	4m Consolidated tailings pond – peat zone (CTW)	4m CT + sand substrate, CT water from dyke seepage [b]	55 ±13[b]
	1986	Natural Wetland (NW)	Surface runoff and CT water [b]	47 ±13[b]

[a] Daly, 2007; [b] Farwell *et al*., 2009 - open water mean 1998-2004; [c] Leung *et al.*, 2003; [d] Golder, 2002; nd, no data

Reference sites

Bill's lake (BL) was constructed in 1996 with 20-50 cm saline sodic overburden (Golder, 2002). Shallow wetlands (SW) was used as a storage area for unprocessed West Interceptor Ditch (WID) water from muskeg drainage. It was constructed in 1993 (Syncrude, SCL lease 17) (Farwell *et al*., 2009). The substrate in Peat pond (PP) consists of 80 cm of a clay-loam mixture and at least 20 cm of a peat and mineral mixture and capped with unprocessed water (Daly, 2007). High sulphate (HS) was constructed in 1985 (Suncor Lease 86) and consists of lean oil sands mixed with overburden mainly consisting of peat (Farwell *et al.*, 2009). South west sands beaver pond (SSBP) is a natural pond formed prior to mining activites on the Syncrude lease site and has not been impacted by mining processes. Its estimated age is at least a few decades (Daly, 2007).

OSPM sites

The substrate in Syncrude CT pond (SCT) or "Mike's pond" (SCL Lease 17) consists of clay and it was capped with CT processed water in 1997 (Ganshorn 2002). Test pond 9 (TP9) was constructed with clay, MFT and capped with processed water from Mildred lake settling basin in 1992 (Daly, 2007; Leung *et al*., 2003). Four meter consolidated tailing wetland (CTW) was constructed in 1998 (Suncor Lease 86); it contains 4 m of CT and sand as well as CT water from dyke seepage (Farwell *et al*., 2009; Daly, 2007). The CT substrate was uncapped except in small areas of muskeg peninsulas (20 to 60 cm thick). There is a continuous inflow of fresh tailings effluent pumped into the adjacent wetland that leads to CTW and exits into another wetland with a residence time of 30 days in this area (Daly, 2007). Natural wetland (NW) (Suncor Lease 86) was created in 1986 and receives surface water runoff and CT water (Farwell *et al*., 2009). NW is adjacent to a settling basin and receives a continuous supply of CT water from dyke seepage (Farwell *et al*., 2009).

2.3.2 Water Chemistry

Temperature, conductivity, pH, and dissolved oxygen were measured using an Orion Model 1230 field meter (Thermo Fisher Scientific, Waltham, MA) at the time of collection of water and biological samples. Samples for TN and TP were collected in 250 ml glass bottles and refrigerated at 4ºC and then shipped to Biogeochemical Analytical Service Laboratory at the University of Alberta (Edmonton, Alberta, Canada) for analysis. Samples for DOC and DIC concentration and their associated $\delta^{13}C$ signatures were collected in 250 mL TraceClean amber

borosilicate glass bottles with a Teflon®-lined closure. Samples were preserved with 5 % W/V $HgCl_2$ and the remaining headspace was topped off with water from the site. Samples were stored at approximately 4°C and shipped to the University of Waterloo, Waterloo, Ontario. Samples were filtered using a 0.45 μm polyethersulfone Nalgene® syringe filter into 40 mL TraceClean amber borosilicate glass vials, sealed with open-top caps with polytetrafluroethylene (PTFE)/rubber septa (flexseal disc, 22 mm, 5/50 mL) (Chromatographic Specialties Inc, Brockville, ON, Canada) and shipped to G.G. Hatch Stable Isotope Laboratory at the University of Ottawa (Ottawa, Ontario). DOC and DIC concentration samples were run on an OI Analytical Aurora Model 1030W. $\delta^{13}C$ signatures were analyzed using the TIC-TOC Analyser coupled with a continuous flow Finnigan Mat DeltaPlusXP isotope ratio mass spectrometer.

2.3.3 Biological Samples

Plankton and periphyton samples collected from the various field sites were preserved as described below at the on lease facility and then shipped to the University of Waterloo (Waterloo, Ontario) for analysis of chl *a*, dry weight and stable isotopes.

Plankton samples were collected in 2007 (June 30-July 3), 2008 (June 18-19, July 9-10, and July 30-31) and 2009 (July 21) to measure chl *a*, TSS and stable isotopes. Samples were collected in 250 ml glass amber bottles and held in a cooler until processed at the facility. A volume of 250-500 ml was filtered onto GF/F filters, which were then placed into 20 ml glass scintillation vials wrapped in aluminum foil, and frozen at -20ºC for chl *a* analysis. Plankton samples for TSS (>0.7μm) were collected by filtering 500 ml of water from each site onto pre-weighed filters, they were dried at 60ºC for 24 hours. For plankton stable C and N isotope analysis, 100-500 ml of water was filtered onto pre-combusted QMA quartz or GF/F filters and dried for 24 hours. Filters were stored at room temperature until analysis.

In 2007 and 2008, artificial substrates were deployed to collect periphyton from each of the study wetlands to measure chl *a*, dry weight and stable isotopes. In 2007, three 6" x 4" glass plates were suspended in each of three white, round 20 L buckets, placed randomly in each of the nine wetlands. Eight holes were drilled into each bucket to allow water circulation. Samples were collected on July 5-6, July 23-24, and August 6-7 in 15-18 day intervals. No samples were collected in NW and CTW for 2007 due to the low water levels, limiting complete submersion of the artificial substrates. In 2008, artificial substrates consisting of 8" x 11" acetate sheets, suspended from bamboo sticks were deployed approximately 20 cm below the water surface and randomly placed into each wetland to collect periphyton. Three artificial substrates were placed in each wetland and samples were collected on July 9-10 and July 30-31, 2008 after a 21-day

exposure period. Periphyton samples were prepared by scraping the material from a predetermined area of each artificial substrate. Samples for chl *a*, dry weight and stable isotope analysis were filtered and stored as described for plankton.

Samples were analyzed for chl *a* on a Turner Designs model 10AU fluorometer (Turner Designs, Sunnyvale, CA) after the filters were extracted in 90% acetone for 24 hours at -20ºC. Samples were corrected for phaeophytin after an acidified reading (Smith *et al*., 1997). Pre-weighed filter samples used to measure dry weight (periphyton and plankton) were dried at 60ºC for 24 hours and re-weighed using a Mettler Toledo AG245 analytical balance (Mettler Toldedo, Columbus, OH).

Stable carbon and nitrogen isotope analysis of dried plankton and periphyton material collected on pre-combusted filters was conducted at the Environmental Isotope Laboratory, Department of Earth Sciences, University of Waterloo (Waterloo, ON, Canada). Approximately 1-10 mg of the sample was ground and measured into tin capsules (5 x 3.5 mm) (SerCon Ltd., Cheshire, United Kingdom). Samples were analyzed for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios using the Thermo-Finnegan Delta Plus Continuous Flow Isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled to a Carlo Erba Elemental Analyzer (Thermo Fisher Scientific, Italy) using the formula:

$$(R_{sample}/R_{standard})-1) \times 10^3 = \delta\ ^{13}C \text{ or } ^{15}N\ (‰),$$

where $R = ^{13}C/^{12}C$ or $^{15}N/^{14}N$. Standard reference materials included carbonate rock Vienna Pee Dee Belemnite (IAEA) for carbon and atmospheric air (National Institute of Standards and Technology) for nitrogen. Data were normalized to a precision of ± 0.2 ‰ for carbon analysis and ± 0.3 ‰ for nitrogen analysis using laboratory standards of sucrose, cellulose and graphite for carbon and ammonium sulphate for nitrogen analysis.

2.3.4 Statistical Analysis

Plankton data for chl *a*, TSS, and $\delta^{13}C$ and $\delta^{15}N$ isotope values from 2007 to 2009 were grouped to compare reference vs. OSPM sites. To evaluate differences in DOC and DIC concentration, and $\delta^{13}C$ of DOC and DIC, data from 2007 to 2009 were grouped according to reference and OSPM sites. Periphyton chl *a*, TSS, and $\delta^{13}C$ and $\delta^{15}N$ isotope values were analyzed separately by year in order to compare reference and OSPM sites (2007 and 2008). For comparisons between reference and OSPM sites, only sites with data for the same sample dates are presented as mean ±SE.

Statistical differences between reference and OSPM sites were analyzed using a paired sample T-test at 95 % confidence level. Linear regressions were conducted to determine if there was a

relationship between phytoplankton or periphyton dependent variables (chl *a*, dry weight or stable isotopes) and independent environmental variables such as TN, TP, DOC and DIC concentration, conductivity and NA concentration using Systat with a significance level of $p<0.05$ (SYSTAT® version 10).

2.4 Results

2.4.1 Water Chemistry Parameters

Basic water chemistry parameters were collected for 9 wetlands from 2007 – 2009 (Table 2.2). In general, the temperature ranged from 19.3 to 20.9 ºC while pH levels ranged from 6.7 to 8.1 and varied little between reference and OSPM sites. DO levels varied between sites and ranged from 82.2 to 106.0% saturation. Conductivity also varied between sites and ranged from 869 to 4462 µS/cm, however, SCT had conductivity 2 times higher than the other OSPM sites. There were no significant differences in conductivity between reference and OSPM sites ($p=0.454$; Table 2.2; Appendix A). TN levels were more variable at OSPM sites (range, 842 - 2953 µg/L) than the reference sites (986 – 1240 µg/L). TP levels ranged from 14.1 to 56.4 µg/L. NW had the highest levels of TN and TP, probably due to the continuous influx of processed water. There were no significant differences in TN ($p=0.330$) and TP ($p=0.651$) concentration between reference and OSPM sites (Table 2.2; Appendix A).

Table 2. 2 Mean ± SE water chemistry parameters for 2007-2009 for reference and OSPM sites.

Sites	pH	DO (%)	Conductivity (µS/cm)	TN (µg/L)	TP (µg/L)	TN:TP
Reference						
BL	6.7 ±0.1	85.0 ±0.4	869 ±16	1240 ±51	32.7 ±8.4	38
SW	7.6 ±0.03	104.3 ±0.3	1271 ±238	986 ±87	17.2 ±5.3	57
PP	7.0 ±0.2	92.1 ±7.2	2367 ±365	1213 ±66	20.1± 2.6	60
HS	7.1 ±0.2	82.2 ±2.0	2313 ±400	1230 ±56	16.0 ±1.7	77
SSBP	7.4 ±0.1	86.0 ±0.8	956 ±5.5	1076 ±69	19.6 ±1.5	55
OSPM						
SCT	7.6 ±0.1	106.0 ±0.6	4462 ±139	842 ±34	16.7 ±1.7	50
TP9	8.1 ±0.1	103.7 ±0.8	2304 ±29	1243 ±62	23.4 ±6.2	53
CTW	7.4 ±0.1	72.9 ±1.3	1851 ±36	1687 ±205	14.1 ±3.5	120
NW	7.6 ±0.1	71.0 ±0.3	1610 ±29	2953 ±574	56.4 ±14	52

Although both DOC and DIC concentrations from 2007-2009 varied between sites, in general dissolved carbon levels were higher in OSPM vs. references sites (Fig. 2.1; Table 2.3). Mean DOC concentrations of OSPM sites (range, 70-110 mg/L) were higher compared to reference sites (range, 30-80 mg/L) (Fig. 2.1a; Table 2.3). Mean DIC concentrations were also higher in OSPM sites (range, 90-210 mg/L) relative to reference sites (range, 60-80 mg/L) (Fig. 2.1b; Table 2.3). There were significant differences in both DOC and DIC concentrations in reference sites compared to OSPM sites ($p \leq 0.003$; Table 2.3; Appendix A). However there were no significant differences in $\delta^{13}C$ of DOC or DIC between reference and OSPM sites (Fig. 2.1 c, d; Table 2.3; Appendix A).

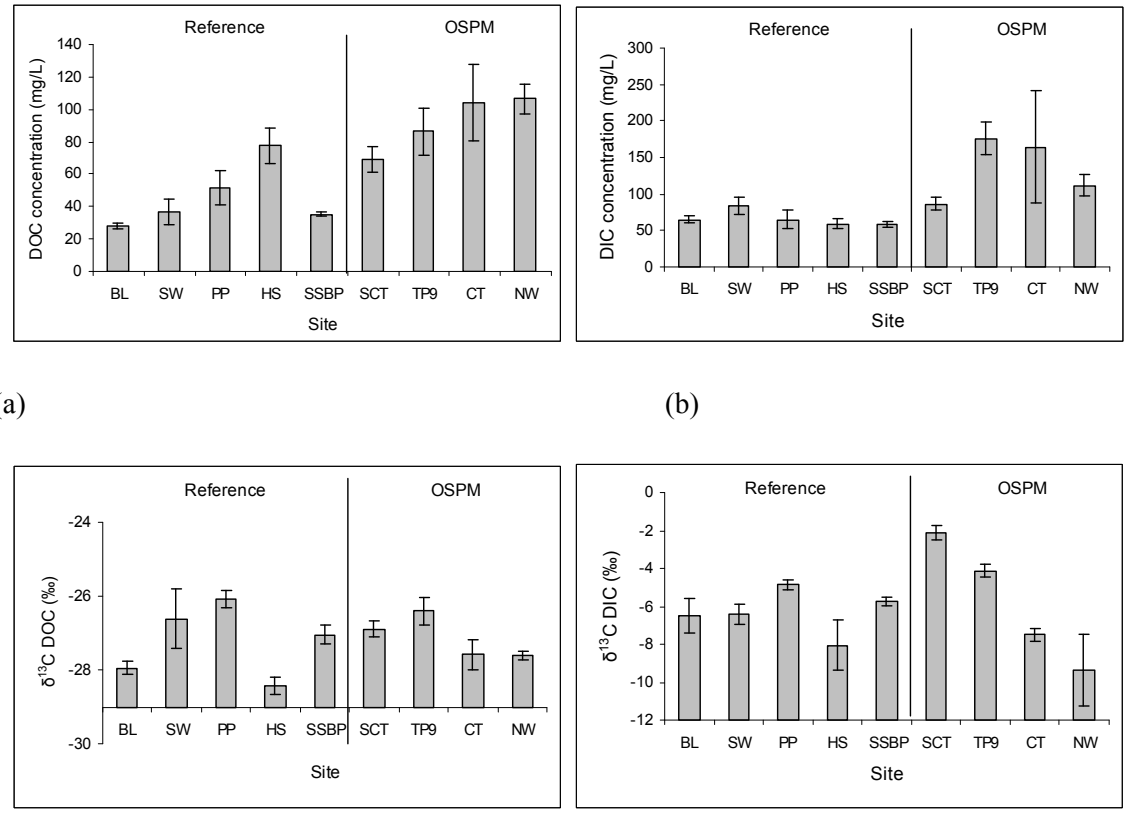


Figure 2. 1 DOC concentrations (a), DIC concentrations (b), $\delta^{13}C$ of DOC (c) and $\delta^{13}C$ of DIC (d) for reference and OSPM study sites from 2007 – 2009 (n=5).

Table 2. 3 Mean ± SE for DOC and DIC concentrations and stable C isotope values for reference and OSPM sites sampled in 2007-2009 (n=5).

	Mean ± SE[a]			
Status	**DOC (mg C/L)**	**DIC (mg C/L)**	**$\delta^{13}C$ DOC (‰)**	**$\delta^{13}C$ DIC (‰)**
Reference	38.9 ±4.9	71.1 ±6.2	-26.9 ±0.4	-5.9 ±0.4
OSPM	87.4 ±7.3	124.7 ±14.2	-27.0 ±0.2	-5.2 ±1.1

[a] Mean ± SE were calculated only for sites with data for the same sampling periods. Due to missing data, SSBP and HS (reference) and CTW (OSPM) were not included in this summary.

2.4.2 Plankton and Periphyton Growth Estimates

Measurements of chl *a* and TSS were used to estimate community growth of plankton (Fig. 2.2 a,b). The majority of the wetlands have plankton chl *a* values under 8 µg/L; indicative of oligotrophic status (Fig. 2.2 a). The highest chl *a* estimates were observed in an OSPM site, NW (12.0 µg/L) as well as two reference sites, BL (5.8 µg/L) and PP (8.8 µg/L). The same sites also had the highest estimates of TSS (NW, 59.2 mg/L; BL, 23.1 mg/L; PP, 17.4 mg/L) in addition to an OSPM site, TP9 (21.6 mg/L) (Fig. 2.2 b). In general, there were no significant differences between reference and OSPM sites for either chl *a* or TSS (Table 2.4; Appendix A). However, linear regression analysis indicated significant positive correlations for both estimates of plankton growth (chl *a* and TSS) and nutrients for the wetland sites. Levels of chl *a* ($r^2 = 0.546$, $p = 0.036$) and TSS ($r^2 = 0.814$, $p = 0.002$) increased as TN concentrations increased. Similarly, chl *a* ($r^2 = 0.702$, $p = 0.009$) and TSS ($r^2 = 0.940$, $p = 0.000$) were positively correlated with TP concentrations. There were no significant correlations for plankton chl *a* or TSS vs. conductivity, NA, DOC or DIC concentrations (Appendix A).

Measurements of chl *a* and dry weight were used to estimate community growth of periphyton from artificial substrates (Fig. 2.2 c-f). As different methods and exposure periods were used in 2007 vs. 2008, the data are presented for each year. Chl *a* values for periphyton were below 4 mg/m^2 for the majority of the sites in both 2007 and 2008 except for BL (2007:4.6 ±2.2; 2008:16.2 ±9.1 mg/m^2) and SSBP (2007:6.2 ±4.1 mg/m^2) and PP (2008: 5.0 ±0.3) (Fig. 2.2 c-f). Different trends were evident between years for estimates of periphyton growth using dry weight. In 2007, dry weight estimates were highest in two reference sites, SW (557.3 ±78.6 mg/m^2) and SSBP (765.7 ±203 mg/m^2) whereas in 2008, dry weight estimates were highest in some reference (BL, SW, and HS) and OSPM (CTW) sites. In general, there were no significant differences for either chl *a* or dry weight between reference and OSPM sites (Table 2.4; Appendix A). Also, there were no linear correlations between either estimates of periphyton growth (chl *a* and dry weight) and nutrients (TN or TP), conductivity (except periphyton chl *a* 2007), NA or dissolved carbon concentrations (DOC, DIC) for the wetland sites for 2007 or 2008 (Appendix A).

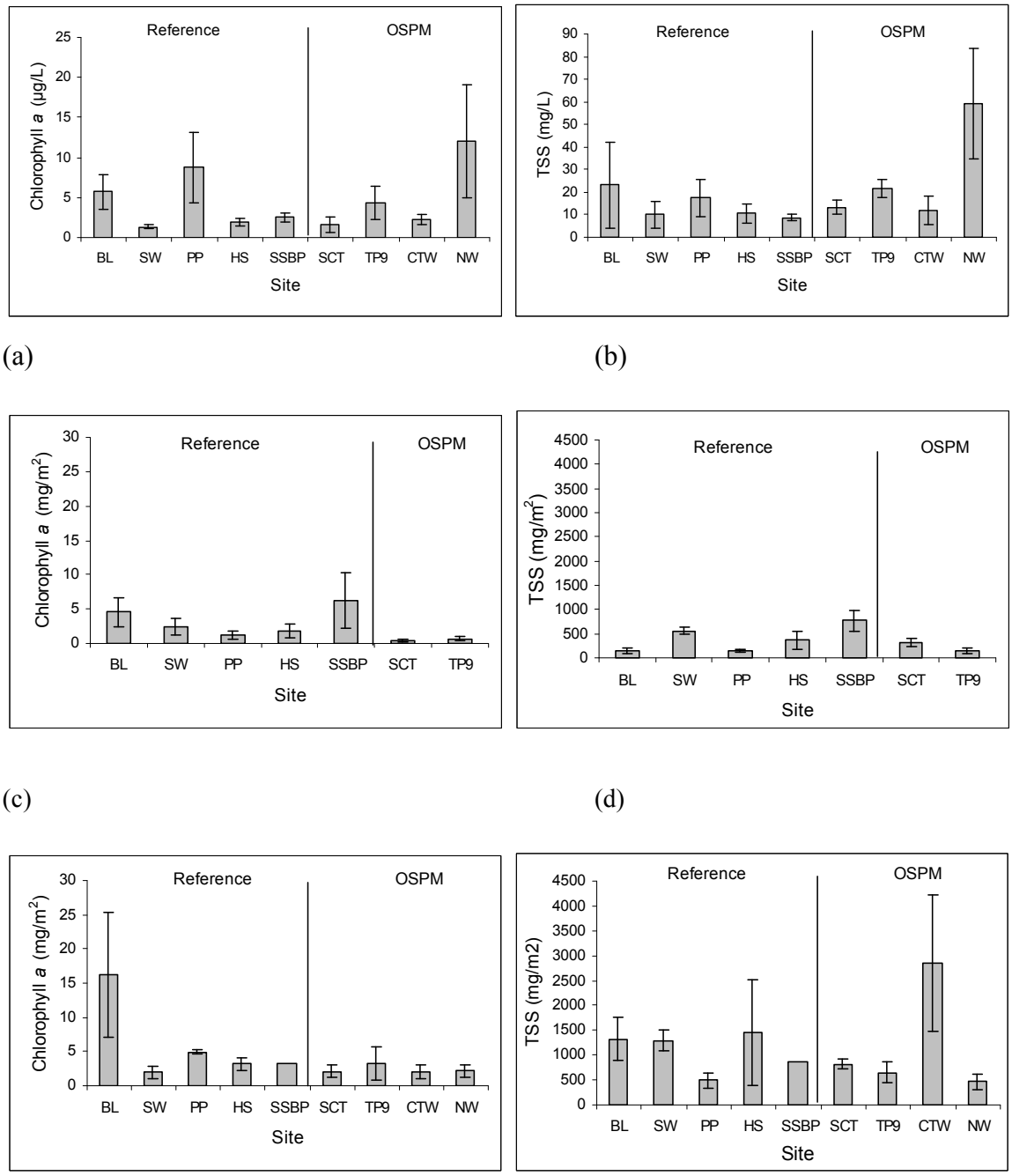


Figure 2. 2 Mean ± SE of plankton chl *a* (a) and TSS (b) and periphyton chl *a* (c) and dry weight (d) in 2007 and periphyton chl *a* (e) and dry weight (f) in 2008.

Table 2.4 Mean ± SE for plankton and periphyton growth estimates collected from reference and OSPM sites.

