The Effects of Nutrient and Peat Amendments on Oil Sands Reclamation Wetlands: A Microcosm Study

by

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AUTHOR'S DECLARATION

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

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Abstract

Oil sand mining operations in Alberta, Canada produce large quantities of process water and mature fine tailing (MFT) during the bitumen extraction process. Wet landscape reclamation is one of the reclamation strategies proposed to utilize process water and MFT in the creation of aquatic reclamation environments that are economically and environmentally acceptable.

In the interest of utilizing nutrient enrichment and peat amendment to improve aquatic flora and fauna colonization in new oil sands aquatic reclamation, this microcosm study was designed to assess the phytoplankton and periphyton growth (summer 2008), as well as benthic invertebrate colonization (summer 2009). Peat amendment significantly increased the growth of phytoplankton and periphyton by providing sufficient nutrients (total nitrogen, total phosphorus and dissolved organic carbon) to the system. In reference wetland, benthic invertebrate colonization was significantly increased by utilizing sand as bottom substrate and decreased by MFT/Sand mixture as bottom substrate. In OSPM-affected wetland, benthic invertebrate colonization was not affected by utilizing MFT/Sand as bottom substrate. In comparison to OSPM-affected wetlands, reference wetland had larger number of benthic invertebrate families and higher total abundance.

In this research, experimental microcosms were constructed in three reclamation wetlands with different types of reclamation materials as the bottom substrates (sand, MFT + sand) and amendments (nutrient and/or peat) added to optimize growing conditions for phytoplankton and periphyton, thus creating a biological detrital layer over unfavourable substrates to enhance benthic invertebrate colonization. The growth estimates of phytoplankton and periphyton on MFT + sand without amendment were low in comparison to the control (water only, no substrate). In comparison to sand, MFT + sand had higher growth estimates at OSPM-affected sites, but lower growth estimates at reference site. The growth estimates of phytoplankton and periphyton on MFT + sand were significantly increased with peat amendment. Nutrient (nitrogen and phosphorus) enrichment insignificantly improved the phytoplankton and periphyton growth. Peat amendments elevated the concentrations of nitrogen, phosphorus and dissolved organic carbon in the system and maintained these high concentrations throughout the experiment period. Nutrient enrichment only temporarily (less than 3 weeks) elevated nitrogen and phosphorus levels as the nutrients added were quickly utilized by the system.

Benthic invertebrate colonization was assessed in the following year. Sand treatments had increased total abundance and numbers of families of benthic invertebrate compared to the mature sediments of the reference wetland. In oil sand process material (OSPM)-affected wetlands, sand treatments had slightly lower abundance and fewer numbers of families in comparison to the mature sediments. In comparison to sand treatments, MFT + sand treatments had decreased total abundance in the reference wetland but not in OSPM-affected wetlands that received MFT input during its construction. Peat amendment and nutrient enrichment had no impact on benthic invertebrate total abundance or composition.

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Chapter 1. Introduction

1.1 Thesis Overview

Wet landscape reclamation options use various oil sand process materials (OSPM) and oil sand process water (OSPW) to construct aquatic reclamation environments that are economically and environmentally acceptable. Studies have examined potential strategies to ensure the health of aquatic flora and fauna in constructed wetlands, ponds and/or lakes.

1.1.1 Objectives

The goal of this study was to determine the utilization of nutrient enrichment and peat amendment as strategies to improve flora and fauna colonization in new oil sands aquatic reclamation. The first objective was to determine the effects of nutrient enrichment and peat amendment on phytoplankton and periphyton growth in microcosms constructed using various types of OSPM and OSPW which vary in naphthenic acid (NA) and polycyclic aromatic compound (PAC) concentrations and salinity. To accomplish this objective, dry weight and chlorophyll *a* (Chl *a*) of phytoplankton and periphyton were measured in closed microcosms containing various types of OSPM and OSPW with and without nutrients and/or peat (Year 1, Closed microcosm study). During the Year 1 growing season (2008), phytoplankton and periphyton biomass contributes to the development of a biological detrital layer over OSPM substrates in closed microcosms. At the end of the 2008 growing season, the microcosms were opened to the surrounding aquatic system to allow colonization of flora and fauna. The second objective (Year 2, Open microcosm study) was to assess the effects of nutrient and/or peat amendments that potentially enhanced the biological detrital layer over low quality substrates (OSPM), on benthic invertebrate colonization.