		***Mean ± SE*[a]**	
Source	**Status**	**Chl a (µg/L)**	**Dry Weight (mg/L)**
Phytoplankton (2007-2009)[1]	**Reference**	3.7 ±1.1	13.9 ±4.1
	OSPM	4.6 ±1.6	24.6 ±6.2
		(mg/m^2)	**(mg/m^2)**
Periphyton (2007)[2]	**Reference**	2.7 ±0.9	283.4 ±75
	OSPM	0.6 ±0.2	223.0 ±62
Periphyton (2008)[3]	**Reference**	7.7 ±3.6	1038 ±218
	OSPM	2.7 ±1.4	733 ±108

[a] Mean ± SE were calculated only for sites with data for the same sampling periods. Due to missing data: [1]SSBP was excluded from calculations for phytoplankton; [2]SSBP, HS were excluded from calculations for periphyton (2007) and [3]SSBP, HS, CTW, and NW were excluded from calculations for periphyton (2008).

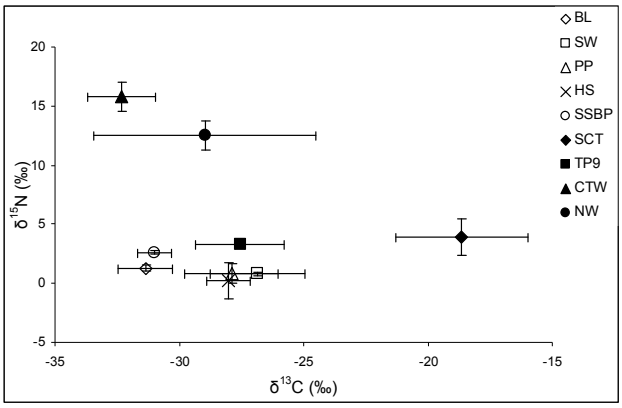
2.4.3 Stable Isotope Analysis of Plankton and Periphyton Biomass

Filtered water samples were analyzed to determine $\delta^{13}C$ and $\delta^{15}N$ values of plankton for samples with sufficient material to allow detection. Mean $\delta^{13}C$ of plankton for all sites were between -32 to -26 ‰ except for SCT (-19‰) which was more ^{13}C enriched relative to the other sites (Fig 2.3 a). Comparisons between reference and OSPM sites indicated no significant differences in $\delta^{13}C$ values of plankton (p=0.193; Table 2.5). In contrast, mean $\delta^{15}N$ of plankton from OSPM sites was significantly higher than reference sites (p=0.002; Fig 2.3 a; Table 2.5). Reference sites had mean $\delta^{15}N$ for plankton of 0.2 to 2.6‰, while some OSPM sites had mean $\delta^{15}N$ values of 3.3-4 ‰ (SCT, TP9) and other sites (CTW, NW) had ^{15}N enriched values as high as 12.5-15.8 ‰ (Fig. 2.3 a). Although not significant, plankton C:N ratios were higher in OSPM vs. reference sites (Table 2.5). There was a significant positive correlation between $\delta^{15}N$ of plankton and TN ($r^2 = 0.509$, $p = 0.031$) but not TP ($r^2 = 0.119$, $p = 0.363$) (Appendix A).

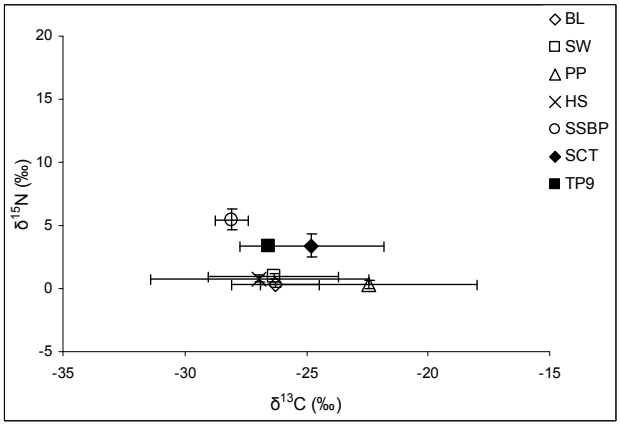
Samples were analyzed to determine $\delta^{13}C$ and $\delta^{15}N$ values of periphyton separately for 2007 and 2008 (Fig. 2.3 b, c). In order to compare isotope data between years only sites sampled in both years were used to calculate mean values for reference and OSPM site comparisons (Table 2.5). In 2007, mean $\delta^{13}C$ values of periphyton ranging from -28.1 to -22.4‰ were not significantly different between reference and OSPM sites (p=0.067; Fig.2.3 b; Table 2.5). Periphyton from OSPM sites had higher $\delta^{15}N$ values (SCT, 3.4 ±0.9‰; TP9, 3.4 ±0.1‰) as did the reference site, SSBP (5.5±0.8‰). On average, $\delta^{15}N$ values were significantly higher (^{15}N enriched) for OSPM vs. reference sites (p=0.007). Periphyton C:N ratios were higher in OSPM vs.

reference sites, although not significant (Table 2.5). There was no correlation between $\delta^{15}N$ of periphyton and nutrients (TN, $r^2 = 0.150$, $p = 0.391$; TP, $r^2 = 0.052$, $p = 0.622$) (Appendix A) however it should be noted that the two sites with the highest TN (CTW and NW) were not sampled in that year.

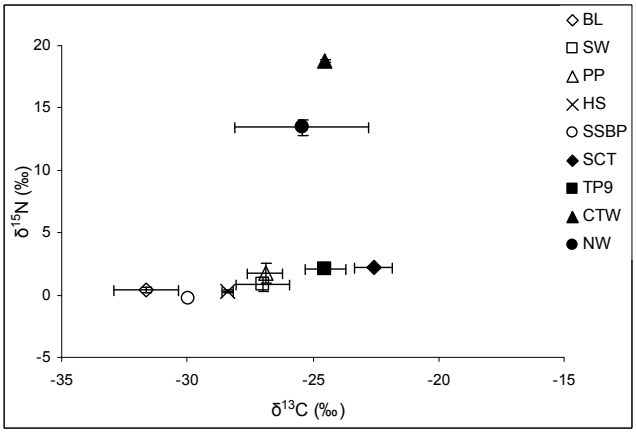
In 2008, microcosms incubated for a longer exposure period, had periphyton that were significantly ^{13}C depleted for reference sites (-31.6 to -26.9‰) relative to OSPM sites (-25.4 to -22.6‰) ($p=0.011$; Fig. 2.3 c; Table 2.5). $\delta^{15}N$ values of periphyton were low for reference sites (0.3-1.8‰) while some OSPM sites had slightly ^{15}N enriched periphyton (TP9 and SCT, 3.4‰) and other OSPM sites (CT and NW, range 13-18‰) had highly ^{15}N enriched periphyton. $\delta^{15}N$ values of periphyton were significantly ^{15}N enriched in OSPM vs. references sites ($p=0.003$). As in 2007, periphyton C:N ratios in 2008 were higher in OSPM vs. reference sites but not significantly different (Table 2.5; Appendix A). There was a positive correlation between $\delta^{15}N$ of periphyton and TN concentration ($r^2 =0.508$, $p =0.031$) but not for TP concentration ($r^2 = 0.093$, $p = 0.426$) (Appendix A).



(a)



(b)



(c)

Figure 2. 3 Plankton $\delta^{13}C$ and $\delta^{15}N$ isotope values for sampling periods in 2007-2009 (a), $\delta^{13}C$ and $\delta^{15}N$ isotope values for 2007 (b) and 2008 (c) periphyton grown on artificial substrates (open shapes – reference sites; closed shapes – OSPM sites).

Table 2. 5 Mean ± SE of $\delta^{13}C$ and $\delta^{15}N$ for plankton and periphyton collected from reference and OSPM sites.

		Mean ± SE		
Sample	**Status**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**C:N**
Plankton (2007-2009)	Reference	-29.1 ±0.8	1.3 ±0.4	11.0 ±1.1
	OSPM	-26.7 ±1.9	9.7 ±2.0	13.6 ±1.8
Periphyton (2007)	Reference	-26.2 ±1.1	1.7 ±0.6	15.7 ±2.4
	OSPM	-25.5 ±1.7	3.4 ±0.5	29.7 ±16
Periphyton (2008)[1]	Reference	-28.6 ±0.7	0.7 ±0.3	12.0 ±1.4
	OSPM	-23.6 ±0.7	2.2 ±0.1	14.1 ±1.6

[1]To allow comparison between years for periphyton, CTW and NW were omitted from the mean calculation of 2008 values.

2.4 Discussion

2.4.1 Estimates of Phytoplankton and Periphyton Growth

Phytoplankton Community Growth

Estimates of phytoplankton community growth in oil sands reclamation from chl *a* and TSS measurements indicated that OSPM sites support low phytoplankton growth that was similar to reference sites (Table 2.4) although there was high variability, both temporal and spatial. Mean estimates of phytoplankton community growth, measured as chl *a*, ranged from 1.3 to 8.8 µg/L for reference sites and 1.6 to 12.0 µg/L for OSPM sites in 2007-2009. Earlier microcosm studies (July 11-18, 1997) of natural phytoplankton communities grown under non-limiting nutrient conditions (in nutrient medium) in various types of oil sands process water (OSPW) found similar chl *a* levels (OSPW, 4.7 – 9.9 µg/L; control, 9.0 µg/L) and even higher levels (21.8 µg/L) in settling basin water with NA concentrations greater than 50 mg/L (Leung *et al*., 2001). Hayes (2005) found that natural phytoplankton communities grown in a nutrient medium at varying concentrations of extracted oil sands NAs resulted in a stimulatory effect on the *in vivo* fluorescence of chl *a* at NA concentrations between 24 to 50 mg/L. The stimulatory effect may be a function of the utilization of organic compounds within the NA extract by more tolerant taxa or a NA-induced physiologically increase in the fluorescence yield of chl *a* (Hayes, 2005). In the

current study, NA concentrations in OSPM sites ranged from 25 to 55 mg/L (mean values) yet not all OSPM sites had elevated chl *a*. NW, with a NA concentration of 47 mg/L (Farwell *et al*., 2009), had the highest chl *a* and TSS levels of all reference and OSPM sites even with high temporal variability. In contrast, sites with 55 mg/L NA concentrations (SCT and CTW) had the lowest chl *a* concentrations (mean values) of all the OSPM sites, which could be a function of inhibition at higher NA concentrations. Growth rates, calculated from *in vivo* fluorescence of chl *a*, were found to be impaired at NA concentrations greater than 50 mg/L based on NA extract incubations (Hayes, 2005). However, chl *a* measurements at day 0, 7, 10 and 14 for nutrient-amended microcosms containing natural phytoplankton communities exposed to extracted NA concentrations (0,25,50,100 mg/L) at 0, 3, 7 g/L salt concentrations showed no evidence of chl *a* suppression by NAs or NAs and salts. While growth rates may be influenced by elevated NAs (>50 mg/L) under controlled nutrient-amended conditions, the slight differences in phytoplankton community growth estimates (chl *a* and TSS) among and within reference and OSPM sites in this study suggests that other factors, such as nutrients may be important (Hayes, 2005).

Previous studies of natural phytoplankton communities showed that changes in phytoplankton community composition were correlated to NA and major ion concentrations in oil sands reclamation and in naturally saline systems (Leung *et al*., 2003; Hayes, 2005). Cyanobacteria (nitrogen-fixing species) were dominant in reference systems and Chlorophyta and other phyla were dominant in systems characterized by high NA concentration and conductivity (Leung *et al*., 2003) or high conductivity (~3500 µS/cm) (Evans and Prepas, 1996). Concentrations of NAs and major ions were correlated to phytoplankton community composition (accounted for 40% of the variability) in natural and reclaimed ponds/lakes within the oil sands leases (Leung *et al*., 2003). Leung *et al*. (2003) determined that there was little ecological effect on phytoplankton communities at NAs < 6.5 mg/L and conductivity < 800 µS/cm. Field surveys of 30 water bodies in the oil sands region, including sites on lease, were used to define threshold effect concentrations for phytoplankton community composition of 30 mg/L NAs and 1000 µS/cm for conductivity (Hayes, 2005). Although phytoplankton species composition was not evaluated in this study, based on these previous studies, reference sites with lower conductivity or sites with lower NA concentration and conductivity probably have different species composition than the high NA and high conductivity OSPM sites.

Interestingly, previous surveys of the oil sand region found no significant correlation between mean total phytoplankton biomass and TN and TP or even NAs and major ions (Leung *et al*., 2003; Hayes, 2005). However, both NAs and major ions were identified as significant variables controlling phytoplankton species composition (Leung *et al*., 2003; Hayes, 2005). In

addition, TP was identified as an important variable influencing species composition in microcosms with water from an active settling basin (Mildred Lake, Syncrude lease: also correlated with high NA concentrations, > 50 mg/L) despite nutrient amendments to these microcosms (Leung *et al*., 2001). In the survey of 10 water bodies on the oil sands lease, there was high mean phytoplankton biomass for both sites with the highest NA concentrations, dominated by Chlorophyta, and the reference site, dominated by Cyanobacteria (Leung *et al*., 2003) yet biomass was not correlated to TP, TN, NA or major ions.

In the current study, mean TP and TN ranged from 14-56 µg/L and 840-2950 µg/L, respectively, and both were found to be positively correlated to mean plankton chl *a* values (linear regression: TN, r^2 =0.55, p=0.036; TP, r^2 =0.70, p=0.009). A similar positive relationship was found for mean TSS values (TN, r^2 =0.81, p=0.002; TP, r^2 =0.94, p=0.000). In general, elevated macronutrients, could explain slightly higher chl *a* and TSS in both reference (BL and PP) and OSPM sites (TP9 and NW). Although not significant, plankton C:N ratios were higher in OSPM vs. reference sites, suggesting nitrogen deficiency. Microcosm studies generally found that adding nutrients associated with either natural peat material or inorganic N and P (NH_4NO_3; KH_2PO_4) could stimulate phytoplankton growth (measured as chl *a*) in microcosms with different sediments (MFT, sand) and capped with reference water or OSPW of varying chemical compositions (Chen, 2011). This suggests that macronutrients (TP or TN or both) are important limiting factors of phytoplankton community growth in oil sand reclamation.

Periphyton Community Growth

Similar to phytoplankton community growth, estimates of periphyton community growth (chl *a* and dry weight) on artificial substrates indicated low periphyton growth with no significant differences in growth estimates between reference and OSPM sites. There were lower periphyton growth estimates in 2007 (mean chl *a* 0.4 - 6.2 mg/m^2) relative to 2008 (mean chl *a* 1.95 - 16.2 mg/m^2) which is to be expected due to a shorter colonization period in 2007 (15-18 days) vs. 2008 (21 days). Similar to chl *a*, periphyton dry weight in 2007 (mean 138 -766 mg/m^2) was lower than in 2008 (mean 464 - 2843 mg/m^2). Estimates of periphyton chl *a* in this study were similar to chl *a* estimates ranging from 2.7 to 5.6 mg/m^2 for periphyton collected from artificial substrates in closed microcosms containing 3 different types of OSPW (average of three 20 day incubations from June to Aug. 2008; Chen 2011). In a biofilm transfer study, chl *a* and total dry weight initially increased in OSPM treatments but after the first year, biomass was similar to control treatments (Frederick, 2011).

Artificial substrates (glass rods) deployed in two Alberta lakes reported mean chl *a* values that ranged from 1.06 to 1.6 μg/cm^2 (Goldsborough, 1991). Periphyton chl *a* and dry weight have also been found to vary with the length of incubation. In a study by Azim *et al.* (2003) periphyton collected on glass slides had chl *a* levels of 128.2 ± 28.6 mg/m^2 and biomass of 63 ± 1.5 g/m^2 after 2 weeks. However after 3 weeks, biomass increased to 221.6 ± 39 mg/m^2 while chl a levels decreased 79.8 ± 23.5 mg/m^2 (Azim *et al*., 2003), these levels are much higher in comparison to oil sands reclaimed sites in this study.

Periphyton growth from artificial substrates, as described by chl *a* and dry weight, tended to be lower in OSPM sites than reference sites for both years, although this difference was not significant. This could be a function of reduced rates of production for both heterotrophs and autotrophs colonizing the artificial substrates. In laboratory studies, Hayes (2005) examined the effects of NA concentrations (extracted material) on natural phytoplankton communities that differed in NA exposure histories. All phytoplankton communities had increased lag phases correlated to NA concentrations and reduced growth rates at greater than 50 mg NA/L (Hayes, 2005). Also, growth rates of bacterioplankton (measured as leucine incorporation) in terms of production were 5 times higher in reference sites than OSPM sites for a similar suite of sites (Daly, 2007) examined in the current study. Assuming a similar response for species that colonize substrates, algae and bacteria colonizing artificial substrates in high NA waters at OSPM sites would likely produce lower standing crop biomass on artificial substrates than periphyton communities in reference sites possibly due to reduced growth rates.

In general, compared to phytoplankton and macrophyte studies related to oil sands reclamation, this study is one of the few studies to examine the impact of reclamation strategies on photosynthetic periphyton growth (Chen, 2011; Frederick, 2011). Periphyton biomass on emergent and submerged macrophytes could contribute significant dietary sources for grazing consumers in wetland reclamation and littoral zones of end pit lakes. While there was no significant difference in periphyton growth estimates between OSPM and reference wetlands based on artificial substrate colonization (this study), there appears to be less macrophyte biomass available for colonization in OSPM wetlands. A review of carbon standing stocks by Kovalenko *et al*. (2013) indicated that submerged macrophyte biomass was lower in OSPW wetlands vs. reference wetlands. Reduced surface area for colonization by microbes and algae associated with low macrophyte biomass could in theory contribute to the observed lower macroinvertebrate trophic diversity in OSPM vs. reference wetlands (Kovalenko et al., 2013).

Although there was no correlation between periphyton community growth estimates and TN or TP in the present study, a previous study of periphyton growth indicated that the addition

of peat to microcosms containing MFT and sand increased periphyton chl *a*, suggesting that periphyton are nutrient-limited (Chen, 2011). Stoichiometric analysis showed higher C:N ratios for epiphytic bacteria in OSPM vs. reference sites but there were no significant differences in seston or algae (Kovalenko *et al.,* 2013). In the current study, there were trends of higher C:N ratios in plankton and periphyton from OSPM vs. reference sites suggesting possible nitrogen limitation. While not the focus of the current study, peat amendments have been considered to enhance biological activity in low productivity waters of OSPM reclamation (Chen, 2011; Kovalenko *et al.,* 2013). In these field studies, as with the current study, the effects of grazing pressure on periphyton biomass may be a factor. As a result, future laboratory studies would be beneficial to assess the value of peat amendments to primary production of OSPM in the absence of grazing.

2.4.2 Stable Carbon and Nitrogen Isotopes of Phytoplankton and Periphyton

Stable Nitrogen Isotopes

In general, there was significant ^{15}N enrichment of plankton and periphyton in OSPM vs. reference sites yet there were no significant differences between plankton and periphyton community growth, nutrients or C:N ratios in OSPM vs. reference sites. The lack of difference between OSPM and reference site comparisons could be a function of the wide range of water chemistry parameters that exist within the OSPM sites, some often similar to reference sites while others have elevated parameters (TN and TP) due to the type of reclamation or in two cases, due to inputs (dyke seepage) into the wetlands from OSPW sources (NW and CTW). As a result, a better understanding of N dynamics in oil sands reclamation is derived from correlations between parameters (e.g. chl *a*, dry weight or isotopes vs. nutrients).

For plankton, both estimates of community growth (chl *a* and TSS) and $\delta^{15}N$ were positively correlated with TN; found in elevated levels in some of the OSPM sites (CTW and NW). In other studies, plankton was found to be more ^{15}N enriched based on sites with increasing NA concentrations (high [NA], 6.3‰; low [NA], 2.9‰), indicative of OSPM, however the correlation between $\delta^{15}N$ and nutrient levels was not examined (Elshayeb, 2006). Other studies have found that the $\delta^{15}N$ of plankton increased with primary production from oligotrophic lakes to eutrophic lakes in Florida (Gu et al., 1996). Also, algal $\delta^{15}N$ signatures were ^{15}N enriched (13.2‰) downstream of a sewage treatment plant and pulp mill discharge compared to upstream samples (2.7-7.8‰) (Wayland and Hobson, 2001).

In theory, if the rate of growth is low, there is preferential uptake of the lighter isotope ^{14}N of NH_3 or NO_3 by algae (Pennock *et al*., 1987; Cifuentes *et al*., 1989) which would result in ^{15}N depletion of algae. In contrast, at higher growth rates, the demand for N is greater thus algae uptake both ^{14}N and ^{15}N and biomass becomes ^{15}N enriched. In the current study, the positive correlation between $\delta^{15}N$ and TN concentration for plankton suggests the importance of increased TN levels on ^{15}N enrichment of primary production and explains the ^{15}N enrichment of invertebrates in OSPM reclamation (Farwell *et al.*, 2009; Murchie & Power, 2004). Similar to phytoplankton, $\delta^{15}N$ values of periphyton were positively correlated with TN for collections made in 2008 but not in 2007. The difference between years is likely due to the lack of sampling at the sites with OSPW recharge and higher TN levels (i.e. CTW and NW) in 2007 and the shorter incubation period in 2007. Daly (2007) also found higher $\delta^{15}N$ values of microbial biofilm from OSPM (7.3‰) vs. reference (-1.9‰) sites. Videla *et al*. (2009) was able to simulate the ^{15}N enrichment of bacteria observed in the field by growing bacteria on oil sands NAs supplemented with a renewed source of mineral medium (NH_4Cl), suggesting the importance of N inputs from recharge zones on ^{15}N enrichment of periphyton. In oil sands reclamation, ammonium levels ranged from 0.3 to 1.7 mg/L, while NO_2 and NO_3 levels measured from reference and OSPM sites were low (<0.1 mg/L) in these systems (Daly, 2007). In addition to recharge via seepage, sites constructed with MFT or CT densify over time, which acts as a source of nitrogen, releasing ammonia into the overlying water that may undergo nitrification prior to utilization by the biota in these systems (Farwell *et al*., 2009). Further study will examine nutrient concentrations (TN and TP) and N species under controlled laboratory conditions to better understand potential differences in N dynamics in different materials used to construct oil sands wetlands (Chapter 3).

Stable N isotopes of plankton and periphyton suggest that ^{15}N enrichment of algae could be a useful indicator of nutrient inputs, perhaps indicative of OSPM seepage into natural systems, for oil sands regional monitoring programs. Identifying indicators of OSPM influences for off lease sites is particularly important as the toxic compounds in OSPM also occur naturally in eroded riverine systems in the oil sands region thus defining exposure to OSPM in natural systems is a challenge. Studies of riverine systems have documented increases in $\delta^{15}N$ values of primary and secondary consumers with nutrient inputs from sewage (Wayland and Hobson, 2001). Farwell *et al*. (2009b) found site-specific trends in $\delta^{15}N$ values of fish potentially attributed to inputs from municipal or industrial effluent discharge in the Athabasca River in the oil sands region.

Stable Carbon Isotopes

The sources and concentrations of dissolved carbon contributing to the DOC and DIC pools in oil sands reclamation are a function of the type of water (fresh vs. expressed water during consolidation) and tailings used in construction, inputs from surface water runoff and groundwater sources and in-situ production (DIC from respiration, DOC from plants and animals) and utilization (DIC for photosynthesis; DOC for microbial production). In this study, both DOC and DIC concentrations were significantly elevated (2-fold) in OSPM sites compared to reference sites; the same trends were observed in 2006 for a similar suite of sites (Videla, 2007). Seasonal data revealed higher DOC concentrations in June, 2006 and decreasing concentrations in July, 2006, possibly reflecting increased microbial activity (Videla, 2007). In 2005, bacterioplankton studies of a similar suite of oil sands reclamation sites (as in the current study) found bacterioplankton biomass had a weak positive correlation to DOC concentration (Daly, 2007). Bacterioplankton biomass was 1.5 ± 0.4 µg C/L (mean ± SE) for reference sites (PP, SW, HS, SSBP) and 3.9 ± 0.9 µg C/L (mean ± SE) for OSPM (SCT, NW, CTW, TP9) (data summarized from Daly, 2007). Increased microbial biomass and thus microbial respiration may explain the elevated concentrations of DIC in OSPM sites.

There were consistent trends of $\delta^{13}C$ enrichment of plankton and periphyton in OSPM sites relative to reference sites (although not significant, except for periphyton in 2008), yet there were no differences in the $\delta^{13}C$ of DOC or DIC between sites. Similar trends were found for plankton samples collected in 2005 (Daly, 2007). Mean plankton $\delta^{13}C$ values were similar for 2007-2009 data (-28.5 ±0.8 ‰; this study) and earlier 2005 data (-28.8 ±1.1 ‰; Daly, 2007) for reference sites. In comparison, plankton were ^{13}C enriched for 2007-2009 data (-26.7 ±1.9 ‰; this study) and the 2005 data (-25.8 ±1.5 ‰; Daly, 2007) for OSPM sites. In general, DIC $\delta^{13}C$ was also slightly more enriched in OSPM vs. reference sites in this study and in 2006 data (reference, -7.5 ± 0.8‰; OSPM, -3.8 ± 1.0‰; Videla, 2007). The OSPM site (SCT) had the more ^{13}C enriched DIC in this study, which may in part explain why this site had the most ^{13}C enriched plankton and periphyton in both this study and Daly's study (2007). Species of *Chara* (macroalgae) and *Potamogeton* (submerged aquatic plant) were also ^{13}C enriched at SCT (also referred to as Mike`s pond; Daly, 2007) relative to the other OSPM sites indicating utilization of ^{13}C enriched DIC. Daly (2007) found that OSPM sites with poor organic soil had $\delta^{13}C$ DIC close to aqueous CO_2 (0‰) which indicated that atmospheric CO_2 was the primary source of DIC, whereas sites with more depleted $\delta^{13}C$ DIC were dominated by microbial respiration.

Plankton and periphyton samples include both heterotrophic and autotrophic biomass. Stable C isotopes for heterotrophic biofilm had similar trends of $\delta^{15}N$ and $\delta^{13}C$ enrichment in

OSPM vs. reference sites (Daly, 2007) as plankton and periphyton in the current study. For the same reference sites (PP, SW, HS and SSBP), mean plankton $\delta^{13}C$ values were similar for 2007-2009 data (-28.5 ±0.8 ‰; this study) and earlier 2005 data (-28.8 ±1.1 ‰; Daly, 2007) and both were slightly ^{13}C enriched (approximately 1 ‰) relative to heterotrophic biofilm (-29.7 ±1.0 ‰; Daly, 2007). For both reference and OSPM sites, plankton was ^{13}C depleted relative to periphyton which is consistent with trends of $\delta^{13}C$ depletion in planktonic algae vs. benthic algae (Hecky and Hesslein, 1995).

In theory, differences in phytoplankton species composition as described by Hayes (2005) and Leung *et al.* (2003) could influence the isotope signatures of community biomass depending on species dominance. Studies have recorded differences in stable C and N isotopes at the species level for aquatic plants and at the genus level for bacteria inhabiting the same environment. There was some evidence of $\delta^{13}C$ differences (3‰), less for $\delta^{15}N$ differences (>1‰), between species of Potamogeton at the same reference site in an oil sands reclamation study (Daly, 2007), however this requires further study. Velinsky and Fogel (1999) suggested that differences in inorganic carbon fractionation by two different microbial species resulted in very different $\delta^{13}C$ POC values in an anoxic fjord system. In another study, marine diatoms were more ^{13}C enriched than naked flagellates due to isotope fractionation differences associated with the mechanism of DIC uptake, whereby diatoms utilize active transport of HCO_3^- versus diffusion of dissolved CO_2 by naked flagellates (Fogel *et al*., 1992). In the current study, species differences as previously reported, the dominance of cyanophytes (nitrogen-fixing species) in reference sites vs. chlorophytes in OSPM sites (Leung *et al.*, 2003), might contribute to differences in plankton $\delta^{13}C$ values. The use of monocultures in laboratory studies to evaluate isotope trends associated with oil sands reclamation materials would eliminate species differences as a factor.

2.5 Conclusions

In conclusion, estimates of plankton and periphyton community growth were low but similar in reference and OSPM reclamation wetlands. Plankton growth estimates were positively correlated with TN and TP concentrations but periphyton growth estimates were not which may be a function of other factors such as grazing or light limitation. Both plankton and periphyton C:N ratios suggest slight N limitation in OSPM vs. reference waters. The use of stable isotope analyses revealed consistently higher $\delta^{15}N$ of plankton and periphyton in OSPM wetlands than reference wetlands, and $\delta^{15}N$ of plankton and periphyton were positively correlated to TN but not TP concentrations. Increased growth stimulated by higher TN and TP concentrations could explain the use of both ^{14}N and ^{15}N, resulting in ^{15}N enriched plankton and periphyton in OSPM

wetlands. Stable N isotopes of plankton and periphyton suggests that ^{15}N enrichment of algae could be useful to detect nutrient inputs associated with OSPM sources in nutrient-limited tributaries as part of an oil sands regional monitoring program. While there were trends of $\delta^{13}C$ enrichment for plankton and periphyton for OSPM sites relative to reference sites, the C sources (DOC and DIC) were not distinctly unique to OSPM.

Chapter 3 Algal Growth and Stable Isotopes in Oil Sands Aquatic Reclamation: A Microcosm Study

3.1 Overview

In this study, I examined phytoplankton growth and stable isotopes in microcosms containing water-soluble fractions (WSF) of oil sands process material (OSPM: ie. mature fine tailings, MFT and consolidated tailings, CT) and an amendment material (peat-mineral overburden) The results of this study showed maximum phytoplankton community growth (measured as chl *a*) when WSFs of peat were combined with either CT or MFT. In general, WSFs of peat had the highest concentration of total nitrogen (TN) which suggests the importance of N from peat to enhance phytoplankton community growth in oil sands reclamation. However, WSFs also promoted the unfavourable growth of filamentous algae, highest at intermediate concentrations of peat and CT WSFs. The data also showed that increased turbidity due to fine clay particles associated with OSPM inhibited phytoplankton and filamentous algae growth at higher WSF percentages of MFT. This suggests that, regardless of nutrient amendments (peat), turbidity associated with fine clay particles could be an important factor limiting phytoplankton growth in end pit lakes. In addition, stable N isotopes of plankton and filamentous algae suggested that ^{15}N enrichment of algae could be a useful indicator of nutrient inputs, including sources in OSPM, for oil sands regional monitoring programs.

3.2 Introduction

In northern Alberta, oil sands mining activities affected over 76,000 hectares of land in 2011 (Alberta Environment, 2012). Of this total area, there are currently 1150 hectares of permanent aquatic reclamation (Alberta Environment, 2012) which represents only a small fraction of the reclamation that will take the form of wetlands and lakes (referred to as end pit lakes) in the future. There are numerous approaches to aquatic reclamation; many of these strategies could utilize semi-solid (referred to as oil sands processed material, OSPM) and/or liquid waste, generated during the extraction of bitumen from oil sands ore, as fill in open pits. The choice of fill material will influence the water quality of the system as well as the species composition and productivity of all trophic levels. In this study, the impacts of the water soluble fractions (WSFs) of two different types of OSPM, potentially used as fill material in wetland or lake reclamation, on phytoplankton community growth were examined.

Bitumen extraction produces a slurry waste that is pumped into tailings ponds and held prior to use in oil sands aquatic reclamation. The larger coarse particles settle out first, forming sand beaches and dykes, while the fine tailings require an extended ageing period to densify, creating mature fine tailings (MFT) below a surface layer of processed water. Fine tailings are composed of 85% water, 13% clay, and 2% bitumen (FTFC, 1995). Early advances in technology produced consolidated or composite tailings (CT) by treating MFT with a coagulant aid, gypsum ($CaSO_4$), to accelerate the precipitation of clay particles from processed water (Whelly, 1999). Both MFT and CT are processed materials that are candidates as fill material to line the bottom of end pit lake or wetland reclamations. In all likelihood the fill material will be capped with clean water or diluted processed water. The WSFs of MFT and CT will contribute to the water quality of the overlying water that supports phytoplankton community growth.

There are numerous opportunistic and constructed ponds/wetlands that have been studied over the years that differ in water and sediment quality due to the characteristics of the fill material and water cap (for examples, see Farwell *et al*., 2009) as well as potential surface and groundwater inputs. Naphthenic acids (NAs) and salinity have been identified as factors influencing phytoplankton community composition in both regional and on oil-sands lease studies (Leung *et al*., 2003; Hayes, 2005). Salinity (measured as conductivity) and NAs at greater than 1000 µS/cm and 30 mg/L, respectively, appear to alter phytoplankton species composition (Hayes, 2005). Notably, total phytoplankton biomass was not correlated to any of the measured parameters including salinity, NAs, TN and TP (Leung *et al*., 2003; Hayes, 2005), yet there were clear differences in biomass between sites suggesting that other biological (grazing), chemical

(other toxic chemicals or limited micronutrients) or physical (turbidity associated with clay in MFT) factors may affect phytoplankton biomass. In the present study, the first objective was to determine the extent to which the WSFs of MFT and CT support phytoplankton growth in laboratory microcosms without confounding factors such as grazing.

There is also an interest in exploring the potential benefits of using muskeg overburden (a peat-mineral mixture) to enhance ecosystem development in oil sands aquatic reclamation. A recent multi-year field study by Kovalenko *et al*. (2013) found that the peat-mineral amendment did not improve submerged macrophyte biomass in wetlands containing OSPM relative to reference wetlands, although the amendment was beneficial for emergent plant growth. In a survey of reclamation wetlands, phytoplankton and periphyton community growth estimates, measured as chl *a* and dry weight biomass, were similar among OSPM and reference wetlands (Chapter 2). Although there was high temporal and spatial variability in this field assessment, the data suggested that higher growth estimates may be due to higher nutrient levels in some systems (Chapter 2). The second objective of the present study was to determine if nutrients present in the WSF of a natural source of peat-mineral mixture (muskeg overburden) could promote higher phytoplankton growth. In end pit lake reclamation, phytoplankton biomass is a very important source of energy for higher trophic levels. But, perhaps equally important is the biomass that settles to the bottom, adding a layer of biological material over less favorable OSPM such as MFT or CT.

Various field studies have examined the $\delta^{13}C$ and $\delta^{15}N$ of producers and consumers to establish trends in carbon utilization and nitrogen-defined trophic levels in oil sands reclamation (Ganshorn, 2002; Daly, 2007; Farwell *et al*., 2009; Elshayeb, 2006; Chapter 2). Isotope research reported extreme $\delta^{15}N$ enrichment in benthic invertebrates associated with some OSPM sites, particularly CT sites (Farwell *et al*., 2009), beyond the expected ^{15}N enrichment associated with trophic position at the level of benthic invertebrates. This $\delta^{15}N$ enrichment was not associated with alterations in food web structure at the OSPM sites (Elshayeb, 2006), but was indicative of differences in nitrogen dynamics at the base of the food web relative to reference sites. Similar ^{15}N enriched trends at OSPM sites have been found for microbial biofilm (Daly, 2007), plankton and periphyton (Chapter 2) and plants (Daly, 2007). This ^{15}N enrichment was thought to be a function of elevated levels of nitrogen, mainly NH_4^+ slowly released from processed material such as MFT (Farwell *et al*., 2009) and N conversion processes such as nitrification (Daly, 2007). Laboratory studies were able to produce ^{15}N enrichment in microbial biomass grown in media containing an oil sands NA extract; in this case, cultures were supplemented with nutrients regularly, characteristic of the potential release of nutrients from MFT (Videla *et al*., 2009). In

the present study, algal biomass was analyzed for stable C and N isotopes to establish isotope trends associated with C and N sources from MFT, CT and nutrient-rich peat.

The objectives of this study were to measure phytoplankton community growth (measured as chl *a*) in laboratory microcosms containing WSFs of MFT and CT and determine if macronutrient levels (TN and TP) in the WSF of peat improved phytoplankton growth. To further the understanding and interpretation of stable C and N isotope trends influenced by oil sands material in field studies, stable C and N isotopes of algae were analyzed to demonstrate the assimilation of C and N sources from oil sands OSPM (MFT and CT) and peat in a controlled environment.

3.3 Materials and Methods

3.3.1 Source of Materials

The substrates used to generate WSFs in this study were collected from two oil sands leases in Alberta, Canada. Peat (stock piled overburden) and MFT (source; West in Pit, an active settling basin) were acquired from the Syncrude lease. The CT was collected from the top 20 cm of a CT reclamation site referred to as 4 m CT wetland (no peat zone) on the Suncor lease. All material was collected in 20 L plastic containers, shipped to the University of Waterloo (Waterloo, Ontario, Canada) and stored at room temperature.

Water collected for the purpose of either phytoplankton inoculation or dilution in WSF microcosms was collected from the Syncrude lease. The source of the phytoplankton inoculum was water collected from Bill's Lake, a reference site constructed in 1997 of non-processed water (Kovalenko *et al*., 2013). Reference/dilution water used to prepare treatments of selected WSF percentages was collected from Shallow Wetland (SW), a reference site constructed in 1992, lined with a post-mining mixture of clay/sand and capped with non-processed water (Kolalenko *et al*., 2013). Water from SW has a low NA concentration (1.4 ±0.8 mg/L), pH of 8.6 ±0.7 and conductivity of 622 ±126 µS/cm (Farwell *et al*., 2009). Both water supplies were collected in 20 L carboys, transported to the University of Waterloo and stored at room temperature.

3.3.2 Preparation of Water Soluble Fractions of Peat, MFT, and CT

Water soluble fractions (WSFs) were prepared separately for peat (muskeg/overburden), MFT and CT. To generate the WSFs, 600 g of peat, MFT, or CT and 20 L of milli-q water were added to glass tanks (41 x 20 x 25 cm), mixed frequently for one week, and allowed to settle for

another two weeks. The tanks were wrapped in aluminum foil to omit light. After two weeks, the overlying water (referred to as WSF) was collected by siphon and filtered with glass fiber filters (1μm pore size; Pall Life Sciences) for all WSFs. The WSF of MFT had to be pretreated by centrifugation for 15 minutes in 250 mL polypropylene centrifuge bottles (Nalge Nunc International, Rochester, NY, USA) at 6000 rpm in a Sorvall® RC-5B Refrigerated Superspeed centrifuge (Thermo Fisher Scientific, Waltham, MA, USA) with the Sorvall® SLA 1500 rotor (Thermo Fisher Scientific, Waltham, MA, USA) and then filtered with glass fiber filters (1μm pore size). In experiment 3 only, batches of peat and MFT WSFs were prepared as described above and then 50 ml of solution containing nutrient media (Fraquil; $CaCl_2$• dH_2O, $MgSO_4$•$7H_20$, $NaHCO_3$, Na_2SiO_3•$9H_2O$, $NaNO_3$, K_2HPO_4, Vitamin solution, and a trace metals solution from stock solutions; Morel *et al*., 1975) was added to each bulk preparation of WSF.

3.3.3 Preparation of Microcosms

Various concentrations of WSFs of peat, MFT or CT were prepared to provide treatments along a gradient of WSFs. For each type of WSF, there were 5 treatments each containing a percentage of WSF (0 %, 25%, 50%, 75% and 100%). Water from SW was used as the reference (0%)/dilution water to prepare the treatments. In experiments 1 and 2, there were treatments containing WSF percentages of peat only and CT only (Exp. 1) or MFT only (Exp. 2). In addition, mixtures of peat and CT (Exp. 1) or MFT (Exp. 2 and 3) at peat:CT or MFT ratios of 100:0, 75:25, 50:50, 25:75 and 0:100 were prepared. The treatment water (900 ml) was then added to microcosms (1 L clear glass jars) and inoculated with 100 ml of water from Bill's Lake to promote phytoplankton growth. A total of 4 (Exp. 1 and 2) or 6 (Exp. 3) microcosm containers per treatment were prepared at the start of the experiment to allow one container to be sacrificed per treatment per sampling period. The microcosms were held at 20°C and a photoperiod of 16:8 hour light:dark in a Conviron incubator (Conviron, Winnipeg, MB) for 3 (Exp. 1 and 2) or 5 (Exp. 3) weeks. Jars were mixed daily to maintain dissolved oxygen levels.

3.3.4 Sampling and Analytical Protocols for Water and Algal Samples

Samples were collected for water quality at the start (day 0) and at the end of the experiment (week 3 or 5). Temperature, conductivity, pH, and dissolved oxygen were measured using an Orion Model 1230 field meter (Thermo Fisher Scientific, Waltham, MA). Samples for TN, TP, DOC, and DIC concentration as well as $\delta^{13}C$ of DOC and DIC were collected for Exp. 1 and 2. In addition, samples were collected for NO_2, NO_3, and NH_4^+ concentration on day 0 and

week 3 in experiment 1. Only TN and TP were collected for Exp. 3. Samples for TN, TP, NO_2, NO_3, and NH_4^+ concentration were collected in 20 ml glass scintillation vials, refrigerated at 4ºC and then shipped to the Biogeochemical Analytical Service Laboratory at the University of Alberta (Edmonton, Alberta, Canada) for analysis. Water samples (250 mL) for DOC and DIC $\delta^{13}C$ and concentration were placed in 250 mL trace clean amber borosilicate glass bottles (VWR), refrigerated at 4°C and then filtered as described in Videla *et al*. (2009). Samples were filtered through a 25 mm, 0.45 μm polyethersulfone Nalgene® syringe filter (Nalge Nunc International, Rochester, NY, USA) into 40 mL TraceClean amber borosilicate glass vials (Chase Scientific Glass Inc., Rockwood, TN, USA). The caps were lined with 22 mm polytetrafluoroethylene-rubber (Chromatographic Specialties Inc., Brockville, ON, Canada). The samples were refrigerated and sent to the G.G. Hatch Isotope Laboratory at the University of Ottawa (Ottawa, Ontario, Canada) for analysis. DOC and DIC concentrations were measured on an OI Analytical Aurora Model 1030W TOC Analyser with a 2% precision. $\delta^{13}C$ values for DOC and DIC were measured on a continuous flow Finnigan Mat DeltaPlusXP isotope ratio mass spectrometer. NA concentrations were analyzed for 100% MFT WSF and 100% CT MFT by FT-IR Spectroscopy (Jivraj *et al*., 1995).

Samples were collected to determine plankton growth on day 0 and then once a week for the duration of the exposure (week 3 or 5). In many of the microcosms, filamentous algae were observed by week 1 or 2; if present, the filamentous algae were first removed by a sieve. Volumes of sieved water were collected for measurements of chl *a* (100 ml), total suspended solids (TSS, 200 ml), nutrients (20 ml each) and stable isotopes (~600 ml; see below). Water for chl *a* analysis was filtered using 47 mm Whatman GF/F glass fiber filters (0.7 μm pore size) and frozen at -20°C in 20 mL glass scintillation vials covered with aluminum foil. Before the analysis, the samples were extracted with 20 mL of 90% acetone at -20°C for 24 hours. Chl *a* samples were measured on a Turner Designs model 10AU fluorometer (Turner Designs, Sunnyvale, CA) against pure chl *a* (Yentsch and Menzel, 1963). Water analyzed for TSS was filtered using pre-weighed 47 mm Whatman GF/F glass fiber filters (0.7μm pore size), the filter was dried for 24 hours at 60ºC and then re-weighed. The filamentous algae collected by sieve (as described above) were dried at 60ºC for 24 hours and weighed. All filters and dry weights were measured on a Mettler Toledo AG245 analytical balance (Mettler Toldedo, Columbus, OH).

3.3.5 Stable Carbon and Nitrogen Analysis

About 600 ml of treatment water was centrifuged for 15 minutes in 250 mL polypropylene centrifuge bottles at 6000 rpm in a Sorvall® RC-5B Refrigerated Superspeed

centrifuge with the Sorvall® SLA 1500 rotor. The supernatant was discarded and the pellet dried at 60ºC for 48 hours. Stable isotope analysis of dried plankton and filamentous algae were conducted at the Environmental Isotope Laboratory, Department of Earth Sciences, University of Waterloo (Waterloo, Ontario, Canada). Ground, dried samples (1-10 mg) were placed into tin capsules (5 x 3.5 mm) (SerCon Ltd., Cheshire, United Kingdom). Samples were analyzed using the Thermo-Finnegan Delta Plus Continuous Flow Isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled to a Carlo Erba Elemental Analyzer (Thermo Fisher Scientific, Italy). Stable carbon and nitrogen values were measured using the formula:

$$(R_{sample}/R_{standard})-1) \times 10^3 = \delta^{13}C \text{ or } ^{15}N \text{ (‰)},$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Standard reference materials included carbonate rock Vienna Pee Dee Belemnite (IAEA) for carbon and atmospheric air (National Institute of Standards and Technology) for nitrogen. There was a standard error of ± 0.2 ‰ for carbon analysis and ± 0.3 ‰ for nitrogen analysis. Laboratory standards of sucrose, cellulose and graphite for carbon and ammonium sulphate for nitrogen analysis were used to normalize the data.