Oil Sands Mining in Northern Alberta

1.2 Overview

The oil sands deposits in the Athabasca River basin of northeastern Alberta is the largest hydrocarbon reserves in the world, with an estimated reserve of 869 billion barrels of bitumen (FTFC 1995). Orinoco (Venezuela) is the second largest with 700 billion barrels of bitumen reserve, and Utah (U.S.A.) the third, with 25 billion barrels (FTFC 1995). A recent report indicates that Alberta oil sand industrial production is at 504,500 barrels of conventional light, medium and heavy cruel oil per day (bbl/d, 1 barrel = 159 L) (Alberta Energy and Utilities Board 2008-2009). The production, presently accounting for over 20 % of Canada's petroleum, is projected to increase by over 50 % in a few years (Leung et al. 2003).

Figure 1.1 Athabasca oil sands deposit.

1.2.1 Mining Process

Surface or open-pit mining technology is one of the methods currently used to access the Athabasca oil sands. First, the overburden is removed to expose the oil sands which are then excavated and transported using truck and shovel method to the extraction facility (FTFC 1995). The bitumen within the sandy soil is extracted via the Clark hot water extraction process (FTFC 1995). In this process, oil sands are digested in large tumblers with 80°C water mixed with caustic soda (sodium hydroxide, NaOH) to produce a slurry mixture. Bitumen is separated as a froth, which floats to the surface and the coarse sands settle to the bottom. The remaining slurry consists of a combination of fine silts and clay, residual bitumen, and organic compounds (NAs and PACs), known as mine tailings. The bitumen froth product is then collected and upgraded to light sweet synthetic crude oil. The tailings are pumped into settling basins.

1.2.2 Tailings Waste

The extraction process produces a large volume of waste material including coarse sand and process water containing fine clays and silts (fine tailings). To extract bitumen from 1 m^3 of oil sands, 3 m^3 of water is required, resulting in an estimated 4 m^3 of waste material (Holowenko et al. 2002). OSPW, stored in settling basins prior to reclamation, changes over a period of years as the fine tailings settle to the bottom and become more compact, creating a significant layer of material referred to as mature fine tailings (MFT). MFT consists of 65% water, clay (less than 22 μm) and residual bitumen (Boerger et al. 1992). MFT can be further treated with the addition of tailings sands and gypsum (calcium sulfate, $CaSO_4$) to generate consolidated tailings (CT). It is estimated that billions of m^3 of MFT will need to be reclaimed as mining operations cease (MacKinnon et al. 2005).

1.2.3 Reclamation Strategies

Based on the regulations set by provincial and federal governments, oil sands companies are held responsible for 1) reclamation of the land to equal or greater productive capacity than the pre-disturbed landscape, and 2) zero discharge of wastes. To achieve such goals, reclamation strategies would include both dry landscapes (upland forests and lowland grassy plains) and wet landscapes (wetlands, ponds and lakes).

Wet landscape reclamation options use the mined pits in part to create wetlands, ponds and/or lakes referred to as end-pit lakes. Reclamation options use various quantities of OSPW and OSPM to construct aquatic reclamation environments that are economically and environmentally acceptable. Over the years, oil sands companies have constructed a wide variety of experimental reclamation wetlands and ponds (Farwell et al. 2009) to study the impacts of OSPW and OSPM on aquatic flora and fauna. For example, some reclamation wetlands were constructed with a base of MFT and capped with either OSPW or non-OSPW (muskeg surface runoff water). Other experimental wetlands were constructed with consolidated tailings (CT) release water or CT substrate. The long-term goal of these reclamation strategies is to create a healthy and sustainable aquatic ecosystem.