3.4 Results

Water quality parameters and estimates of plankton and filamentous algae growth, measured as chl *a* or dry weight, respectively, were determined for microcosms containing varying proportions of the WSF of a) CT or MFT only, b) peat only, and c) mixtures of peat and CT or MFT (3 week exposures; Experiments 1 and 2). These parameters were also measured for nutrient - amended treatments containing mixtures of WSFs of peat and MFT (5 week exposure; Experiment 3).

3.4.1 Microcosm Experiment 1: Water Soluble Fractions of Consolidated Tailings and Peat

Water Quality Parameters

Water temperature and pH ranged from 20-21°C and 7.1 to 9.2, respectively. The pH of 100% WSF of peat was lower than 100% WSF of CT. The DO levels ranged from 88 to 199 % saturation (7.5-8.2 mg/L); lower in peat vs. CT treatments. Conductivity was higher in reference/dilution water (760 µS/cm) relative to 100% WSFs of peat (108 µS/cm) and CT (242 µS/cm). The NA concentration of 100% CT was 11.1 mg/L.

The WSFs of peat had higher TP concentration than the WSFs of CT and both WSFs had higher TP concentration than the reference/dilution water (0%) (Table 3.1). The 100% WSF of

CT had TN concentrations similar to the reference/dilution water but 100% peat WSF had higher (2 times) TN concentration. TN:TP ratios ranged from 11 to 21 for WSF treatments compared to >35 for reference/dilution water. Concentrations of $NO_2 + NO_3$ were lower in 100% WSF of peat relative to the reference/dilution water and 100% WSF of CT. Concentrations of NH_4^+ were higher in the 100% WSF of peat (>10 times) compared to the reference/dilution water and 100% WSF of CT. DOC and DIC concentrations ranged from 7 to 70 mg/L and 2 to 80 mg/L, respectively. The reference/dilution water and the WSFs of CT had lower DOC and higher DIC concentrations relative to comparable WSFs of peat.

Table 3. 1 Nutrient concentrations in microcosms with different WSF percentages of peat, CT, and peat:CT mixtures (Exp. 1).

WSF Treatment (%)	TN (µg/L)		$NO_2 + NO_3$ (N µg/L)		NH_4^+ (N µg/L)		TP (µg/L)		TN:TP		DOC (mg/L)		DIC (mg/L)	
	Day 0	Week 3	Day 0	Week 3	Day 0	Week 3	Day 0	Week 3	Day 0	Week 3	Day 0	Week 3	Day 0	Week 3
Peat														
0	1110	527	377	13	36	86	31	8	35.8	65.9	19.71	16.98	79.23	44.69
25	1520	702	349	8	266	94	76	21	20.0	33.4	29.26	26.82	56.61	31.33
50	2120	916	335	8	546	90	101	34	21.0	26.9	40.48	36.17	35.45	19.9
75	2690	1190	341	11	869	68	139	48	19.4	24.8	50.39	45.31	18.36	6.36
100	3280	2240	309	119	1070	475	182	72	18.0	31.1	69.64	68.31	3.27	BDL
CT														
0	1090	541	395	6	26	35	29	15	37.6	36.1	18.71	17.04	75.59	47.35
25	1150	509	442	7	23	60	58	10	19.8	50.9	15.29	15.78	62.45	36.83
50	1140	533	532	5	33	25	74	23	15.4	23.2	12.99	13.95	44.34	22.41
75	1190	644	579	6	69	30	101	81	11.8	8.0	11.19	11.91	28.81	18.49
100	1140	722	543	5	78	26	nd	nd	Nd	nd	7.94	10.83	18.88	9.4
Peat:CT														
100:0	3290	2290	304	98	1,040	535	180	80	18.3	28.6	68.78	65.52	2.19	BDL
75:25	2810	1210	385	27	836	63	159	42	17.7	28.8	54.99	46.98	5.42	BDL
50:50	2200	1030	471	10	590	69	151	56	14.6	18.4	30.08	28.86	7.98	BDL
25:75	1680	880	578	10	354	81	nd	nd	Nd	nd	15.79	18.23	12.13	2.68
0:100	1210	696	679	11	96	19	109	55	11.1	12.7	7.24	8.66	16.36	6.76

nd, no data available; BDL, below detection limit of 2 mg/L

Estimates of Algal Growth

The WSFs of CT and peat supported higher plankton growth than the reference/dilution water (Table 3.2 and Fig. 3.1). Maximum chl *a* levels were observed for 75% WSF of CT and 100% WSF of peat, ≥ 5 times higher than the reference/dilution water. The WSFs of combined peat and CT at a ratio of 50:50 had the highest average chl *a* values (38 µg/L) over the 3 week incubation.

Estimates of TSS, which includes organic and inorganic suspended solids, were lower for treatments containing the WSFs of peat relative to CT for day 0 values and weekly values (Fig. 3.1); however TSS values were highly variable. For treatments containing WSFs of CT, TSS levels on day 0 provided an estimate of residual clay (clearly visible in CT microcosms), that increased with increasing WSF percentages of CT.

Growth of filamentous algae was evident by week 2 and increased in week 3 in all treatments (Fig. 3.1 and Table 3.2). Dry weight estimates were similar for reference/dilution water and lower percentages of WSFs of peat or CT, decreasing at higher percentages of peat or CT. For combined treatments containing WSFs of peat and CT, dry weight increased with percentage CT WSF although estimates were still lower than the reference/dilution water.

Regression analyses of mean growth estimates for plankton (chl *a*) and filamentous algae (dry weight) and TN and TP indicated the importance of TP in controlling algal growth (Fig. 3.2). TP values were positively correlated with plankton chl *a* (r^2 =0.63, p=0.001) and negatively correlated with filamentous algal dry weight (r^2 = 0.86, p=0.000). There was no significant trend between TN and plankton chl *a* (r^2 =0.17, p=0.12) however filamentous algal dry weight was negatively correlated to TN (r^2 = 0.70, p=0.000). In this study, day 0 TSS was used as an indicator of CT-associated fine clay particles to determine if elevated TSS affected algal growth. There were no significant trends associated with day 0 TSS for either plankton chl *a* (r^2 =0.22, p=0.09) or filamentous algal dry weight (r^2 =0.01, p=0.81) suggesting that low day 0 TSS (< 100 mg/L) was not a significant factor controlling algal growth.

Table 3. 2 Mean ± SE for plankton and filamentous algae growth estimates in WSFs of peat, CT and peat:CT mixtures following a 3 week exposure (Exp. 1).

WSF Treatment (%)	Plankton Chl *a* (µg/L)[1]	Filamentous Algae (dry wt mg/L)[2]
Peat		
0	3.1 ±2.8	61.2 ±3.6
25	5.6 ±5.8	60.3 ±17.0
50	10.6 ±6.7	45.0 ±12.2
75	10.5 ±9.2	37.4 ±7.6
100	16.3 ±10.0	8.5 ±2.1
CT		
0	0	79.0 ±12.9
25	0	88.5 ±12.2
50	0.5 ±6.3	71.6 ±17.9
75	31.2 ±7.1	54.4 ±4.9
100	13.3 ±7.2	47.8 ±13.8
Peat:CT		
100:0	23.7 ±15.2	10.1 ±0.6
75:25	10.1 ±2.9	21.9 ±4.3
50:50	38.4 ±13.9	32.6 ±5.4
25:75	35.3 ±19.8	32.0 ±4.5
0:100	13.3 ±7.6	37.2 ±7.2

[1] phytoplankton chl *a* mean ± SE values were calculated as the average of weeks 1, 2 and 3 data, adjusted using the day 0 chl *a* measurements for each treatment.
[2] filamentous algae was not detected on day 0 or week 1, dry weight mean ± SE values were calculated as the average of week 2 and 3.

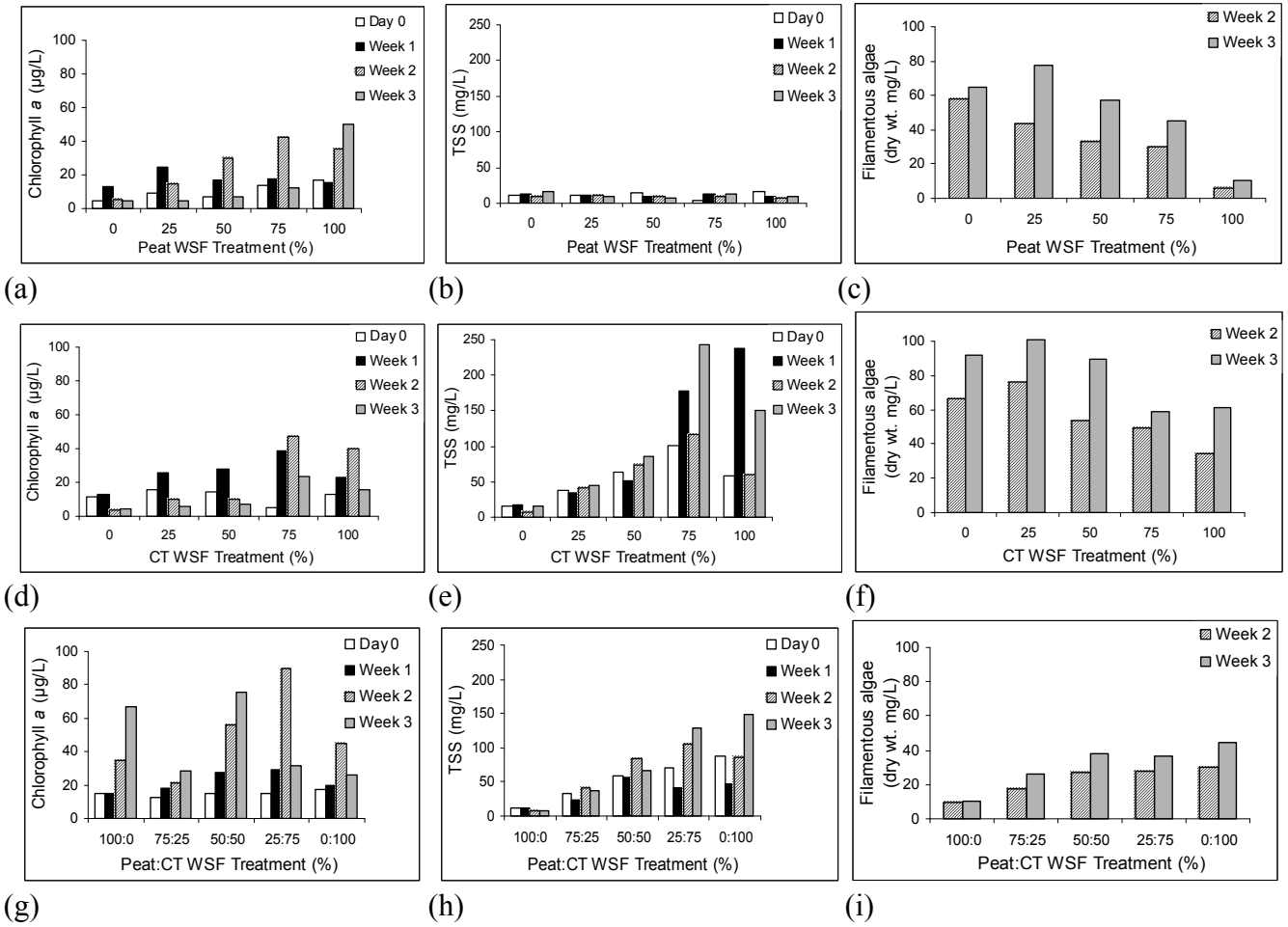


Figure 3. 1 Plankton chlorophyll *a* (a, d, g), TSS (b, e, h), and filamentous algae dry weight (c, f, i) in WSFs of peat, CT, and peat:CT mixtures sampled on day 0, week 1, 2 and 3 (Experiment 1).

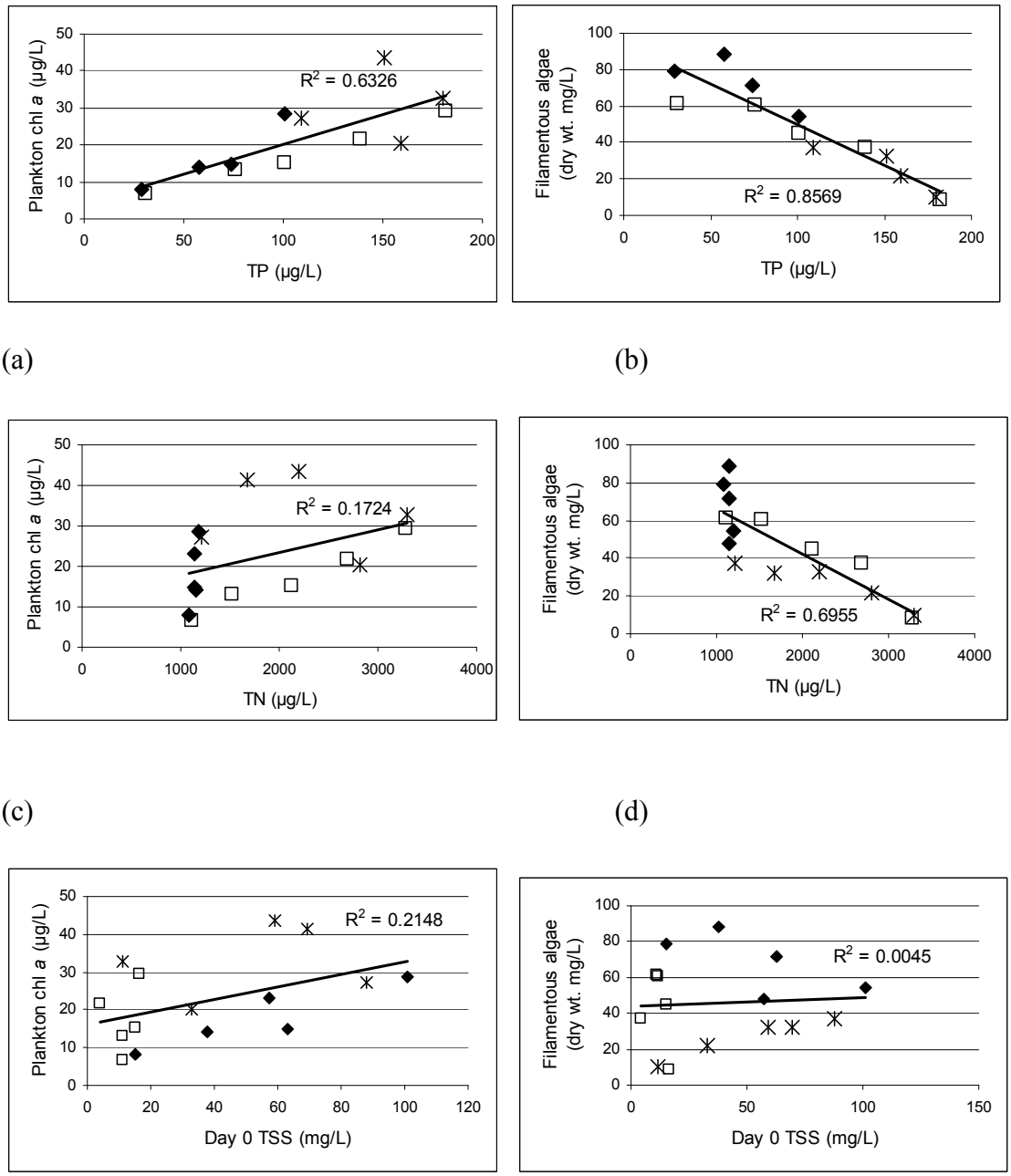


Figure 3. 2 Linear regression of plankton chl *a* or filamentous algae dry weight and TP (a,b), TN (c,d) and TSS (e,f) for various WSF treatments of peat (open square), CT (closed diamond) and peat:CT mixture (star) (Experiment 1).

3.4.2 Microcosm Experiment 2: Water Soluble Fractions of Mature Fine Tailings and Peat

Water Quality Parameters

Microcosms were held at room temperature (20-21°C). In general, pH ranged from 7.1-9.1; the lowest pH was associated with 100% WSF peat. Conductivity (104 – 778 μS/cm) decreased with increasing percentage of the WSF for both peat and MFT. Conductivity was more than 3 times higher in the 100% WSF of MFT than peat. Dissolved oxygen levels were similar for WSF peat and MFT exposures (70 – 80% saturation; 6-7 mg/L) and lower in the peat:MFT mixture exposure (44-72% saturation; 3.7-6.0 mg/L). The NA concentration for 100% WSF of MFT was 11.5 mg/L, similar to the 100% WSF of CT. NA concentrations were not determined for the WSFs of peat.

Nutrient data (day 0) showed trends of increasing TN and TP concentrations with increasing percentages of the WSFs of both peat and MFT (Table 3.3). At the highest concentration (100 %), the WSF of peat had higher TN and lower TP (>3 times lower) relative to the WSF of MFT. The TN:TP ratios ranged from 4-5 for 100% WSF MFT and 25-29 for 100% WSF peat. DOC concentrations increased with increasing percentage WSF of peat. Both the reference/dilution water and the WSFs of MFT had lower levels of DOC relative to peat WSFs. The reference/dilution water had higher DIC concentrations than the WSFs of both peat and MFT.

Estimates of Algal Growth

Both WSF treatments of MFT and peat had higher plankton community growth compared to the reference/dilution water (Table 3.4 and Fig. 3.3a-i). Maximum average plankton chl *a* estimates were 2 times greater at 75% WSFs of peat (35 μg/L) compared to MFT (15 μg/L) (Table 3.4). Treatments with combined WSFs had the highest average chl *a* values at a peat:MFT ratio of 50:50.

Similar to the CT experiment, TSS levels were lower in WSFs of peat than MFT (Fig. 3.3). Visual observations of reduced transparency in MFT WSFs and day 0 TSS values (as high as 1035 mg/L; Fig. 3.3e) that increased with percentage MFT WSF were indicative of the high clay content associated with MFT. Increased turbidity at 100% WSF of MFT could explain the

lower chl *a* values relative to lower percentages of MFT WSFs in both MFT only and peat:MFT treatments.

Table 3. 3 Nutrient concentrations in microcosms with different WSF percentages of peat, MFT, and peat:MFT mixtures (Exp 2; 3 week exposure) and nutrient-amended peat:MFT mixtures (Exp. 3; 5 week exposure).

WSF Treatment (%)	TN (µg/L)		TP (µg/L)		TN:TP		DOC (mg/L)		DIC (mg/L)	
	Day 0	Week 3 or 5	Day 0	Week 3 or 5	Day 0	Week 3 or 5	Day 0	Week 3	Day 0	Week 3
Peat										
0	904	516	4	7	226.0	73.7	17.98	17.89	65.39	60.41
25	2200	763	38	56	57.9	13.6	29.15	28.17	53.49	33.16
50	2950	1030	69	26	42.8	39.6	40.67	40.2	32.35	21.42
75	3680	2130	109	45	33.8	47.3	54.14	52.06	16.29	6.04
100	4040	2510	140	57	28.9	44.0	72.59	68.99	BDL	BDL
MFT										
0	1190	516	4	7	297.5	73.7	17.08	18.12	69.04	64.23
25	1620	290	184	114	8.8	2.5	16.3	17.19	66.4	53.5
50	2170	316	338	212	6.4	1.5	14.24	18.12	52.9	43.87
75	2410	367	401	251	6.0	1.5	12.2	14.27	38.93	38.87
100	2660	2320	559	309	4.8	7.5	11.1	11.8	28.87	29.9
Peat:MFT										
100:0	3890	2270	159	44	24.5	51.6	68.77	65	4.29	BDL
75:25	3350	2360	260	130	12.9	18.2	58.26	39.35	12.87	BDL
50:50	2960	2210	347	182	8.5	12.1	nd	24.42	nd	9.84
25:75	2870	3820	459	258	6.3	14.8	24.77	15.77	29.16	20.17
0:100	2540	2730	598	298	4.2	9.2	15.22	11.86	39.35	31.45
Peat:MFT (nutrient-amended)										
100:0	3590	2010	322	67	11.1	30.0	nd	nd	nd	nd
75:25	4080	3750	nd	131	nd	28.6	nd	nd	nd	nd
50:50	3830	1810	462	198	8.3	9.1	nd	nd	nd	nd
25:75	3590	1810	550	231	6.5	7.8	nd	nd	nd	nd
0:100	1650	2190	573	249	2.9	8.8	nd	nd	nd	nd

nd, no data available; BDL, below detection limit of 2 mg/L

Table 3. 4 Mean ± SE for plankton and filamentous algae growth estimates in WSFs of peat, MFT, and peat:MFT mixtures (Exp. 2), and nutrient-amended peat:MFT mixtures (Exp. 3) following a 3 week exposure.

WSF Treatment (%)	Plankton Chl a (µg/L)[1]	Filamentous algae (dry wt mg/L)[2]
Peat		
0	0	23.7 ±5.9
25	12.1 ±3.2	45.5 ±21.0
50	17.3 ±4.2	32.4 ±12.1
75	35.2 ±13.0	31.6 ±15.8
100	27.0 ±12.3	12.9 ±6.2
MFT		
0	0	32.9 ±6.0
25	13.5 ±9.5	3.7 ±3.7
50	10.6 ±8.9	0
75	15.0 ±8.8	0
100	0	0
Peat:MFT		
100:0	17.4 ±4.5	8.5 ±2.9
75:25	39.5 ±28.4	7.4 ±5.0
50:50	59.7 ±33.1	3.2 ±2.4
25:75	16.3 ±8.0	1.1 ±0.8
0:100	0	0.3 ±0.3
Peat:MFT (nutrient-amended)		
100:0	30.5 ±15.3	23.9 ±0.3
75:25	86.3 ±48.6	12.1 ±2.9
50:50	180.6 ±103.7	2.2 ±0.7
25:75	163.7 ±96.2	11.2 ±6.9
0:100	41.5 ±28.1	0

[1] phytoplankton chl *a* mean ± SE values were calculated as the average of weeks 1, 2 and 3 data, adjusted using the day 0 chl *a* measurements for each treatment.
[2] filamentous algae were not detected on day 0, dry weight mean ± SE values were calculated as the average of weeks 1, 2 and 3.

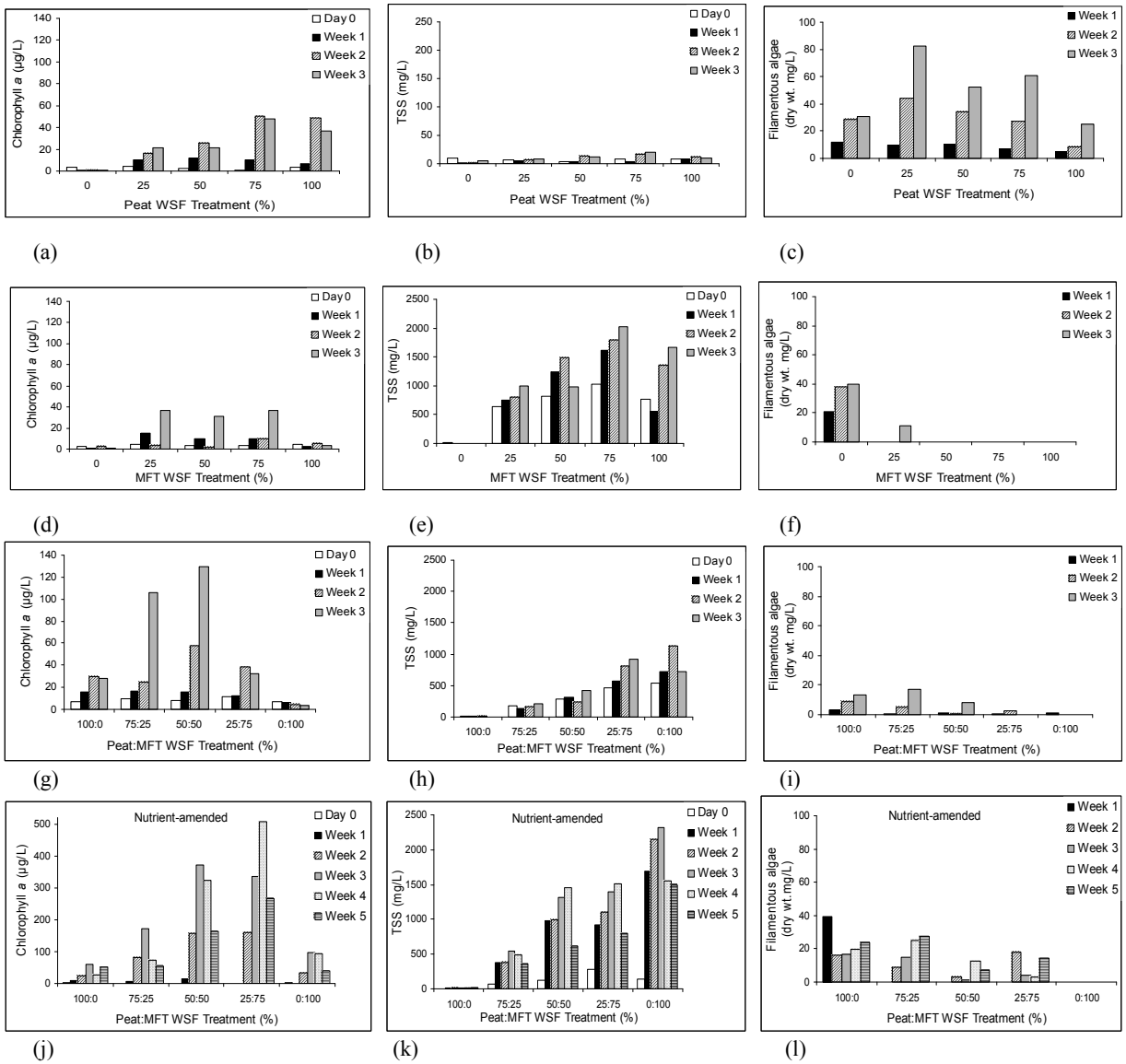


Figure 3. 3 Plankton chlorophyll *a* (a, d, g, j), TSS (b, e, h, k), and filamentous algae dry weight (c, f, i, l) in WSFs of peat, MFT, peat:MFT mixtures, and nutrient-amended peat:MFT mixtures sampled on day 0, week 1, 2, and 3 (Experiment 2 and 3).

Filamentous algae were observed in reference/dilution water and the WSFs of peat by week 1 however there was only limited growth in all treatments with WSFs of MFT during the 3 week period (Fig. 3.3 and Table 3.4). Overall, the maximum average dry weight was 46 mg/L for peat (25% WSF), approximately 11 times higher than the dry weight (4 mg/L) of 25 % WSF of MFT (Table 3.4). For combined treatments, dry weight decreased with an increase in percentage MFT WSF. The decrease in growth of filamentous algae could be attributed to the elevated TSS associated with MFT. At 100% WSF, day 0 TSS estimates were approximately 10 times greater for MFT than CT treatments (see regression analysis for MFT WSFs in section 3.4.3).

3.4.3 Microcosm Experiment 3: Nutrient-amended Water Soluble Fractions of Mature Fine Tailings and Peat

Water Quality Parameters

Nutrient-amended microcosms were held at room temperature (21°C). Water pH ranged from 9.0-9.6; the lowest pH was associated with 100% WSF peat. Conductivity (182 – 464 μS/cm) was more than 2 times higher in 100% WSF of MFT than peat. Dissolved oxygen was 38-107% saturation (3.3-9.0 mg/L), lowest at 25:75 peat:MFT. There were higher TN concentrations in peat WSFs, at least 2 times higher than 100% WSF of MFT. Concentrations of TP increased with percentage WSF of MFT (Table 3.3). The TN:TP ratio ranged from 3 (100% WSF MFT) to 11 (100% WSF peat).

Estimates of Algal Growth

Nutrient-amended WSF treatments of peat and MFT mixtures had higher plankton community growth than non-nutrient amended treatments in experiment 2 (Fig. 3.3j-l). In this 5 week exposure, maximum phytoplankton community growth was observed on week 4 at higher percentages of MFT WSFs compared to week 3 at higher percentages of peat WSFs. In order to compare with experiment 2 (3 week exposure), average chl *a* values for experiment 3 were reported based on data from weeks 1, 2, and 3 in Table 3.4. The 50:50 peat:MFT WSF treatment had the highest average chl *a* (180 μg/L) over the 3 week period. At 100% MFT, average chl *a* values were 4 times lower than the chl *a* estimates for 50:50 peat:MFT WSF but similar to 100% peat WSF.

The day 0 TSS concentration was low in 100% peat WSF (3.5 mg/L) but generally increased along the MFT gradient, indicative of fine clay suspended particles. The highest day 0

TSS level (25:75 peat:MFT, 275.0 mg/L) was lower than values reported for peat:MFT WSFs in Experiment 2.

Nutrient-amended microcosms supported filamentous algal growth for all treatments with the exception of 100% MFT WSF (Fig. 3.3 l and Table 3.4). Average dry weight estimates were highest in 100% peat WSF (24 mg/L). Average dry weight over 3 weeks was higher (2 times at 100% peat WSF) in the nutrient-amended treatments compared to non-amended treatments (Experiment 2).

Regression analyses of growth estimates and macronutrients (TN and TP, day 0), combined for Exp. 2 and 3, showed some different relationships compared to Exp. 1, likely due to the confounding factors of elevated TSS and TP concentrations associated with MFT (Fig. 3.4). For Exp. 2 and 3, TSS was positively correlated to TP (r^2 =0.563, p= 0.001) but not TN (r^2 =-0.035, p= 0.50) (data not shown). Unlike Exp. 1, TP values were not correlated to plankton chl *a* (r^2 =0.17, p= 0.08) (Fig. 3.4). However, there was a negative correlation between TP and filamentous algal dry weight (r^2 =0.62, p= 0.000), similar to Exp. 1. TN concentration was weakly correlated to plankton chl *a* (r^2 =0.20, p= 0.05) but there was no correlation between TN concentration and filamentous algal dry weight (r^2 =0.02, p= 0.72). There was a non-linear relationship between day 0 TSS and plankton chl *a* levels (r^2 =0.02, p= 0.54, Fig. 3.4 e). At low to moderate TSS levels (<300 mg/L), plankton chl *a* levels were highly variable, but at higher TSS (> 400 mg/L, day 0) there was a trend of reduced plankton chl *a*. For filamentous algae, some of the highest dry weight estimates were reported at low TSS levels while at TSS levels >100 mg/L, in most cases, dry weight estimates were lower (≤ 10 mg/L). There was a negative correlation between TSS and filamentous algae growth (r^2 =0.43, p= 0.002).

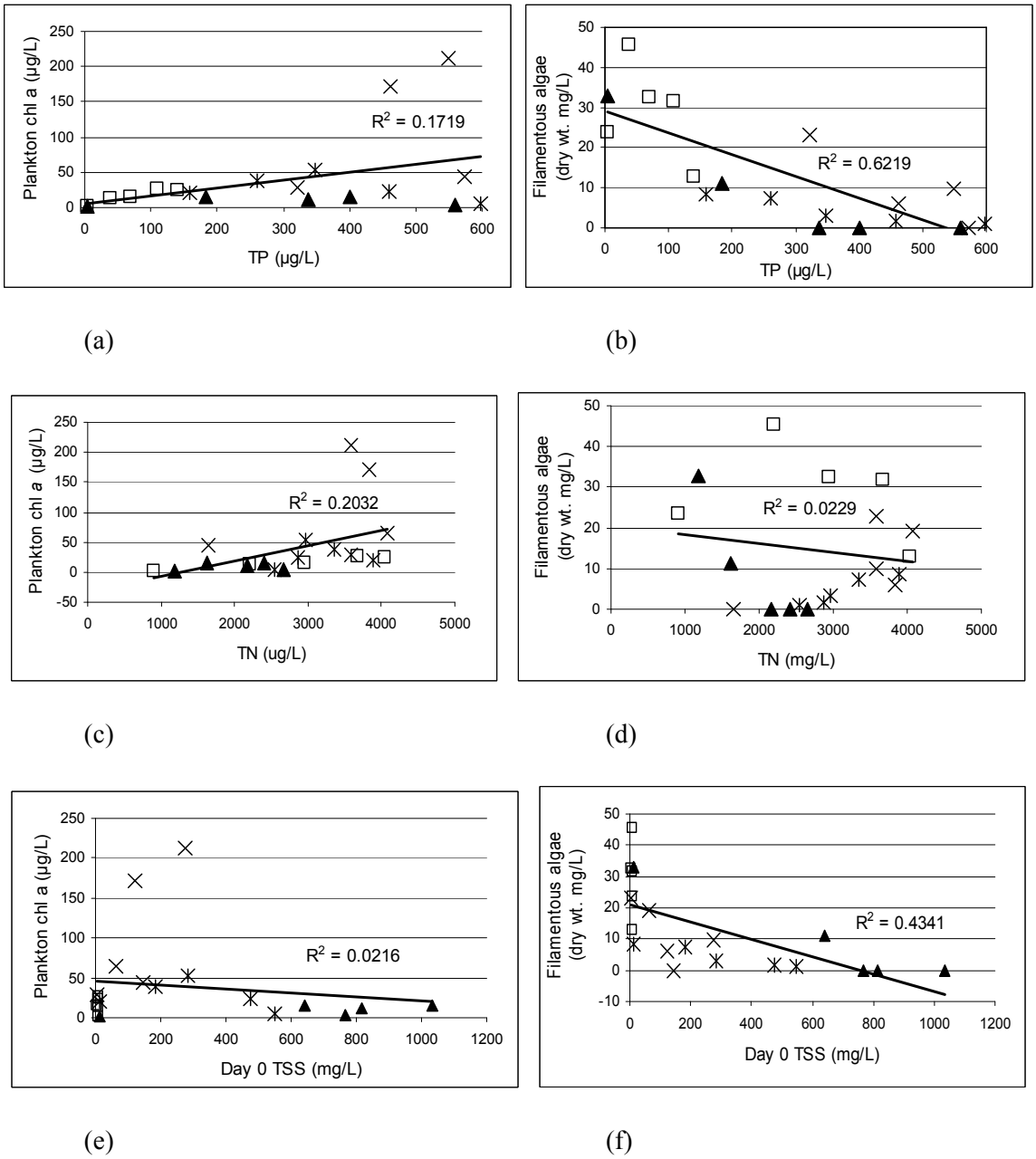


Figure 3. 4 Linear regression of plankton chl *a* or filamentous algae dry weight and TP (a,b), TN (c,d), and TSS(e,f), for various WSF treatments of peat (open square), WSF MFT (closed triangle), peat:MFT mixture (star), and peat:MFT mixture (x) with nutrient amendment (Experiment 2 and 3).

3.4.4 Stable Carbon and Nitrogen Isotope Analysis

Stable C and N isotope values of algal samples were determined for all WSF treatments where sufficient material allowed detection. In this section, stable isotope trends for all three experiments will be presented for a) filamentous algae and b) TSS which included autotrophic and heterotrophic biomass. $\delta^{13}C$ and $\delta^{15}N$ values are considered to be ^{13}C or ^{15}N depleted or enriched when values decrease or increase, respectively.

The substrates used to generate WSFs had the following isotope values: CT substrate, $\delta^{13}C$ of -27.95‰ and $\delta^{15}N$ of 6.77‰; MFT substrate, $\delta^{13}C$ of -26.63‰ and $\delta^{15}N$ of 3.64‰; peat substrate, $\delta^{13}C$ of -25.94‰ and $\delta^{15}N$ of 0.10‰. Peat contained the highest proportion of N (0.34 %) and had a C:N ratio of 42. For both MFT and CT substrates, the proportion of N was lower and the C:N ratio was higher (MFT, 0.09 % N, C:N ratio, 81: CT, 0.14 % N; C:N ratio, 88).

Stable Carbon and Nitrogen Isotopes of Filamentous Algae

Stable isotope trends for DOC and filamentous algae were similar for both WSF treatments of peat and CT (Experiment 1; Fig. 3.5 and Table 3.5). As percentage WSF increased, DOC was more ^{13}C depleted (day 0; range 2 ‰) however DIC was more variable and generally more ^{13}C depleted at higher WSFs of peat compared to CT (Table 3.5). Algae were more ^{13}C and ^{15}N enriched at higher percentages of WSFs relative to the reference/dilution water; this was more pronounced for WSFs of CT (Fig. 3.5 and Table 3.5). In general, filamentous algae were more ^{13}C (2-3 ‰) and ^{15}N (9-10 ‰) enriched at 100% WSF of CT compared to peat.

In experiment 2, filamentous algae from the WSFs of peat were more ^{13}C and ^{15}N enriched than the reference/dilution water, similar to experiment 1 (Fig. 3.6a-f and Table 3.6). However, there are limited data for WSFs of MFT due to minimal growth of filamentous algae in these treatments. While $\delta^{13}C$ data were highly variable (75:25 peat:MFT), the $\delta^{15}N$ values showed trends of ^{15}N enrichment at higher percentages of WSFs of MFT. Filamentous algae were more ^{15}N (5-8 ‰) enriched at intermediate ratios of peat:MFT (50:50, 25:75) than 100% WSF of peat. In contrast, filamentous algae were more ^{15}N depleted with increased percentage WSF of MFT for the nutrient-amended treatments (Experiment 3; Fig. 3.6g,h and Table 3.6). Filamentous algae were more ^{13}C enriched (3 ‰) and ^{15}N (8 ‰) depleted at higher MFT WSF percentages (peat:MFT, 25:75) than 100% peat WSF for nutrient-amended treatments.

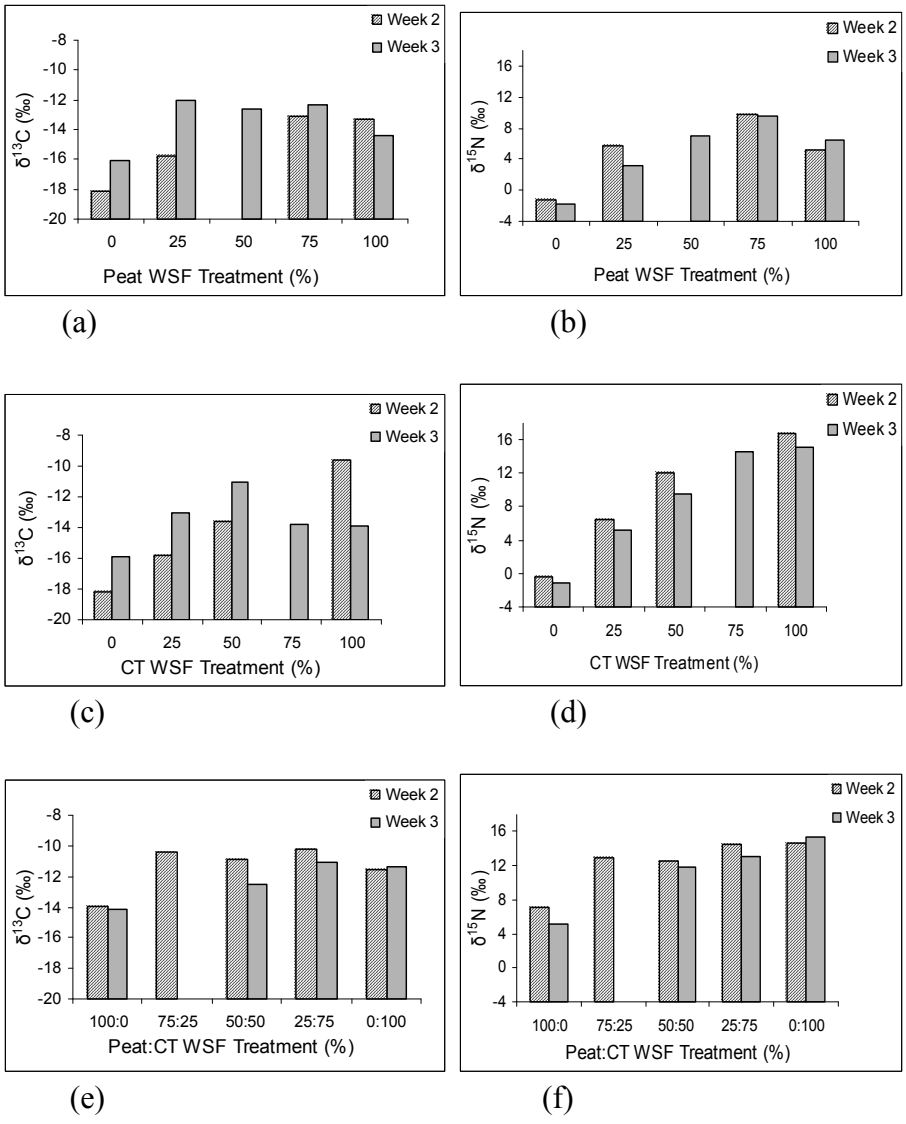


Figure 3. 5 Stable C and N isotope of filamentous algae in WSF peat (a,b), WSF CT (c,d), and peat:CT mixtures (e,f) sampled on week 2 and 3 (Experiment 1).

Table 3. 5 Mean ± SE for $\delta^{13}C$ of DOC and DIC, and $\delta^{13}C$ and $\delta^{15}N$ of filamentous algae in WSFs of peat, CT, and peat:CT mixtures following a 3 week exposure (Exp. 1).

WSF Treatment (%)	DOC (‰)	DIC (‰)	Filamentous Algae (‰)[1]	
	$\delta^{13}C$	$\delta^{13}C$	$\delta^{13}C$	$\delta^{15}N$
Peat				
0	-24.70 ±0.9	-2.63 ±3.7	-17.07 ±1.0	-1.56 ±0.3
25	-25.55 ±0.2	-3.63 ±2.1	-13.88 ±1.9	4.41 ±1.2
50	-25.93 ±0.2	-3.98 ±1.0	-12.61	6.93
75	-26.24 ±0.4	-7.16 ±4.2	-12.72 ±0.4	9.64 ±0.1
100	-26.43 ±0.2	-4.41	-13.83 ±0.5	5.80 ±0.7
CT				
0	-24.92 ±0.1	-2.70 ±2.4	-17.04 ±1.1	-0.77 ±0.3
25	-25.02 ±0.1	-3.08 ±1.9	-14.46 ±1.4	5.83 ±0.7
50	-25.55 ±0.01	-3.21 ±1.6	-12.31 ±1.3	10.79 ±1.3
75	-25.40 ±0.3	-2.77 ±0.3	-13.78	14.58
100	-26.92 ±1.3	-1.86 ±0.9	-11.74 ±0.1	15.93 ±0.8
Peat:CT				
100:0	-26.52 ±0.1	-6.00	-14.05 ±0.13	6.08 ±0.96
75:25	-26.45 ±0.1	-3.78	-10.39	12.82
50:50	-26.26 ±0.5	-1.60	-11.67 ±0.81	12.18 ±0.35
25:75	-26.47 ±1.0	-7.34 ±4.5	-10.62 ±0.44	13.73 ±0.66
0:100	-27.23 ±1.7	-3.90 ±0.9	-11.48 ±0.08	15.05 ±0.34

[1] filamentous algae were not detected on day 0 and week 1, filamentous algae mean ±SE values were calculated as the average of weeks 2 and 3

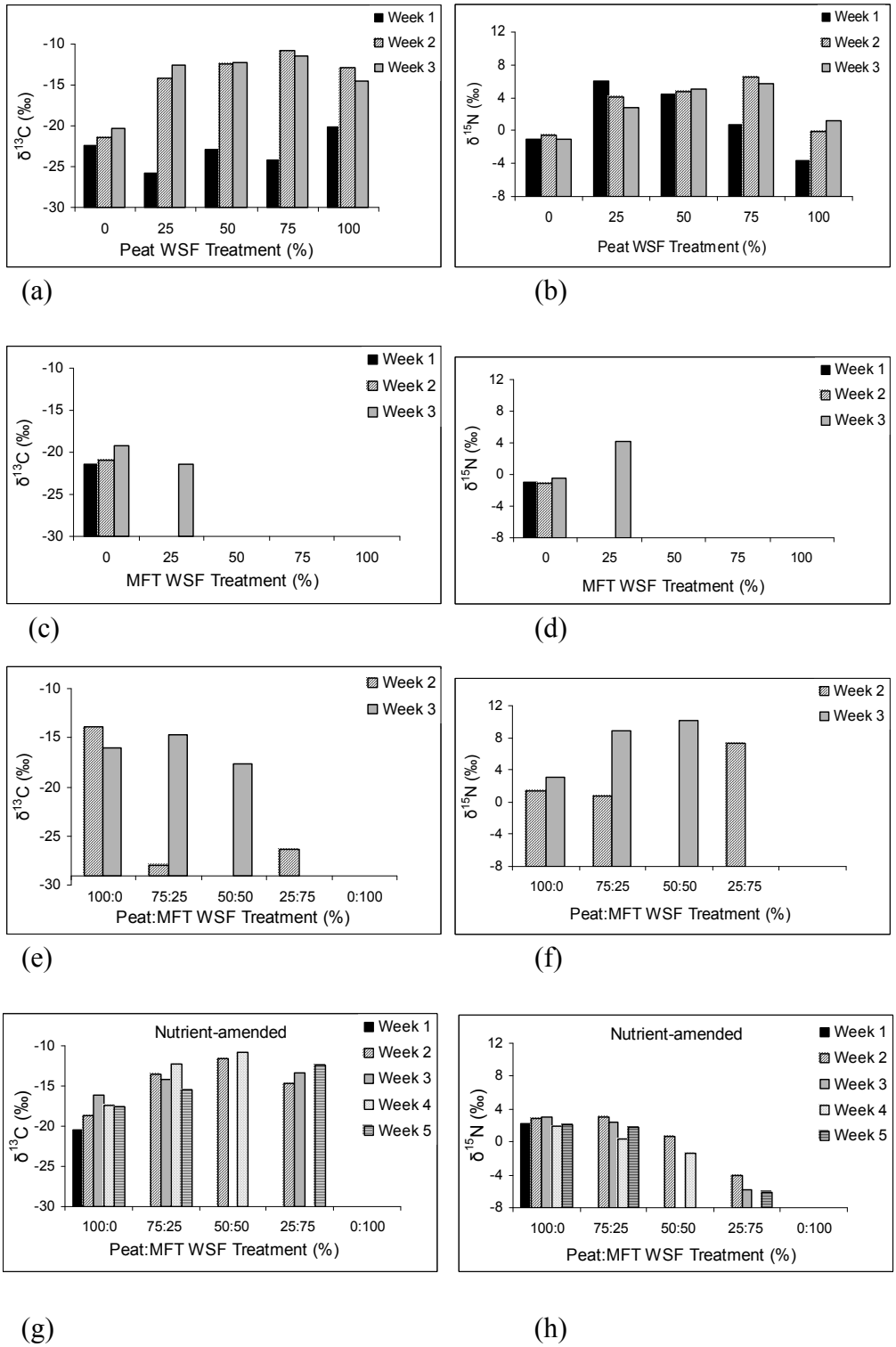


Figure 3. 6 Stable C and N isotopes of filamentous algae in WSF peat (a,b), WSF MFT (c,d), Peat:MFT mixture (e,f) (Experiment 2), and nutrient-amended peat:MFT mixture (g,h) (Experiment 3).