Aquatic organisms in oil sands reclamation may be exposed to oil sands constituents associated with OSPW and/or OSPM depending on the reclamation strategy utilized. There are elevated levels of major cations and anions, particularly Na^+ (675 mg/L tailings pond water, TPW), SO_4^{2-} (210 mg/L, TPW), Cl^- (300 mg/L, TPW) and HCO^{3-} (Nelson et al. 2000). In comparison, reference sites on and off of the oil sands deposit have lower concentrations of Na^+ (<34 mg/L), SO_4^{2-} (<28 mg/L), Cl^- (<11 mg/L) and HCO^{3-}(<154 mg/L) (Farwell et al. 2009). There are a wide variety of organic compounds such as PACs and NAs which are classified as the principal toxic components of OSPW and OSPM. Naphthenic acids (NAs) are a group of low molecular weight (< 500 amu) saturated aliphatic and alicyclic carboxylic acids naturally found in bitumen (Clemente and Fedorak 2005). This group of compounds is found at elevated levels in OSPW due to increased water solubility under alkaline conditions produced during the bitumen extraction process (MacKinnon and Boerger 1986; FTFC 1995). Generally reference sites on and off of the oil sands deposit have NAs concentrations of <1.2 mg/L (Farwell et al. 2009) compared to 80-100 mg/L for TPW (Nelson et al. 2000). Other naturally occurring organic compounds include alkylated PACs, a group of non-polar, hydrophobic compounds consisting of two or more fused benzene rings with alkyl-substitution. These compounds have a high affinity to particulates with higher concentrations in MFT (136 µg PAHs/g, Madill et al. 1999; 4.72-374 µg PAHs/g, Ganshorn 2002) relative to OSPW (3.3 µg PAHs/L TPW, Madill et al. 1999).

1.3 Impact of Oil Sands Reclamation on Aquatic Flora and Fauna

1.3.1 Aquatic Flora

A study examining the effects of OSPW on phytoplankton communities showed significant community effects in fresh (younger) OSPW with elevated NA concentrations compared to aged OSPW with lower NA concentration (Leung et al. 2003). NA concentration and salinity (conductivity) were also found to be highly correlated to the phytoplankton community structure. Studies of phytoplankton community composition have identified thresholds for NA of > 20 mg/L (Leung et al. 2001) and 24-50 mg/L (Hayes 2005). Species such as: *Botryococcus braunii, Cosmarium depressum, Navicula radiosa,* and *Ochromonas spp.* were suggested as possible indicator species of NA pollution (Leung et al. 2003).

The growth of three macrophytes (*Myriophyllum spicatum, Potamogenton richardsonii,* and *Chara vulgaris*) on different oil sands reclamation materials (including tailings sand, CT and natural sediment) has been examined (Luong, 1999). There was a positive correlation between plant growth and the percentage silt in the sediments. The addition of inorganic nutrients (fertilizer spikes) to these sediments had little effect on macrophyte growth. However, the addition of organic matter (peat) to tailing sand increased growth significantly. Results suggested that sediment texture (including organic matter content) and nutrient content together determine the suitability of sediments for plant growth. Increasing salinity of the overlaying water lowered *M. spicatum and P. richardsonii* growth. Tailing sand amended with organic matter was suggested as the most appropriate substrate for macrophyte establishment in the littoral zone of the water-capped lake (Luong, 1999).

In a field microcosm study, the addition of peat had a negative impact on the growth of macrophytes (*Chara*) in wetlands that contained some organic matter, and no impact on wetlands with little or no organic content (Baker 2007). Due to the chemical complexity (salinity and NAs concentrations) and physical complexity (wetland depth, turbidity) of oil sands reclamation wetlands, the impact of nutrient enrichment on macrophyte growth was inconclusive.

Earlier reclamation strategies had used various types of amendments in an attempt to enhance the rate and type of aquatic floral and faunal colonization. Experimental ponds (i.e., Syncrude Lease 17, 1989 Test ponds) were amended with either biota inoculums from local wetlands or inorganic phosphorus and nitrogen (Farwell et al. 2009). Unfortunately there is little information available on the success of these amendments following construction.

1.3.2 Aquatic Fauna

A study determined the effects of OSPW on zooplankton (McCormick, 2000). The study showed that total zooplankton biomass was greatly reduced in the presence of pond water with newer tailings. Both zooplankton community structure (measured as Percent Model Affinity) and total zooplankton biomass were strongly correlated with NA concentration. An average concentration of NA that would be considered as "no effect" on the zooplankton community structure was calculated at 5 mg/L. The study also found that in the ponds monitored, with community structures varied, the total zooplankton biomass remained similar, suggesting that adaptation of zooplankton communities in those ponds. Two possible indicator species: *Daphnia pulex* and *Brachionus rubens* were recommended (McCormick, 2000).