Table 3. 6 Mean ±SE for $\delta^{13}C$ of DOC and DIC, and $\delta^{13}C$ and $\delta^{15}N$ of filamentous algae in WSFs of peat, MFT and peat:MFT mixtures (Exp. 2) and nutrient-amended peat:MFT mixtures (Exp. 3) following a 3 week exposure.

WSF Treatment (%)	$\delta^{13}C$ DOC (‰)	$\delta^{13}C$ DIC (‰)	Filamentous Algae (‰)[1]	
Peat			**$\delta^{13}C$**	**$\delta^{15}N$**
0	-24.83 ±0.1	-2.72 ±0.4	-20.90 ±0.6	-0.81 ±0.3
25	-25.12 ±0.2	-3.67 ±0.7	-13.38 ±0.9	3.42 ±0.7
50	-25.83 ±0.01	-4.63 ±1.1	-12.31 ±0.1	4.87 ±0.2
75	-25.98 ±0.1	-8.37 ±4.8	-11.15 ±0.3	6.10 ±0.4
100	-26.30 ±0.08	BDL	-13.70 ±0.8	0.58 ±0.6
MFT				
0	-24.66 ±0.01	-3.60 ±1.1	-20.09 ±0.8	-0.82 ±0.3
25	-24.91 ±0.1	-3.09 ±1.3	-21.56	4.17
50	-24.80 ±0.1	-2.67 ±0.9	BDL	BDL
75	-25.82 ±0.1	-2.50 ±0.6	BDL	BDL
100	-26.26 ±0.1	-2.52 ±0.2	BDL	BDL
Peat:MFT				
100:0	-26.38 ±0.02	-8.56 ±0.4	-15.00 ±1.1	2.24 ±0.9
75:25	-26.34	-15.17	-21.33 ±6.62	4.82 ±4.1
50:50	-26.51	-3.11	-17.67	10.09
25:75	-26.80 ±0.2	-3.56 ±2.8	-26.34	7.37
0:100	-26.68 ±0.1	-5.07 ±1.7	BDL	BDL
Peat:MFT (nutrient-amended)				
100:0	nd	nd	-17.46 ±1.3	2.93 ±0.1
75:25	nd	nd	-13.80 ±0.3	2.72 ±0.3
50:50	nd	nd	-11.62	0.66
25:75	nd	nd	-14.07 ±0.7	-4.98 ±0.9
0:100	nd	nd	BDL	BDL

[1] filamentous algae mean ±SE values were calculated as the average of weeks 2 and 3; nd, no data available; BDL, below detection limit due to limited or no biomass

Stable Carbon and Nitrogen Isotopes of Plankton

Limited isotope data were available for plankton, which includes autotrophic and heterotrophic production, due to detection limits for either C or N in these samples. Larger test volumes would be beneficial for generating sufficient weight of material for stable isotope analyses. No data are available for Exp. 1 or peat WSF treatments in Exp. 2. Plankton were ^{13}C depleted (2 ‰) in 100% MFT WSF relative to the reference/dilution water however there was no difference in $\delta^{13}C$ values of plankton for peat:MFT treatments regardless of nutrient supplementation (Experiments 2 and 3; Table 3.7 and Fig. 3.7). In experiment 2, there was ^{15}N enrichment (4 ‰) of plankton at higher percentages of WSFs for MFT only and peat:MFT WSF treatments however there was little difference in the $\delta^{15}N$ values of plankton in the nutrient-amended treatments.

Table 3. 7 Mean ±SE for $\delta^{13}C$ and $\delta^{15}N$ of TSS (including plankton) in WSFs of MFT and peat:MFT mixtures (Exp. 2) and nutrient-amended peat:MFT mixtures (Exp. 3) following a 3 week exposure.

WSF Treatment (%)	TSS (‰)[1]	
	$\delta^{13}C$	$\delta^{15}N$
MFT		
0	BDL	BDL
25	-26.62 ±0.3	5.89 ±0.2
50	-27.23 ±0.1	7.23 ±0.3
75	-27.31 ±0.07	5.89 ±0.3
100	-28.64 ±0.2	9.46 ±0.8
Peat:MFT		
100:0	BDL	BDL
75:25	-27.79 ±0.1	4.23 ±2.3
50:50	-27.79 ±0.3	5.74 ±0.6
25:75	-28.07 ±0.2	6.50 ±0.3
0:100	-28.18 ±0.01	8.82 ±0.5
Peat:MFT (nutrient-amended)		
100:0	BDL	BDL
75:25	-27.33 ±0.3	3.27 ±0.4
50:50	-27.40 ±0.7	4.18 ±0.7
25:75	-26.93 ±0.8	3.41 ±0.9
0:100	-27.25 ±0.5	4.05 ±0.8

[1]TSS mean ± SE values were calculated as the average of weeks 1, 2, and 3; BDL, below detection limit due to insufficient biomass

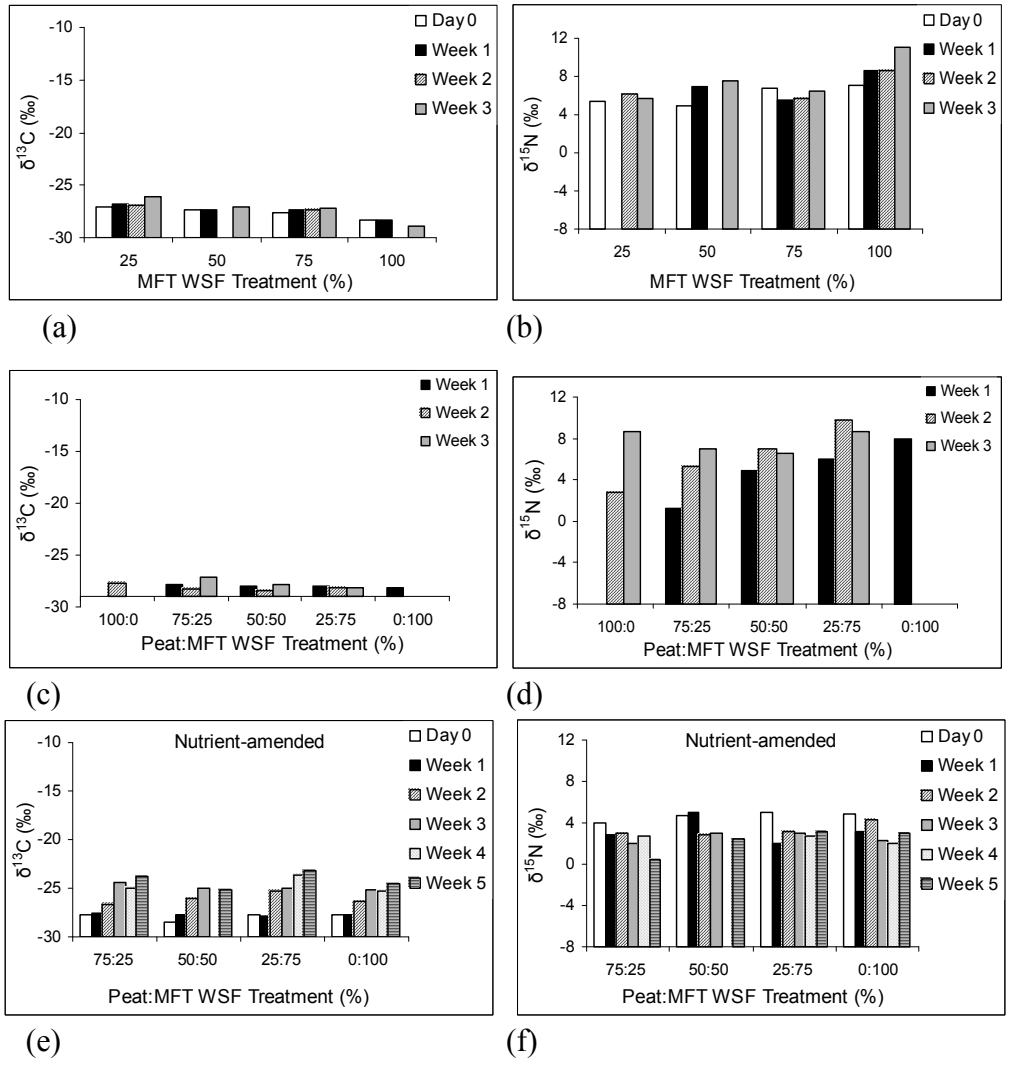


Figure 3. 7 Stable C and N isotopes of the TSS in WSF MFT (a,b), WSF peat:MFT (c,d) (Experiment 2), and nutrient-amended peat:MFT mixture (e,f) (Experiment 3).

3.5 Discussion

3.5.1 Growth of Plankton and Filamentous Algae

Plankton Growth

In all cases, the WSFs of the different sources (peat, MFT or CT) were able to stimulate greater plankton growth relative to reference/dilution water based on mean chl *a* levels (Exp. 1 and 2). In general, mean chl *a* levels increased with increasing WSF of peat or CT and had the highest mean chl *a* levels at ≥75% WSF. In contrast, the WSF of MFT promoted growth at intermediate concentrations (25% to 75% WSF) but chl *a* levels were reduced at 100% WSF of MFT, similar to chl *a* levels in the reference/dilution water. High levels of residual suspended fine clay particles in the WSF of MFT, even after centrifugation and filtration (1μm pore size), contributed to increased turbidity at higher concentrations of MFT WSFs as indicated by elevated levels of TSS at day 0 (Exp. 2). Lower day 0 TSS levels were reported for peat WSFs (Exp. 1 and 2), CT WSFs (Exp. 1) and nutrient-amended MFT WSFs (Exp. 3). The reduction in chl *a* at 100% WSF MFT is more likely a function of turbidity associated with fine clay particles in MFT and not a function of NA toxicity since Leung et al. (2001) reported elevated chl *a* levels in microcosms containing MFT settling basin water with elevated NA concentrations (>50 mg/L); NA concentrations 4x higher than reported in the present study. Chen (2011) also attributed lower algal growth in some field microcosms containing a MFT and sand mixture to increased turbidity associated with fine clay particles from MFT. Earlier studies of experimental oil sands reclamation sites have also reported high turbidity due to the use of MFT to line a reclaimed pond (Demonstration pond, Syncrude) (Gould, 2000). Turbidity was suggested to be an important factor in limiting growth of phytoplankton and macrophytes in this benthic invertebrate assessment (Gould, 2000). Turbidity will likely be a critical factor in the initial success of oil sands reclamation involving MFT; limiting phytoplankton resources and contributions to biological detritus via sedimentation. Although the fine clay particles may settle out to some degree, turbidity was still a visible issue in a MFT- constructed pond (Test Pond 7, Syncrude) 10 + years after construction (Farwell et al., 2009); thus resuspension of fine clay particles in reclamation could be a chronic issue. This is the only publication, to the best of the author's knowledge, to provide quantitative measures of the potential impact of oil sands suspended fine clay particles, contributing to turbidity, on phytoplankton community growth.

Estimates of plankton growth (chl *a*) indicated elevated growth at intermediate ratios of peat:MFT or CT which is likely a function of nutrient availability. Each WSF differed in macronutrient concentrations; WSFs of peat had higher TN and WSFs of MFT had higher TP

than the other WSFs. The combination of higher TP from MFT and higher TN from peat could explain the elevated mean chl *a* at intermediate concentrations of peat:MFT mixtures. Unfortunately due to possible light limitation caused by the co-factor, elevated MFT-associated fine clay particles (measured as TSS on day 0), it is difficult to interpret the importance of TP for plankton growth at increasing concentrations of the WSF of MFT. Leung et al. (2001) observed the highest chl *a* values in MFT-associated microcosms with TP concentrations of 183-215 µg/L relative to <70 µg/L for other microcosms and identified TP as an important factor affecting phytoplankton species composition in those nutrient-amended microcosms.

Both types of nutrient amendment (as organic or inorganic supplements) in this study enhanced phytoplankton growth in microcosms with OSPM WSFs. Other microcosm studies, using Fraquil media to resolve possible nutrient limitation, have reported high chl *a* values in microcosms containing oil sands processed water or oil sands NA extracts (Leung *et al*. 2001; Hayes, 2005). Certainly, the benefits of macronutrients from organic or inorganic amendments or from OSPM material (high TP from MFT) are evident based on the current microcosm study however the advantage is likely short-lived based on field studies of aged reclamation sites. In field assessments, phytoplankton community biomass differed among sites, but was not correlated to macronutrients (TN and TP) or NAs and major ion concentrations, parameters of interest in oil sands aquatic reclamation (Leung *et al*., 2003; Hayes, 2005). In the field component of this study (Chapter 2), phytoplankton community growth estimates (measured as chl *a*) were similar in aged OPSM wetlands relative to reference wetlands but there was a positive correlation between TN and TP concentration and phytoplankton chl *a* levels. In this microcosm study, macronutrient concentrations (both TN and TP) in the WSFs were higher and supported greater plankton chl *a* than reference and OSPM reclamation sites (Chapter 2). In some cases however, there are reclamation sites with recharge of OSPM water, as is the case with CT water recharge in Natural Wetland (NW); the renewal of macronutrients from CT seepage at this site likely explains the higher plankton chl *a* than other oil sands reclamation sites (Chapter 2).

Growth of Filamentous Algae

Filamentous algae had the highest biomass (dry weight) at low WSF levels (25%) of CT and peat, decreasing with increasing percentage WSF. Similarly, growth of filamentous algae decreased with increasing percentage WSF of MFT and in some cases, there was minimal or no growth at 100% WSF MFT. Decreased growth of filamentous algae is likely due to light limitation associated with increased plankton growth and/or suspended clay particles in the WSFs. Although there are no data available on the toxicity of OSPM WSF on filamentous algae, the

trend of decreasing growth at higher WSFs including peat WSFs indicated that toxicity is likely not the primary limiting factor. The strong negative correlation between dry weight and TP suggests that TP plays a role in limiting the growth of filamentous algae however this interpretation is complicated by the presence of TSS as both parameters increase at higher WSFs, particularly for MFT. A study by Mullineaux (1993) showed that light-state transitions in cyanobacteria species were inhibited by 0.2-0.4 M phosphate. Another study on benthic microalgae showed that the saturation threshold for phosphorus effects was 25 µg/L of soluble reactive phosphorus (SRP) (Hill and Fanta, 2009).

Although there are numerous studies on phytoplankton (Leung *et al*., 2003; Hayes, 2005) and macrophyte growth (references in Kovalenko *et al*., 2013) in oil sands reclamation, there is little information on filamentous algae. Daly (2007) documented the presence of filamentous algae in a CT-constructed wetland (e.g. 4 m-CT, Suncor lease), the same location as the source of CT used in the current study; however filamentous algae were not reported for other reference or OSPM sites in Daly`s (2007) study. The presence of filamentous algae at this CT-field site only (Daly 2007) is consistent with the higher (> 3 times) filamentous algae dry weight estimates for 100% WSF of CT relative to 100% WSFs of either peat or MFT in the current study. The growth of filamentous algae in the present study and reports of filamentous algae in nutrient-amended field microcosms (Chen, 2011) suggests the need to consider filamentous algae growth in future studies that assess peat-derived or inorganic nutrient amendments as reclamation strategies. Proliferation of filamentous algae is the least favorable result in terms of enhancing primary production in oil sands reclamation, particularly in end pit lakes, since it is not a valued food resource for either pelagic or benthic secondary production, it results in increased competition for nutrients, and if biomass accumulation is significant, oxygen-consuming decomposition of filamentous algae could contribute to reduced dissolved oxygen levels critical for fish survival.

3.5.2 Stable Isotopes of Plankton and Filamentous Algae

Nitrogen Stable Isotopes

In general, the WSFs of the different sources (peat, MFT or CT) resulted in ^{15}N enrichment of filamentous algae. There was also ^{15}N enrichment of plankton in MFT WSF only; unfortunately, isotope data were limited for plankton in some tests (peat and CT) due to the low quantities available for isotope analyses. In general, ^{15}N enrichment was more pronounced for filamentous algae than plankton for treatments that had both data sets available. The $\delta^{15}N$ variability of algae may be due to a number of factors including the $\delta^{15}N$ values of N sources and

species that are influenced by isotope fractionation associated with N processes (volatilization of NH_3) and rate of growth.

Plankton or filamentous algae tended to be more ^{15}N enriched at higher percentages of MFT or CT compared to peat. The differences in the $\delta^{15}N$ of algae in OSPM WSFs vs peat WSFs could be a function of the sources and/or N species of the substrates used to generate WSFs. The stable N isotope composition of the substrates used to create the WSFs varied from low $\delta^{15}N$ for peat (0.1 ‰) to higher $\delta^{15}N$ for MFT (3.6 ‰) and CT (6.8 ‰). Both MFT and CT contained process-added NH_4^+ from bitumen upgrading and raw sewage that were discharged into the settling basin (Farwell *et al*., 2009). Under alkaline conditions in the settling basin, NH_4^+ is converted to NH_3 and NH_3 volatilization is possible:

(high pH, CT and MFT) $\mathbf{H_3O^+ + NH_3 \rightleftharpoons H_2O + NH_4^+}$ ***(low pH, peat)***

Volatilization of NH_3 results in significant isotope fractionation (Heaton, 1986; Macko and Ostrom, 1994), leading to ^{15}N enriched NH_3 for conversion to $NO_2 + NO_3$ or uptake. Volatilization of NH_3 was thought to influence the ^{15}N enrichment of NH_3 from sewage (Wayland and Hobson, 2001, Jordan *et al*., 1997). The ^{15}N enrichment of plants (Jones *et al*., 2001) and algae (Wayland and Hobson, 2001) has been observed in association with elevated nutrients downstream of sewage discharge and in sewage treatment ponds, respectively. Isotope fractionation associated with the volatilization of NH_3, producing ^{15}N enriched N species for uptake by algae and the low NH_4^+ concentrations of WSFs of CT (indicative of pH dependent conversion to NH_3 and subsequent NH_3 volatilization) relative to peat, could explain the ^{15}N enriched algae in the WSFs of CT compared to peat. Although no data are available for NH_4^+ concentrations of MFT, a similar explanation is possible. Thus, the $\delta^{15}N$ of algae is lower at higher NH_4^+ concentrations (e.g. WSF of peat). Similarly, Gu *et al.* (1993) found that the $\delta^{15}N$ of plankton was inversely related to NH_4^+ concentration. Also, the higher $\delta^{15}N$ values of the substrate and algae associated with CT compared to MFT could be a function of the process used to create CT. The mixing of MFT and gypsum (Ca_2SO_4) to create CT, resulting in increased pH (pH is higher in WSFs of CT vs. MFT) and aeration, could further increase conversion of NH_4^+ to NH_3 and volatilization of NH_3 in CT substrates causing higher $\delta^{15}N$ values of the CT substrate and algae grown in WSFs of CT. Isotope analyses of N species (NH_3, NH_4^+, $NO_2 + NO_3$) in addition to concentrations are needed to further our understanding on N isotopes in oil sands reclamation.

Differences in the $\delta^{15}N$ values of both plankton and filamentous algae from nutrient-amended (Exp. 3) compared to non-amended (Exp.2) microcosms may reflect differences in the

N source of the nutrient media. The nutrient-amended treatments (Exp.3) had lower $\delta^{15}N$ values of 3.3 to 4.2‰ for plankton and -5.0 to 2.9 ‰ for filamentous algae compared to 4.2 to 8.8 ‰ for plankton and 2.2 to 10.1 ‰ for filamentous algae in similar peat:MFT treatments without Fraquil medium (Exp. 2). Both plankton and filamentous algae also had trends of ^{14}N depletion over time in nutrient-amended treatments yet often there were trends of ^{15}N enrichment over time in non nutrient-amended treatments. This difference could be a function of incorporation of ^{14}N depleted N species from this prepared nutrient medium.

While the source and species of N in the study are likely important factors affecting the $\delta^{15}N$ of filamentous algae for different sources of WSF treatments, there is some evidence to suggest that differences in the rate of growth may also influence the $\delta^{15}N$ of algae particularly at intermediate vs. low or high percentages of WSFs. For example, in the non-amended treatments, filamentous algae biomass and $\delta^{15}N$ values were higher at intermediate percentages of the WSFs of peat (Exp. 2, week 3) but at 0% and 100% WSF, filamentous algae were ^{14}N depleted and had lower biomass. In general, algae favour the uptake of the lighter isotope (^{14}N) of nitrogen species from water (Pennock *et al*., 1987; Cifuentes *et al*., 1989). Under conditions of low growth, the preferential uptake of ^{14}N of NH_3 or NO_3 would result in ^{15}N depletion of algae, yet under optimal growth conditions the demand for N is so great that algae uptake both ^{14}N and ^{15}N indiscriminately, resulting in ^{15}N enriched biomass. Gu *et al.* (1996) found that the $\delta^{15}N$ of plankton increased from oligotrophic lakes to eutrophic lakes as a function of primary productivity. In the current study, greater growth of filamentous algae at intermediate WSF percentages may explain the higher $\delta^{15}N$ values of filamentous algae relative to other WSF percentages in peat treatments (Exp.2). The interpretation of $\delta^{15}N$ trends for TSS is more complicated due to the presence of both bacterial and photosynthetic biomass. Videla *et al*. (2009) found ^{15}N enrichment of bacteria grown on oil sands NAs supplemented with a renewed source of mineral medium (NH_4CI).