Benthic invertebrate community indexes are another important indicator of healthy wetland ecosystems. Assessments of benthic invertebrate communities in oil sands reclamation found significantly lower total abundance of invertebrates in an OSPW-affected site (Demonstration Pond) compared to the off-site nature lakes: Sucker Lake and Kimowin Lake (Gould, 2000). High water turbidity limits colonization of macrophytes and phytoplankton, which in turn, limits the accumulation of organic matter, and therefore, limits food source and habitat for the benthic macroinverterbrates. Aside from water turbidity, other factors such as unsuitable substrates, low levels of toxicity, an early stage of colonization and fish predation were suggested as possible causes leading to lower invertebrate abundance. For example, *Chironomus,* a large benthic Chironomidae, was not found in DP, possibly due to a preference for organic rich sediments in addition to being an important prey item for fish (Gould, 2000).

In other studies of oil sands reclamation wetlands, the detrital abundance was found correlated to abundance of zoobenthos and zoobenthic community richness (Leonhardt 2003). The abundance and taxa richness of benthic invertebrate communities can be influenced by many additional factors, such as water pH, turbidity, fish predation (Whelly 1999), submerged macrophytes (Leonhardt 2003), phytoplankton (Leung et al. 2001; Hayes 2005), and conductivity (Leonhardt 2003).

1.4 Northern Alberta Studies

Data on nutrient levels and Chl *a* measurements for the Oil Sands Central Mixedwood Region (Golder, 2003) in northern Alberta was used to compare OSPM-affected and non-OSPM aquatic systems. Concentrations of total nitrogen (TN) and total phosphorous (TP) were slightly lower for OSPM-affected aquatic systems (Table 1). Chl *a* levels, an index of community growth, were on average lower for OSPM-affected sites (mean, 3.4 µg/L; range, 0.6 to 19.0 µg/L; Hayes, 2005; Gould, 2000; Leung et al. 2003) compared to non-OSPM affected sites in the same region (mean, 29.1 µg/L; range, 1.5 to 371.0 µg/L; Golder, 2003).

Table 1: Summary of nutrient concentrations and Chl *a* measurements from OSPM-affected and non-OSPM aquatic systems in the Oil Sands Central Mixedwood Region.

Water bodies	TP(µg/L)			TN(µg/L)		
	Min.	Max.	Mean	Min.	Max.	Mean
OSPM sites[a]	9.7	180	42.1	401	6100	1477
Non-OSPM sites[b]	12.3	299.1	55.1	483	6558	1522

[a]Hayes, 2005; [b]Golder, 2003

In other studies of wetland lakes in the boreal region of Alberta, Canada, lakes were shallow (mean depth 1.3m), rich in phosphorous (123 µg total P L^{-1}), and relatively low in available nitrogen (18 µg L^{-1} $NH_4 + NO_3$) (Golder 2003). The phytoplankton-dominated lakes (>20 µg L^{-1} Chl *a* concentration) were usually found in hypereutrophic conditions (mean = 205 µg total P L^{-1}), whereas the submerged aquatic vegetation (SAV) dominated lakes (>25% cover) primarily exist in eutrophic and mesotrophic conditions (mean = 82 µg total P L^{-1}) and have lower available N (11 µg L^{-1} $NH_4 + NO_3$) (Golder 2003).

1.5 Factors Influence Primary Production

Nutrient availability in an aquatic system, such as a lake or wetland, has a major influence on the systems' primary productivity (Jones et al., 2004; Norlin et al., 2005; Ventura et al., 2008). The influence of nitrogen (N) and phosphorus (P) on the productivity of an aquatic system has been studied extensively and the influence of other inorganic nutrients, including silica (Si), iron (Fe), potassium (K), and trace metals and vitamins (Roelke et al., 1999; Twomney & Thompson 2001) has also been investigated. Certain nutrients are often more readily available than others in an ecosystem, and the productivity for such ecosystems is likely dependent upon the concentration of the limiting nutrient. However, in most ecosystems, the productivity is not solely determined by a single factor; rather the productivity is determined by the combination of inorganic and organic carbon (C) availability and the dynamic of their composition (Roelke et al., 1999, Vrede et al., 1999).