This microcosm study demonstrated that both the source (e.g. peat, MFT or CT) and percentage of WSF will influence the $\delta^{15}N$ of filamentous algae. The trends of ^{15}N enrichment of filamentous algae and plankton grown in WSFs of OSPM (CT and MFT) vs. peat in this microcosm study are consistent with trends for biota collected from OSPM and reference field sites. The $\delta^{15}N$ values of microbial biofilm (OSPM, 7.3‰; reference, -1.9‰; Daly, 2007), plankton (OSPM, 9.7‰; reference, 1.3‰; Chapter 2) and plants (OSPM, 15.9‰; reference, 6.2‰; Daly, 2007) are consistent with the utilization of ^{15}N enriched N species associated with OSPM, particularly CT. The ^{15}N enriched primary production is consumed, resulted in $\delta^{15}N$ enriched

benthic invertebrates; highest $\delta^{15}N$ values for invertebrates at CT sites followed by MFT sites (Farwell *et al*., 2009). Isotope analyses of N species (NH_4^+, NH_3, as well as $NO_2 + NO_3$) are needed to further our understanding on N isotopes in oil sands reclamation and to use N isotopes of N species as indicators of oil sands processed water seepage in regional studies.

Carbon Stable Isotopes

In general, the WSFs of peat and CT resulted in slight ^{13}C enrichment of filamentous algae compared to the reference/dilution water. Filamentous algae were more ^{13}C enriched in 100% WSF of CT vs. peat in peat:CT WSF treatments (Exp. 1) but were more ^{13}C depleted in higher WSF percentages of MFT vs. 100% peat WSF in peat:MFT WSF treatments, although highly variable (Exp. 2). The ^{13}C depletion at higher percentages of MFT WSFs is consistent with trends of ^{13}C depletion in benthic invertebrates in oil sands reclamation along an increasing gradient of oil sands tailings water and MFT (Farwell *et al*., 2009). In other studies, macrophytes from references sites had $\delta^{13}C$ values in the range of -27.7 to -23.3‰ while OSPM sites had slightly enriched ^{13}C values in the range of -26.1 to -18.2‰ (Daly, 2007). Elshayeb (2006) found no differences in macrophytes, specifically *Typha latifolia* (-28.8 to -28.5‰), at sites categorized by low (0-4 mg/L) to high (>15 mg/L) NA concentrations.

For the plankton data that are available, plankton had lower $\delta^{13}C$ values than filamentous algae. Ventura *et al*. (2008) found seston had the lowest $\delta^{13}C$ relative to epiphytes, sediment biofilm and macrophytes. In general, there was little difference between the $\delta^{13}C$ values of plankton in peat:MFT WSF treatments (Exp. 2 and 3) suggesting that the $\delta^{13}C$ values of the C sources from peat or MFT were similar. The $\delta^{13}C$ values of microbial biofilm (OSPM, -26.2‰; reference, -29.5‰; Daly, 2007) and plankton (OSPM, -26.7‰; reference, -29.1‰; Chapter 2) showed trends of ^{13}C enrichment at OSPM sites relative to reference sites. The lack of distinct $\delta^{13}C$ values for plankton samples among WSFs from OSPM vs. natural muskeg overburden sources limits the use of stable C isotopes for tracing C sources in oil sands reclamation.

3.6 Conclusions

Phytoplankton growth (chl *a*) was highest in treatments with a 50:50 ratio of peat:CT or peat:MFT compared to 100% WSFs of peat, CT or MFT. The results suggest that the addition of peat as an amendment to OSPM (particularly MFT), contributes additional TN that could improve phytoplankton community growth in oil sands reclamation. Reduced chl *a* at higher percentages

of CT and MFT was likely due to increased turbidity associated with fine clay particles from tailings. The addition of nutrient medium (Fraquil) to peat:MFT WSFs provided macro- and micronutrients for greater phytoplankton growth at intermediate ratios of peat:MFT yet at 100% MFT, chl *a* levels were still reduced. The reduction of chl *a* associated with fine clay particles, regardless of nutrient levels, suggests that turbidity may be an important factor influencing phytoplankton growth in end pit lakes constructed with MFT. The WSFs also promoted the unfavourable growth of filamentous algae, highest at intermediate concentrations of peat and CT WSF treatments yet limited in MFT WSF treatments, likely due to increased turbidity from fine clay and biological growth. Such growth is considered unfavourable because filamentous algae are relatively poor food sources for secondary producers. Trends in stable isotopes of plankton and filamentous algae suggested that ^{15}N enrichment of biota could be a good indicator of nutrient-enrichment and may be useful for tracing nutrient inputs from OSPM sources in oil sands monitoring programs for the Athabasca River and tributaries. However, $\delta^{13}C$ values were similar among WSFs, limiting their use for tracing different C sources in oil sands reclamation.

Chapter 4 General Discussion and Conclusions

4.1 Impacts of OSPM on Phytoplankton and Periphyton Community Growth

Chl a and Biomass Estimates

Based on this study and other field and microcosm studies (Chen, 2011; Frederick, 2011; Leung *et al*., 2001 and 2003; Hayes, 2005), there is no indication that the chemical composition of OSPM negatively impact phytoplankton and periphyton community growth based on chl *a*. In fact some studies have reported high chl *a* values associated with elevated levels of NAs and conductivity in water from a settling basin (Leung *et al*., 2001) and in NA extract studies (Hayes, 2005). A stimulatory effect of *in vivo* fluorescence of chl *a* at high NA concentrations (24 - 50 mg/L) was thought to be due to either the utilization of carbon in the NA extract by tolerant taxa or a physiological increase in the fluorescence yield of chl *a* induced by NAs (Hayes, 2005). In the current microcosm study (Chapter 3), while chl *a* increased at intermediate percentages of OSPM, the concentrations of NAs were relatively low (<12 mg/L) compared to NA concentrations associated with stimulatory effects reported by Hayes (2005). Further study is required to better understand the potential NA extract-induced stimulation, as indicated by chl *a* (Hayes, 2005) at elevated NA concentration to properly interpret both field and laboratory data used to monitor and evaluate oil sands remediation and reclamation strategies. If, in fact, tolerant phytoplankton species are able to utilize carbon in the NA extract as suggested by Hayes (2005), this would benefit current aquatic reclamation strategies. Unfortunately, since the $\delta^{13}C$ values of DOC from the different sources of oil sands reclamation material (peat, MFT and CT; Chapter 3) were similar, there is no way to differentiate potential NA-derived carbon utilization by phytoplankton using stable C isotopes.

This study and other field and microcosm studies (Leung *et al*., 2001 and 2003; Hayes, 2005) have suggested factors that may affect phytoplankton biomass. In general, all the field studies of standing stocks of both phytoplankton and periphyton are difficult to interpret due to potential losses associated with grazing (Chen, 2011; Frederick, 2011; Leung *et al*., 2003; Hayes, 2005). The implications of differing algal species composition in oil sands reclamation in terms of selective feeding by grazers also remains unknown. However, crustacean and rotifer zooplankton biomass estimates were found to be reduced at NA concentrations greater than 20 mg/L in a microcosm study (McCormick, 2000). Thus grazing pressure may be higher in reference and OSPM sites with lower NA concentration (<20 mg/L). Unknown grazing rates

complicate the interpretation of environmental variables (chemical and/or physical factors) of concern in oil sands reclamation.

One of the problems with interpreting environmental data and identifying factors that may positively or negatively influence phytoplankton chl *a* or biomass is that many of these environmental factors are co-variables (e.g. NA concentration and conductivity; turbidity and TP in MFT reclamation). The goal here is to briefly summarize the factors based on current knowledge:

1) Turbidity associated with fine clay from OSPM:

Numerous field studies have observed turbidity issues in oil sands reclamation (Gould, 2000; Farwell et al., 2009; Chen, 2011) and suggested turbidity as a possible negative impact on phytoplankton growth (Hayes, 2005), and yet water clarity/transparency is infrequently documented. In the current laboratory study (Chapter 3), day 0 TSS was used as an indicator of turbidity associated with fine clay in OSPM, unfortunately TSS is a co-variable with TP (TSS was positively correlated to TP, r^2 =0.56). At low day 0 TSS (<100 mg/L) in WSFs of peat and CT, chl *a* increased with TP (r^2 =0.63) and there was no significant correlation with TSS (r^2 =0.22). For WSFs of MFT, chl *a* increased at lower percentages of MFT WSF and decreased at higher percentages. Changes in chl *a* were thought to be driven by TP at lower WSFs and turbidity at higher WSFs of MFT. The major limitation of using chl *a* to estimate phytoplankton community growth is that phytoplankton compensate for light limitation by increasing the chl *a* concentration in the cell (Wassink, 1959). The threshold of possible phytoplankton compensation (resulting in elevated chl *a* production) associated with low light due to clay particles from MFT and CT WSFs or high dissolved humic acids in WSFs of peat requires further study. Calculating biomass based on cell numbers per species and cell dimensions of each species as described in Leung *et al.* (2003) could improve our understanding of the impacts of turbidity on phytoplankton growth.

Regardless of possible low light-induced chl *a* increases in lower WSFs of MFT, high turbidity (>400 mg/L day 0 TSS) in 100% WSF MFT was likely the cause of lower chl *a* indicating reduced phytoplankton community growth (Chapter 3). Turbidity will likely be a critical factor in the initial success of oil sands reclamation involving MFT by limiting phytoplankton growth and thus resources for secondary production and also limiting biological detritus via sedimentation. Wind events and shoreline erosion causing resuspension of fine clay particles could be a long-term issue.

2) TN, TP, major ions or NA concentration:

Phytoplankton biomass was not correlated to TN, TP, major ions or NA concentration based on an oil sand reclamation field survey (Leung *et al*., 2003). Phytoplankton species of Cyanobacteria (nitrogen-fixing species) were dominant in some reference systems with low NA concentration and conductivity while Chlorophyta were dominant in systems with high NA concentration and conductivity (Leung *et al*., 2003). In that case, both Cyanophyta and Chlorophyta dominated sites had the highest phytoplankton biomass reported (Leung *et al*., 2003). The lowest reported TN levels (≤ 1.65 mg/L) at some reference sites could explain the dominance of nitrogen-fixing species (Cyanophyta) and the high phytoplankton biomass at two of the study sites. This could in part explain the lack of correlation between nutrients (TN) and phytoplankton biomass in Leung *et al*. (2003).

The challenge with some of the microcosm studies on phytoplankton community composition (Leung *et al*., 2001; Hayes, 2005) is that nutrient medium was added to create meso-eutrophic conditions to eliminate nutrient-limitation as a variable in order to examine the impacts of NA concentration and salinity. However, by adding nutrients, species composition could be altered, particularly the abundance of nitrogen-fixing species (Cyanophyta). To improve the understanding of the factors influencing phytoplankton growth in different oil sands reclamation strategies, microcosm studies without nutrient medium should examine phytoplankton community growth along gradients of environmental factors such as turbidity, TN, TP, major ions or NA concentration and use measured phytoplankton biomass (Leung *et al*., 2003) to estimate phytoplankton community growth.

Several studies have examined differences in species composition of phytoplankton associated with OSPM and NA extracts (Leung *et al*., 2001 and 2003; Hayes, 2005) however there are no studies that have examined the species composition of the complex consortium of attached and plankton algal species colonizing substrates in oil sands reclamation. Changes in phytoplankton species composition were correlated to NAs and conductivity (Leung *et al*., 2001 and 2003; Hayes, 2005). However, the impact associated with changes in phytoplankton species composition on zooplankton biomass and composition remains unknown.

4.2 Impacts of Peat Amendment on Phytoplankton and Periphyton Community Growth

Aquatic reclamation of oil sands waste has been a subject of much study over the last 15-20 years but it is only recently that studies have closely examined the use of amendments to accelerate biological productivity and colonization in wetland or end pit lake reclamation (Kolavenko *et al*., 2013). Increased microbial biomass from peat-amended OSPM sites vs. reference wetlands (Daly,

2007), increased phytoplankton and periphyton chl *a* in peat-amended MFT microcosms (Chen, 2011), and increased phytoplankton chl *a* in WSFs of peat:CT and peat:MFT (Chapter 3) suggest enhanced heterotrophic and autotrophic growth. Peat amendments also improved development of emergent plant communities, in contrast, submerged macrophyte biomass remained lower in OSPM sites regardless of peat-amendment (Kolavenko *et al*., submitted). This reduced submerged macrophyte biomass could be a function of turbidity as observed for reduced filamentous algae biomass (Chapter 3).

The results of the study of peat, CT and MFT WSF showed maximum phytoplankton community growth (measured as chl *a*) when WSFs of peat were combined with either CT or MFT. In general, WSFs of peat had the highest concentration of total nitrogen (TN) which suggests the benefit of N from peat to enhance phytoplankton community growth in MFT WSFs that have elevated TP concentration. Regardless of the potential carbon or nutrient benefits of peat amendments which could function to enhance phytoplankton and periphyton heterotrophic and autotrophic biomass in the short term, the use of peat to assist in stabilizing sediments (reducing resuspension of clay) could have significant value in terms of long term sustainability. The use of amendments to enhance phytoplankton growth in larger reclamation projects (e.g. end pit lakes) may be important for the initial development of both pelagic and benthic food webs in new oil sands reclamation.

4.3 Applications of Stable Carbon and Nitrogen Isotopes in Oil Sands Reclamation

The WSF experiment provided a better understanding of growth related changes in stable isotopes. $\delta^{13}C$ values of filamentous algae were more ^{13}C enriched with increased growth and more ^{13}C depleted with reduced growth for algae under light limitation (based on filamentous algae). The use of stable C isotopes to trace C from oil sands sources is limited given that the $\delta^{13}C$ of DOC and DIC are similar for OSPM and reference sites. However, as more information is provided on the isotope values of components of these aquatic food webs, it may be possible to better understand C flow via hetertrophic vs autotrophic resources within a given wetland.

Stable N isotopes of plankton and periphyton in the field survey (Chapter 2) and plankton and filamentous algae in the WSF microcosm study (Chapter 3) indicated greater ^{15}N enriched algae in OSPM. The findings suggested that both natural organic matter (e.g. peat) or OSPM could result in ^{15}N enriched algae but that OSPM sources showed greater ^{15}N enrichment of algae, likely due to the isotope fractionation associated with NH_3 volatilization in settling basins and later uptake of ^{15}N enriched NH_3 or $NO_2 + NO_3$ by algae. Isotope analyses of N species (NH_4^+,

NH_3, as well as $NO_2 + NO_3$) are needed to further our understanding on N isotopes in oil sands reclamation and to use N isotopes of N species as indicators of oil sands process seepage in regional studies.

Based on the current knowledge of stable N isotope trends from laboratory and field studies of OSPM, stable N isotopes may be a useful tool to trace the level of exposure to OSPM. For environmental assessments of oil sands reclamation strategies, the use of N isotopes of tissues of amphibians, and aquatic or terrestrial birds and mammals inhabiting OSPM wetland areas would provide valuable information on exposure to OSPM. In this case, the degree of exposure to OSPM is a function of the N isotope values of animal tissues and the dietary items consumed from OSPM reclamation. Animals with more ^{15}N enriched tissues indicate greater exposure to OSPM.

Recommendations for Future Research

Based on the current state of knowledge, the following recommendations are made for future oil sands reclamation research on phytoplankton and periphyton.

1) Use of chl *a* to estimate phytoplankton community growth:

Further study is required to better understand the potential NA extract-induced physiological stimulation of chl *a* as suggested Hayes (2005). If there is physiological-based increases of chl *a* in the presence of elevated NA concentration then the use of chl *a* is limited as a measure of phytoplankton and periphyton community growth in field and laboratory studies to monitor and evaluate oil sands remediation and reclamation strategies.

Also study is required to better understand the effects of clay particles on chl *a* concentrations in phytoplankton and periphyton. The use of chl *a* is a time and cost effective method to estimate phytoplankton community growth however it will have limited use in monitoring and evaluations of oil sands remediation and reclamation strategies if clay particles from OSPM significantly increase chl *a* as a compensation mechanism for limited light.

2) Use of phytoplankton biomass to measure phytoplankton community growth:

The use of phytoplankton biomass (biomass calculated based on cell numbers per species and cell dimensions of each species as described in Leung *et al.* 2003) vs. chl *a* or TSS could improve our understanding of the impacts of environmental factors such as turbidity, TN, TP, NAs and conductivity on phytoplankton community growth, although this method is more costly and time consuming. Microcosm studies without nutrient medium should examine phytoplankton

community growth along gradients of environmental factors such as turbidity, TN, TP, major ions or NA concentration and use measured estimates of phytoplankton biomass (Leung *et al*., 2003) to estimate phytoplankton community growth.

References

Alberta Environment, 2012

Azim, M.E., Verdegem, M.C.J., Mantingh, I., van Dam, A.A., and Beveridge, M.C.M. 2003. Ingeston and utilization of periphyton grown on artificial substrates by Nile tilapia, Oreochromis niloticus L. Aquacult. Res. 34, 85-92.

Chen, H. 2010. The effects of nutrient and peat amendments on oil sands reclamation wetlands: a microcosm study. MSc. Thesis. University of Waterloo, Waterloo, Ontario, Canada.

Cifeuentes, L.A., Fogel, M.L., Pennock, J.R., and Sharp, J.H. 1989. Biogeochemical factors that influence the stable nitrogen isotope ratio of dissolved ammonium in the Delaware Estuary. Geochim. Cosmochim. Acta. 53, 2713-2721.

Clemente, J., and Fedorak, P.M. 2004. Evaluation of the analyses of *tert*-butyldimethylsilyl derivatives of naphthenic acids by gas chromatography-electron impact mass spectrometry. J. Chromatography A 1047, 117-128.

Coffin, R.B., Fry, B., Peterson, B.J. and Wright, R.T. 1989. Carbon isotopic composition of estuarine bacteria. Limnol. Oceanogr. 34, 1305-1310.

Colavecchia, M.V., Backins, S.M., Hodson, P.V., and Parrott, J.L. 2004. Toxicity of oil sands to early life stages of fathead minnow (*Pimephales promelas*). Environ. Toxicol. and Chem. 23, 1709-1718.

Daly, C. 2007. Carbon sources, microbial community production, and respiration in constructed wetlands of the Alberta, Canada oil sands mining area. MSc. Thesis, University of Windsor, Windsor, Ontario, Canada.

Doi, H., Kikuchi, E., Shikano, S. and Takagi, S. 2004. A study of the nitrogen stable isotope dynamics of phytoplankton in a simple natural ecosystem. Aquat. Microb. Ecol. 36, 285-291.

Elshayeb M. 2006. Determining food web impacts on experimental aquatic systems from the disposal of oil sands process-affected waste material. M.Sc. Thesis. University of Waterloo, Waterloo, Ontario, Canada.

Evans, J.C. and Prepas, E.E. 1996. Potential effects of climate change on ion chemistry and phytoplankton communities in prairie saline lakes. Limnol. Oceanogr. 41, 1063-1076.

Farwell, A.J., Nero, V., Ganshorn, K., Leonhardt, C., Cibrowski, J., Mackinnon, M., and Dixon, D.G. 2009a. The use of stable isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) to trace exposure to oil sands processed material in the Alberta oil sands region. Journ. Toxic. Environ. Health. 72, 385-396.

Farwell, A.J., Parrott, J.L., Sherry, J.P., Tetreault, G.R., Van Meer, T., and Dixon, D.G. 2009b. Use of stable carbon and nitrogen isotopes to trace natural and anthropogenic inputs into riverine systems in the Athabasca oil sands region.

Fine Tailings Fundamentals Consortium (FTFC), 1995. Volume I: Clark hot water extraction: fine tailings. Hamza, H. Chapter 1. Alberta Department of Energy, Oil sands and research division. Advances in oil sands tailings research.

Fogel, M.L., Cifuentes, L.A., Velinsky, D.J., and Sharp, J.H. 1992. Relationship of carbon availability in estuarine phytoplankton to isotopic composition. Mar. Ecol. Ser. 82, 291-300.

Frederick, K. 2011. Productivity and carbon accumulation potential of transferred biofilms in reclaimed oil sands-affected wetlands. MSc. Thesis. University of Alberta, Edmonton, Alberta, Canada.

Ganshorn, K.E., 2002. Secondary production, trophic position, and potential for accumulation of polycyclic aromatic hydrocarbons in predatory Diptera in four wetlands of the Athabasca oil sands, Alberta Canada. M.Sc. Thesis. University of Windsor, Windsor, ON, Canada.

Golder Associates Ltd. 2002. Wetland study sites associated with oil sands research and monitoring. Prepared for CONRAD environmental research. Calgary, Alta. Canada.

Goldsborough, G. 1991. A comparison of periphytic algal biomass and community structure on *Scirpus Validus* and on a morphologically similar artificial substratum. J. Phycol. 27, 196-206.

Gould, R.L. 2000. The effects of oil sands mine tailings in a constructed pond on the benthic invertebrate community structure and diet of yellow perch (*Perca flavescens*). MSc. Thesis, University of Waterloo, Waterloo, Ontario.

Gu, B.H., Schell, D.M., Alexander, V. 1994. Stable carbon and nitrogen isotopic analysis of the plankton food-web in a sub-arctic lake. Can. J. Fish. Aquat. Sci. 51, 1338-1344.

Gu, B.H., Schelske, C.L., and Brenner, M. 1996. Relationship between sediment and plankton isotope ratios (delta C-13 and delta N-15) and primary productivity in Florida lakes. Can. J. Fish. Aquat. Sci. 53, 875-883.

Hayes, T.M.E., 2005. Examining the ecological effects of naphthenic acids and major ions on phytoplankton in the Athabasca oil sands region. Ph. D. thesis, University of Waterloo, Waterloo, Ontario, Canada.

Headley. J.V., Akre, C., Conly, M., Peru, K. M., Dickson, L.C., 2001. Preliminary characterization and source assessment of PAHs in tributary sediments of the Athabasca River, Canada. Environ. Forensics 2, 335-345.

Heaton, T.H.E. 1986 Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere – a review. Chem. Geol. 59, 87-102.

Hecky, R.E. and Hesslein, R.H. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. J.N. Am. Benthol. Soc. 14, 631-653.

Herman, D.C., Fedorak, P.M., MacKinnon, M.D. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. Can. J. Microbiol. 40, 467-477.

Hill, W.R. and Fanta, S.E. 2009. Quantifying phosphorus and light effects in stream algae. Limnol. Oceanogr. 54, 368-380.

Holowenko, F.M, MacKinnon, M.D., Fedorak, P.M. 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. Water Res. 36, 2843-2855.

Jivraj, M.N., Mackinnon, M.D., Fung, B. 1995. Naphthenic acid extraction and quantitative analysis with FTIR spectroscopy. Technical report. Prepared for Syncrude Research Centre, Edmonton, Alberta, Canada.

Jordan, T. E., Correll, D. L., and D. E. Weller. 1997. Nonpoint source discharges of nutrients from Piedmont watersheds of Chesapeake Bay. J. Am. Water Resour. Assoc. 33, 631–645.

Kolavenko, K.E., Ciborowski, J.J.H., Daly, C., Dixon, D.G., Farwell, A.J., Foote, A.L., Frederick, K.R., Gardner Costa, J.M., Kennedy, K., Liber, K., Roy, M.C., Slama, C.A., and Smits, J.E.G. Submitted. Carbon flow and food web structure in oil sands reclaimed wetlands.

Leonhardt C.L. 2003. Zoobenthic succession in constructed wetlands of the Fort McMurray oil sands region: developing a measure of zoobenthic recovery. MSc. Thesis. University of Windsor, Windsor, Ontario, Canada.

Leung, S.S., Mackinnon, M.D., and Smith, R.E.H., 2001. Aquatic reclamation in the Athabasca, Canada, oil sands: naphthenate and salt effects on phytoplankton communities. Environ. Toxicol. Chem. 20, 1532-1543.

Leung, S.S., Mackinnon, M.D., and Smith, R.E.H., 2003. The ecological effects of naphthenic acids and salts on phytoplankton from the Athabasca oil sands region. Aquatic Toxicol. 62, 11-26.

Macko, S.A. and Ostrom, N.E. 1994. Pollution studies using stable isotopes. Stable isotopes in Ecology and Environmental Science. London: Blackwell. pp. 45-62.

Madill, B.G. Robert, E.A. Brownlee, P., Josephy, D., Bunce, N.J., 1999. Comparison of the Ames S*almonella* assay and Mutatox genotoxicity assay for assessing the mutagenicity of polycyclic aromatic compounds in pore water from Athabasca oil sands mature fine tailings. Environ. Sci. Technol. 33, 2510-2516.

McCormick, J.K., 2000. The effects of oil sands tailings on zooplankton communities in Northern Alberta. MSc. Thesis, University of Waterloo, Waterloo, Ontario.

Minigawa, M., and Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between ^{15}N and animal age. Geochim. Cosmochim. Acta 48, 1135-1140.

Mollard, F.P.O., Roy, M.C., Frederick, K., and Foote, L. 2012. Growth of the dominant macrophyte *Carex aquatilis* is inhibited in oil sands affected wetlands in Northern Alberta, Canada. Ecolog. Eng. 38, 11-19.

Morel, F. M. M., Westall, J. C., Reuter, J.G., and Chaplick, J. P. 1975. Description of the algal growth media “Aquil” and “Fraquil.” Technical Report 16. Water Quality Laboratory, Ralph

Parsons Laboratory for Water Resources and Hydrodynamics, Massachusetts Institute of Technology, Cambridge, 33 p.

Mullineaux, C.W. 1993. Inhibition by phosphate of light-state transitions in cyanobacerial cells. Photosynth. Res. 38, 135-140.

Murchie, K.J., and Power, M. 2004. Growth- and feeding-related isotopic dilution and enrichment patterns in young-of-the-year yellow perch (*Perca flavescens*). Fresh. Biol. 49, 41-54.

Natural Resources Canada, CanmetENERGY, 2010.

Pennock, J.R. 1988. Temporal and spatial variability in phytoplankton ammonium and nitrate uptake in the Delaware estuary. Estuar. Coast. Shelf Sci. 24, 841-857.

Pennock, D.J., Zebarth, B.J., De Jong, E. 1987. Landform classification and soil distribution in hummocky terrain, Saskatchewan, Canada. Geoderma 40, 297-315.

Peterson, B.J., and Fry. B., 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. 8, 293-320.

US Energy information agency, 2005. http://www.infoplease.com/ipa/A0872964.html. Oil & Gas Journal, Vol. 103, No. 47.

Van den Heuvel, M.R., Power, M., MacKinnon, M., and Dixon, D.G. 1999. Effects of oil sands related aquatic reclamation on yellow perch (*Perca flavescens*). II. Chemical and biochemical indicators of exposure to oil sands related waters. Can. J. Fish. Aquat. Sci. 56, 1226-1233.

Velinsky, D.J. and Fogel, M.L. 1999. Cycling of dissolved and particulate nitrogen and carbon in the Framvaren Fjord. Norway: isotopic variations. Mar. Chem. 67, 161-180.

Videla, P.P. 2007. Examining oil sands dissolved carbon and microbial degradation using stable isotope analysis. MSc. Thesis. University of Waterloo, Waterloo, ON, Canada.

Videla, P., Farwell, A.J., Butler, B.J., and Dixon, D.G. 2009. Examining the microbial degradation of naphthenic acids using stable isotope analysis of carbon and nitrogen. Water Air Soil Pollut. 197, 107-119.

Wassink, E. D. 1959. Efficiency of light energy conversion on plant growth. Plant Physiology 34, 261-356.

Wayland, M. and Hobson, K.A. 2001. Stable carbon, nitrogen, and sulfur isotope ratios in riparian food webs on rivers receiving sewage and pulp-mill effluents. Can. J. Zool. 79, 5-15.

Whelly, M. 1999. Aquatic invertebrates in wetlands of the oil sands region of Northern Alberta, Canada, with emphasis on Choronomidae (Diptera). M.Sc. Thesis. University of Windsor, Windsor, ON, Canada.

Yentsch, C.S. and Menzel, D.W. 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-sea Res. 10, 221-231.

Appendix A

Table 1 Paired sample T-test with 95% confidence comparing the mean values of reference and OSPM data

		Mean ±SE				
Parameter[a]	**Unit**	**Reference**	**OSPM**	**t**	**Df**	**Prob**
TP	µg/L	21 ±3	28 ±10	-0.5	3	0.651
TN	µg/L	1149 ±50	1681 ±458	-1.2	3	0.330
NA	**mg/L**	**4.6 ±3**	**46 ±7**	**-6.6**	**3**	**0.007**
Conductivity	µS/cm	1555 ±327	2556 ±651	-0.9	3	0.454
[DOC] 2007-2009[b]	**mg/L**	**38.9 ±4.9**	**87.4 ±7.3**	**6.1**	**11**	**0.000**
[DIC] 2007-2009	**mg/L**	**71.1 ±6.2**	**124.7 ±14.2**	**-3.8**	**11**	**0.003**
δ^{13}C DOC	‰	-26.9 ±0.4	-27.0 ±0.2	0.2	11	0.818
δ^{13}C DIC	‰	-5.9 ±0.4	-5.2 ±1.1	-0.6	11	0.583
Plankton Chl *a* 2007-2009[c]	µg/L	3.7 ±1.1	4.6 ±1.6	-0.4	19	0.690
Plankton TSS 2007-2009[c]	mg/L	13.8 ±4.1	24.6 ±6.2	-1.4	19	0.167
Plankton C:N ratio		11.0 ±1.0	13.6 ±1.8	-0.964	3	0.406
Periphyton Chl *a* 2007[d]	mg/m^2	2.7 ±0.9	0.6 ±0.2	1.9	5	0.114
Periphyton Chl *a* 2008[d]	mg/m^2	7.7 ±3.6	2.7 ±1.1	1.6	3	0.209
Periphyton Dry weight 2007[d]	mg/m^2	283.4 ±75	229.2 ±62	0.5	5	0.669
Periphyton Dry weight 2008[d]	mg/m^2	1037.5 ±218	732.9 ±108	1.2	3	0.317
Periphyton 2007 C:N ratio [d]		15.7 ±2.4	29.7 ±15.8	-0.925	1	0.525
Periphyton 2008 C:N ratio [d]		11 ±1.5	14.1 ±1.6	-0.911	3	0.429
Plankton 07-09 δ^{13}C	‰	-29.1 ±0.9	-26.7 ±2.9	-1.4	10	0.193
Plankton 07-09 δ^{15}N	**‰**	**1.3 ±0.4**	**9.7 ±2.0**	**-4.5**	**8**	**0.002**
Periphyton 2007 δ^{13}C[e]	‰	-26.2 ±1.1	-25.5 ±1.7	-2.5	4	0.067
Periphyton 2007 δ^{15}N[e]	**‰**	**1.7 ±0.6**	**3.4 ±0.5**	**-5.2**	**4**	**0.007**
Periphyton 2008 δ^{13}C[e]	**‰**	**-28.6 ±0.7**	**-23.6 ±0.7**	**-5.6**	**3**	**0.011**
Periphyton 2008 δ^{15}N[e]	**‰**	**0.7 ±0.3**	**2.2 ±0.1**	**-9.1**	**3**	**0.003**

[a]Mean ± SE were calculated only for sites with data for the same sampling periods. Due to missing sampling date data, the following sites were not included in mean estimates for reference or OSPM sites: [b] SSBP, HS, and CTW; [c]SSBP; [d]SSBP, HS, CTW, and NW; [e]CTW, and NW.

Table 2 Linear regressions of the mean values phytoplankton or periphyton measurements and water quality parameters.

Phytoplankton or Periphyton Measurements	Water Quality Parameters	R^2	P value
Plankton chl a	**TP**	**0.702**	**0.009**
	TN	**0.546**	**0.036**
	NA	0.008	0.835
	Conductivity	0.034	0.633
	[DOC]	0.094	0.503
	[DIC]	0.054	0.617
Plankton TSS	**TP**	**0.940**	**0.000**
	TN	**0.814**	**0.002**
	NA	0.145	0.351
	Conductivity	0.018	0.728
	[DOC]	0.381	0.103
	[DIC]	0.021	0.732
Plankton $\delta^{15}N$	**TP**	0.119	0.363
	TN	**0.509**	**0.031**
Periphyton chl a 2007	TP	0.142	0.404
	TN	0.32	0.736
	NA	0.330	0.233
	Conductivity	0.607	0.039
	[DOC]	0.611	0.066
	[DIC]	0.306	0.255
Periphyton Chl a 2008	TP	0.038	0.642
	TN	0.024	0.714
	NA	0.350	0.122
	Conductivity	0.150	0.302
	[DOC]	0.385	0.101
	[DIC]	0.138	0.364
Periphyton TSS 2007	TP	0.236	0.328
	TN	0.237	0.327
	NA	0.136	0.472
	Conductivity	0.112	0.463
	[DOC]	0.194	0.382
	[DIC]	0.144	0.457
Periphyton TSS 2008	TP	0.213	0.250
	TN	0.009	0.828
	NA	0.097	0.453
	Conductivity	0.030	0.657
	[DOC]	0.002	0.908
	[DIC]	0.076	0.507
Periphyton $\delta^{15}N$ 2007	TP	0.052	0.622
	TN	0.150	0.391
Periphyton $\delta^{15}N$ 2008	TP	0.093	0.426
	TN	0.508	0.031

Table 3 Mean ± standard error for DOC and DIC concentrations and $\delta^{13}C$ isotope values for reference and OSPM sites sampled in 2007-2009.

Age	Status	Organic Level	Sites	Mean ± standard error			
				[DOC] (mg/L)	[DIC] (mg/L)	$\delta^{13}C$ DOC (‰)	$\delta^{13}C$ DIC (‰)
Young	Reference	Low	BL	28.08 ±1.64	64.70 ±4.61	-27.95 ±0.18	-6.46 ±0.91
		High	PP	51.80 ±10.30	65.13 ±13.39	-26.06 ±0.24	-4.84 ±0.27
	OSPM	Low	SCT	69.21 ±7.70	86.28 ±8.88	-26.89 ±0.23	-2.11 ±0.36
		High	CTW	104.06 ±23.82	206.37 ±40.74	-27.66 ±0.29	-7.49 ±0.35
Mature	Reference	Low	SW	36.79 ±7.86	83.55 ±11.85	-26.61 ±0.79	-6.43 ±0.54
		High	HS	77.0 ±11.00	59.29 ±6.58	-28.43 ±0.24	-8.05 ±1.30
			SSBP*	35.39 ±1.50	57.57 ±3.98	-27.04 ±0.25	-5.75 ±0.22
	OSPM	Low	TP9	86.52 ±14.47	176.12 ±22.40	-26.40 ±0.37	-4.12 ±0.31
		High	NW	106.36 ±9.54	111.69 ±14.69	-27.62 ±0.13	-9.34 ±1.90

*All sites were samplde 4 times on June 29-July 3 2007, June 18-19 and July 30-31, 2008, and July 22-23, 2009 except SSBP which was sampled 3 times.

Table 4 Mean ± standard error for phytoplankton Chl *a* values, TSS, carbon and nitrogen isotope values for 2007-2009 samples.

Age	Status	Organic Level	Sites	Mean ± standard error			
				Chl *a*[1] (µg/L)	**TSS[1] (mg/L)**	**$\delta^{13}C$[2] (‰)**	**$\delta^{15}N$[3] (‰)**
Young	Reference	Low	BL	5.75 ±2.17	23.1 ±19.02	-31.35 ±1.09	1.29 ±0.28
		High	PP	8.75 ±4.35	17.4 ±8.03	-27.89 ±1.88	0.84 ±0.82
	OSPM	Low	SCT	1.61 ±1.03	13.3 ±3.21	-18.64 ±2.65	3.94 ±1.54
		High	CTW	2.22 ±0.60	11.9 ±6.21	-32.30 ±1.36	15.81 ±1.27
Mature	Reference	Low	SW	1.32 ±0.22	10.1 ±5.99	-26.87 ±1.91	0.81 ±0.17
		High	HS	1.92 ±0.44	10.7 ±4.28	-28.02 ±0.87	0.21 ±1.53
			SSBP	2.49 ±0.63	8.8 ±1.57	-31.01 ±0.68	2.61 ±0.13
	OSPM	Low	TP9	4.39 ±2.08	21.6 ±3.96	-27.55 ±1.77	3.28
		High	NW	12.01 ±7.11	59.2 ±24.6	-28.98 ±4.45	12.53 ±1.20

*All sites were sampled 5 times on June 29-July 3 2007, June 18-19, Jul 9-10, and July 30-31, 2008, and July 22-23, 2009;[1] SSBP was sampled 4 times;[2] Stable isotope samples were sampled 3 times June 29-July 3 2007, Jul 9-10, 2008, and July 22-23, 2009;[3] Nitrogen isotope sample numbers varied since nitrogen isotope values were not detectable due to low biomass in the samples.

Table 5 Mean ± standard error for periphyton Chl *a* values, TSS, carbon and nitrogen isotope values for 2007 samples.

Age	Status	Organic Level	Sites	Mean ± standard error			
				Chl *a* (mg/m^2) [1]	TSS (mg/m^2) [1]	$\delta^{13}C$ (‰)[2]	$\delta^{15}N$ (‰)[2]
Young	Reference	Low	BL	4.55 ±2.2	154.7 ±60	-26.29 ±1.8	0.31 ±0.2
		High	PP	1.19 ±0.6	138.3 ±31	-22.42 ±4.5	0.34 ±0.3
	OSPM	Low	SCT	0.41 ±0.3	311.0 ±89	-24.79 ±3.0	3.41 ±0.9
Mature	Reference	Low	SW	2.42 ±1.2	557.3 ±79	-26.35 ±2.7	1.00 ±0.2
		High	HS	1.78 ±1.0	363.5 ±183	-26.93 ±4.5	0.80 ±0.3
			SSBP	6.24 ±4.1	765.7 ±204	-28.07 ±0.7	5.47 ±0.8
	OSPM	Low	TP9	0.70 ±0.2	147.3 ±66	-26.56 ±0.1	3.39 ±0.1

*All sites were sampled on July 5-6, 23-24, and August 6-7, 2007.
[1] HS was sampled 2 times
[2] Stable isotope sample numbers varied since some values were not detectable due to low biomass, HS, PP and TP9 had 2 sample dates.

Table 6 Mean ± SEM for periphyton chl *a* values, TSS, carbon and nitrogen isotope values for 2008 samples.

Age	Status	Organic Level	Sites	Mean ± standard error			
				Chl *a* (mg/m) [1]	TSS (mg/m^2) [1]	$\delta^{13}C$ (‰)[1]	$\delta^{15}N$ (‰)[1]
Young	Reference	Low	BL	16.23 ±9.1	1324.4 ±443	-31.63±1.3	0.42±0.3
		High	PP	4.96 ±0.3	491.1 ±146	-26.90±0.7	1.75±0.8
	OSPM	Low	SCT	2.11 ±1.0	815.1 ±99	-22.59±0.8	2.16±0.1
		High	CTW	2.11 ±1.0	2842.6 ±1367	-24.55±0.04	18.77±0.1
Mature	Reference	Low	SW	1.95 ±0.9	1297.1 ±210	-27.00±1.1	0.86±0.6
		High	HS	3.21 ±0.9	1456.8 ±1060	-28.38±0.2	0.29±0.1
			SSBP	3.28	858.6	-29.97	-0.22
	OSPM	Low	TP9	3.28 ±2.4	650.8 ±215	-24.51±0.8	2.14±0.3
		High	NW	2.16 ±0.9	464.0 ±156	-25.44±2.7	13.44±0.6

[1] All sites were sampled in July 9-10, and July 30-31, 2008 except SSBP was sampled only in July 9-10, 2008

Table 7 Stable isotope analysis from phytoplankton sampled in 2007-2009.

Sites	Date	$\delta^{13}C$	$\delta^{15}N$	%C	%N	C:N
Reference						
BL	2007	-32.42	1.56	12.39	0.94	13.2
	2008	-32.46	nd	3.76	nd	nd
	2009	-29.17	1.01	24.52	1.86	13.2
SW	2007	-23.07	0.97	40.19	2.40	16.7
	2008	-29.00	nd	3.15	nd	nd
	2009	-28.54	0.64	3.93	0.70	5.6
PP	2007	-31.66	0.93	30.01	4.99	6.01
	2008	-26.01	2.21	11.72	0.80	14.7
	2009	-26.01	-0.61	7.65	1.00	7.7
HS	2007	-28.89	-1.31	21.18	1.58	13.4
	2008	-27.15	1.74	10.25	0.76	13.5
	2009	nd	nd	nd	nd	nd
SSBP	2007	-32.33	2.60	20.05	1.95	10.3
	2008	-30.63	2.84	4.31	0.72	6.0
	2009	-30.08	2.40	3.32	0.48	6.9
OSPM						
SCT	2007	-23.77	6.79	31.50	2.23	14.1
	2008	-14.93	3.50	8.20	0.66	12.4
	2009	-17.22	1.52	6.68	0.59	11.3
TP9	2007	-26.31	3.28	18.71	1.03	18.1
	2008	-25.29	nd	3.03	nd	nd
	2009	-31.05	nd	3.03	nd	nd
CTW	2007	-30.42	18.26	22.95	1.80	12.7
	2008	-31.55	15.15	3.21	0.44	7.3
	2009	-34.94	14.01	10.05	1.12	9.0
NW	2007	nd	nd	nd	nd	nd
	2008	-24.53	13.73	11.33	0.99	11.7
	2009	-33.42	11.33	30.35	1.74	17.4

* nd – no data were available due to low biomass

Table 8 Stable isotope analysis of periphyton from artificial substrate in 2007.

Sites	Date	$\delta^{13}C$	$\delta^{15}N$	%C	%N	C:N
Reference						
BL	Jul 6	-29.80	0.74	16.68	1.77	9.4
	Jul 24	-25.29	0.01	26.90	2.72	9.9
	Aug 7	-23.79	0.17	28.82	1.70	17.0
SW	Jul 5	-31.04	1.42	21.17	1.56	13.6
	Jul 23	-26.21	0.82	27.64	1.94	14.2
	Aug 7	-21.81	0.76	30.94	1.73	17.9
PP	Jul 6	-26.88	0.66	31.07	1.81	17.2
	Aug 7	-17.97	0.02	24.28	0.80	30.4
HS	Jul 5	-31.43	1.12	26.91	1.52	17.7
	Aug 7	-22.44	0.49	13.01	0.75	17.3
BP	Jul 6	-29.24	4.08	18.38	1.61	11.4
	Jul 24	-28.03	5.42	16.33	1.90	8.6
	Aug 7	-26.93	6.92	19.07	2.05	9.3
OSPM						
SCT	Jul 6	-30.69	2.28	21.82	0.20	109.1
	Jul 23	-21.58	5.16	2.64	0.20	13.2
	Aug 6	-22.10	2.79	8.41	0.59	14.3
TP9	Jul 5	-26.49	3.28	17.11	0.99	17.3
	Aug 6	-26.62	3.50	24.40	2.33	10.5

Table 9 Stable isotope analysis of periphyton from artificial substrate in 2008.

Sites	Date	$\delta^{13}C$	$\delta^{15}N$	%C	%N	C:N
Reference						
BL	Jul 10	-30.35	0.67	7.99	1.02	7.8
	Jul 30	-32.91	0.17	12.07	1.53	7.9
SW	Jul 9	-28.05	1.42	13.73	1.20	11.4
	Jul 30	-25.95	0.31	15.02	0.90	16.7
PP	Jul 10	-27.62	0.97	27.62	1.83	15.1
	Jul 30	-26.18	2.53	23.23	2.30	10.1
HS	Jul 10	-28.59	0.22	14.39	1.11	13.0
	Jul 30	-28.17	0.36	10.49	0.76	13.8
BP	Jul 9	-29.97	-0.22	21.16	2.99	7.1
OSPM						
SCT	Jul 9	-23.34	2.23	11.52	0.69	16.7
	Jul 31	-21.84	2.09	10.31	0.75	13.7
TP9	Jul 9	-25.33	2.46	13.48	0.81	16.6
	Jul 31	-23.68	1.82	11.34	0.72	15.8
CT	Jul 10	-24.59	18.65	17.20	1.19	14.5
	Jul 30	-24.51	18.89	11.23	0.66	17.0
NW	Jul 10	-22.77	14.07	18.90	2.46	7.7
	Jul 30	-28.11	12.81	14.41	1.30	11.1