Different nutrient species, compositions, and concentrations can also alter the primary productivity of an ecosystem. Laboratory experiments using freshwater algae have shown that the effect of different nutrient ratios can be stated as follows: high Si:P and N:P ratios - diatoms favored; low Si and high N:P ratio - green algae favored, and low N - cyanobacteria favored (Tilman, 1986; Kiham and Heckey 1988; Sommer 1989). Additional lake studies indicate that either the SAV-dominated (>25 % area coverage), or clear-water state exists over a wide range of nutrient concentrations, yet it is most often between 50 and 150 $\mu g\ L^{-1}$ TP (Moss et al. 1994). If this threshold is exceeded, lakes are likely to switch to the more turbid phytoplankton state unless N input is low (available N : P ratios < 6:1) (Scheffer & Jeppese, 1998).

Chapter 2. The Impact of Peat Amendment and Nutrient Enrichment on Plankton and Periphyton in Oil Sands Reclamation Wetlands in Northern Alberta, Canada: A Microcosm Study

2.1 Overview

To ensure the development of healthy aquatic ecosystems, different oil sands reclamation strategies were examined to optimize initial phytoplankton and periphyton community growth. In this microcosm study, mature fine tailings (MFT) and process water generated from the extraction of bitumen from oil sands were the main two types of reclamation materials utilized; these materials are known to be elevated in naphthenic acids, polycyclic aromatic compounds and salts. Microcosms were designed to test different substrates (sand, MFT + sand) and were deployed in three experimental reclamation wetlands along a gradient of naphthenic acid and salt concentrations. To optimize algal growth, with the goal of creating a biological detrital layer on reclamation substrates, the addition of nutrients and/or peat as amendment strategies was also examined. Following the initial deployment, the microcosms were monitored during the growing season for water chemistry parameters (total nitrogen, TN; total phosphorus, TP; dissolved organic carbon, DOC; dissolved inorganic carbon, DIC) and estimates of phytoplankton and periphyton growth (dry weight and Chl *a*). Microcosms with MFT + sand as a substrate had reduced phytoplankton and periphyton growth relative to sand treatments. Peat amendments significantly increased the growth of phytoplankton and periphyton for MFT + sand treatments. The increased phytoplankton and periphyton growth with the peat amendment was attributed to the availability of nutrients (TN, TP and DOC) throughout the growing season. In contrast, nutrient-enriched microcosms, receiving a single pulse of nutrients, had a short period of enhanced growth . The study indicated the potential of utilizing peat amendment as a reclamation strategy to promote phytoplankton and periphyton growth to enhance the biological detrital layer of new oil sands aquatic reclamation.

2.2 Introduction

The oil sands deposit in the Athabasca River basin of northeast Alberta is one of the largest hydrocarbon reservoirs in the world. Bitumen reserves in the region are estimated to be as high as 869 billion barrels (FTFC 1995). Currently, there are more than 20 companies involved in the oil sand project, with production at 504,500 barrels of conventional light, medium and heavy cruel oil per day (bbl/d, 1 barrel = 159 L)) (Alberta Energy and Utilities Board 2008-2009). To access the bitumen in the region requires the use of surface mining technology, which involves the removal of overburden (a mixture of muskeg and topsoil) that is stored for later use, typically in terrestrial reclamation. The extraction of bitumen from sand involves the Clark hot water extraction method which generates waste material including coarse sand and soft fine tailings (mature fine tailing, MFT). MFT consists of more than 80% water by volume and contains silt, clay, un-recovered bitumen (Leung et al. 2001) and associated polycyclic aromatic compounds (PACs, 4.72-374 µg/g; Ganshorn 2002). MFT has elevated levels of salt (total ion content >2000 mg/L) and dissolved organic compounds including NAs (70-100 mg/L; Leung et al. 2001). The addition of gypsum (calcium sulfate) to MFT to consolidate the tailings (referred to as consolidated tailings, CT) also increases salinity (MacKinnon et al. 2001). These waste materials may be incorporated into oil sands wet landscape reclamation using a variety of reclamation strategies to create healthy wetlands, ponds and/or lakes (referred to as end-pit lakes) that are environmentally acceptable.

Nutrient enrichment was proposed as a possible strategy to increase phytoplankton and periphyton growth and to generate an initial detrital layer in new oil sands reclamation wetlands. For the wet landscape option, the quality of the substrate used in construction may be poor due to physical (low organic content, coarse material ie. sand) and/or chemical characteristics (elevated levels of PACs i.e., MFT). Nutrient addition (chemical fertilizers) and/or substrate amendments that are a source of nutrients (i.e., peat) may elevate phytoplankton and periphyton production, which could provide a more favorable biological-based detrital layer leading to enhanced aquatic flora and fauna colonization in new oil sands reclamation wetlands.

For northern regions of Alberta, available data on nutrient levels and chlorophyll *a* (Chl *a*) measurements suggests there are slight differences between aquatic systems depending on the influence of oil sands mining activity. Mining-affected and reclaimed aquatic environments of the Oil Sands Central Mixedwood Region had slightly lower total nitrogen (TN) and total phosphorus (TP) (mean TN, 1477 µg/L; mean TP, 42.1 µg/L) compared to unaffected sites of the same region (mean TN, 1521 µg/L; mean TP, 55.1 µg/L) (Gould 2000, Leung et al. 2003, Hayes 2005, Golder 2003). Chl *a* levels, an index of community growth , were also lower for mining-affected and reclaimed sites of the Oil Sands Central Mixedwood Region (mean, 3.4 µg/L; range, 0.6 to 19.0 µg/L) compared to unaffected sites in the same region (mean, 29.1 µg/L; range, 1.5 to 371.0 µg/L) (Gould 2000, Golder 2003, Leung et al. 2003, Hayes 2005).

Although nutrient availability in an aquatic system, such as a lake or wetland, has a major influence on the systems' primary productivity (Jones et al. 2004; Ventura et al. 2008), there are many factors that may influence phytoplankton and periphyton production. Studies in the Alberta oil sand region showed that phytoplankton communities were correlated with NA concentration, salinity and sulfate (SO_4) levels (Hayes 2005). Other studies have suggested the importance of turbidity, carbon dioxide (CO_2), dissolved oxygen (DO), temperature, grazing, submersed macrophyte and zoobenthic communities, and sedimentation on the primary production of an ecosystem (Vrede et al. 1999; Bayley and Prather 2003; Zimmer et al. 2003; Buyukates and Roelke 2005; Bubier et al. 2007; Ventura et al. 2008).

The relationship between nutrients and primary production is complex and dynamic. More than one study suggested that primary production does not respond linearly to nutrient levels (Levine and Schindler 1999; Roelke et al. 1999; Liboriussen and Jeppesen 2006; Lagus et al. 2007). The rate of nutrient uptake by phytoplankton can be influenced by other nutrient species, such as major ions, silica (Si) and iron (Fe) where diatoms were favored when Si: P and N: P ratios were high, green algae were favored when Si was low and the N:P ratio was high, and cyanobacteria were favored when N was low (Roelke et al. 1999). The plankton community composition could also influence the rate and species type (nutrient species such as N, P, Si, DOC and DIC) of nutrient utilization (Piehler et al. 2004; Lagus et al. 2007).

The objectives of this study were to investigate the applicability of using MFT as part of wet landscape reclamation and the use of amendments, specifically the addition of peat and/or nutrients, to promote phytoplankton and periphyton growth in oil sands reclamation wetlands. To achieve this, microcosms were set up in three experimental reclamation wetlands; the water from these wetlands provided a range of concentrations of NAs and salts to test phytoplankton and periphyton tolerance. In each wetland, microcosms were lined with either sand or 50% MFT: 50% sand (high in PACs) and amended with peat (MFT treatments only) and/or nutrients. Estimates of phytoplankton and periphyton growth, measured as Chl *a* and total dry weight, were determined for 3-4 periods during the summer of 2008 and compared to water controls without substrates. These endpoints provided estimates of growth which would contribute to the biological detrital layer following sedimentation. Further research will examine the colonization of benthic invertebrates in these microcosms (see Chapter 3). This study will provide a better understanding of the usefulness of amendments (additions of peat and/or nutrients) as a method to accelerate initial colonization in constructed wetlands.

2.3 Materials and Methods

2.3.1 Study Sites

The field study was conducted in the Athabasca Oil Sands Region, northeast of Fort McMurray (56.66° N 111.21° W), Alberta, Canada on the Syncrude Canada Ltd. lease. Three reclamation sites were chosen for the microcosm study: Shallow Wetland South Ditch (SWSD), Demonstration Pond (DP) and Mike's Pond (MP) (also referred to as Composite Tailings Pond, CTP). These sites were selected based on water quality, particularly NA concentrations. The reference site, SWSD, contained surface runoff water at low NA concentration and conductivity (Table 2.1). In comparison, moderate and high NA concentrations were found in DP and MP, respectively.

SWSD is a reference site that received no process-affected water. It was used to store West Interception Ditch water (muskeg draining water from the west side of the lease). SWSD was constructed in 1993 as one of the six Large Scale Test Ponds. It has a NA concentration in the same range as other surface waters in the region (<2.0 mg/L) (Golder 2003). SWSD has an area of approximately 12 x 220 m with a depth of 2.5 m. Different from DP and MP, the long and narrow shape of SWSD likely reduces the effect of wind on water turbidity in this wetland (Golder 2002).

DP is an OSPM-affected wetland that was also constructed in 1993. It contains 70,000 m^3 of MFT and 70,000 m^3 of surface run-off water. DP is also one of the Large Scale Test Ponds, Pit #11. It is located 20 m from SWSD (site location: 458352E 6326665N). DP has dimensions of approximately 140 x 200 m and a depth of 2.9 m (Golder 2002).

MP is another OSPM-affected wetland that was constructed in 1997 (site location: 458714E 6330045N). It is also known as a CT pond because the water source is CT release water. It has a surface area of approximately 4 ha with dimensions of 125 x 175 m and a depth of 1.5 m (Golder 2002).

Table 2.1 Selected study reclamation wetlands

Wetland	Code	Status	Age in 2008	Description[a]	NAs (mg/L)[a]	Conductivity (µs/cm)[a]
Shallow Wetland South Ditch	SWSD	Reference	15	Storage of unprocessed WID water (muskeg drainage water)	1.4 ± 0.8	892± 51
Demonstration Pond	DP	OSPM	15	70, 000 m^3 MFT capped with 70, 000 m^3 non-OSPW from WID	8.9 ± 2.7	1753 ± 43
Mike's Pond	MP	OSPM	11	CT released water	55 ± 11	4638 ± 274

[a] Farwell et al. 2009 (mean ± standard error). WID = West Intercept Ditch

2.3.2 Microcosm Design

Microcosms were constructed of two open-ended 86L polyethylene garbage bins. Each microcosm unit consisted of a bottom bin, 25 cm in height, positioned in a pre-excavated hole approximately 20 cm deep, leaving 5 cm of the bin above the sediment-water interface. The second bin, 45 cm in height, was then positioned over the bottom bin to create the basic structure of the microcosm. Each microcosm was lined with a linear low density polyethylene bag to contain the experimental substrates and site water to be added later.

Three types of substrate, obtained from Syncrude Canada Ltd., were used (alone or in combination) in the microcosms which represented possible reclamation materials (coarse tailing sand, MFT, and a peat-mineral mixture). There was a total of four substrate treatments for the microcosm experiment: 1) Water - no substrate, water only (control); 2) Sand - 20 cm for tailing sand; 3) MFT/SAND - 20 cm of premixed 50 % v/v MFT and sand mixture; 4) Peat+MFT/SAND - 2 cm of peat overlaid on 20 cm of the premixed 50 % v/v MFT and sand mixture. After the substrates were added, each microcosm was filled with 40L of site water. Water treatments consisted of the control (no nutrient addition) and a nutrient treatment of 426 mg of ammonia nitrate (NH_4NO_3) and 68 mg of potassium phosphate (KH_2PO_4) per 40L of water (N, 3731µg/L; P, 385µg/L). In total, 104 microcosms were deployed, 32 microcosms at SWSD and MP (n = 4 per treatment, ie. 4 substrates x 2 nutrient treatments x 4 replicates = 32) and 40 microcosms at DP (n = 5 per treatment, ie. 4 substrates x 2 nutrient treatments x 5 replicates = 40). All microcosms were deployed between June 10 to 13, 2008 and allowed to settle for three days prior to initial sampling.

2.3.3 Water Chemistry in Microcosms

During the experimental period in the summer of 2008, water samples were collected from each microcosm treatment at three week intervals to monitor the temperature, pH, conductivity, dissolved oxygen (DO), total phosphorus (TP), total nitrogen (TN), dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC). Temperature, pH, conductivity and DO were monitored using a multi-line field meter (Hanna #HI 9828, Italy). Water samples for TN and TP analyses were collected in 250ml TraceCleanTM amber borosilicate glass bottles, stored at 4°C and shipped to the Biogeochemcial Analytical Laboratory, University of Alberta (Edmonton, AB, Canada) for TN and TP determinations using colorimetric methods. For DOC and DIC, water samples were initially collected in 250ml amber borosilicate glass bottles with a Teflon-lined closure, preserved with 5% w/v $HgCl_2$, then topped up with water from the site to eliminate headspace and stored at 4°C. Water samples were then filtered through a 25mm diameter, 0.45µm polyethersulfone Nalgene® syringe filter (Nalge Nunc International, Rochester, NY, USA) into 40mL TraceClean^{TM} amber borosilicated glass vials (Chase Scientific Glass Inc., Rockwood, TN, USA) and sealed with open-top caps containing silicone- Teflon® septa (Chase Scientific Glass Inc., Rockwood, TN, USA). Filtered water samples were stored at 4°C and shipped to the G.G. Hatch Isotope Laboratory, University of Ottawa (Ottawa, ON, Canada) for analyses.

2.3.4 Estimates of Community Growth in Microcosms

Biological samples were collected from each microcosm at three-week intervals from June to August, 2008. Community growth was estimated using four parameters: phytoplankton Chl *a*, periphyton Chl *a*, periphyton dry weight and total suspended solid (TSS). Three 250 ml amber glass bottles were pre-rinsed in microcosm water and submerged at a depth of 10 cm to collect water samples for phytoplankton Chl *a* and TSS measurements. For periphyton sampling, an acetate sheet (210 mm x 297 mm) suspended from a bamboo stick was deployed into each microcosm at approximately 5 cm below the water surface for a period of 20 days. After 20 days, the sheets were removed and placed in sealed plastic bags. All samples were stored in coolers with ice packs and transported to the Syncrude Environmental Complex for processing.

Prior to the processing of the phytoplankton Chl *a* samples, a drop of magnesium carbonate solution (5 g in 250 ml) was added to each of the 250 ml water samples and gently shaken. Samples were then filtered through glass fiber filters (GF/F 47mm). For periphyton samples, both sides of the acetate sheets were scraped, rinsed with distilled water, and filtered through glass fiber filters (GF/F 47mm). Prior to filtration, a drop of magnesium carbonate solution (5 g in 250 ml) was added to the water containing the biofilm. The phytoplankton and periphyton filters were stored in 20 ml scintillation vials, wrapped in aluminum foil and held at -20°C prior to shipping to the University of Waterloo for Chl *a* analysis.

Total suspended solids (TSS) were estimated by filtering known quantities of water through pre-weighed, pre-combusted (450-500 °C for 4 hours) glass fiber filters (GF/F 47mm). For every sampling period, 500 ml of water from each microcosm was filtered to estimate plankton biomass. For periphyton dry weight, the collected biofilm from a known surface area of the acetate sheet was filtered using the same method as described for TSS. All filters were dried at 40°C for 12 hours, stored in labeled plastic petri dishes and shipped to the University of Waterloo.

2.3.5 Laboratory Samples Analyses

Chl *a* analysis was carried out at the University of Waterloo laboratory. A volume of 20 ml of 90:10 acetone-water (CH_3CN, HPLC grade) was added to each of the samples in the scintillation vials. The vials were then re-stored at -20°C over night to extract the Chl *a* into the acetone solution. The solution was transferred into 10 ml cuvettes and measured at an absorbance of 665 nm by spectrophotometer (Turner Designs MOD10-AU, Sunnyvale California). The resulting absorbance was used to calculate Chl *a* concentration.

To determine the periphyton dry weight and TSS, dry filters containing the samples were weighed, using the same analytical balance as the pre-weighed filters (Mettle Toledo #AG245, Switzerland). The difference in weights between the pre-weighed and sample filters was used to calculate periphyton dry weight per m^2 and TSS concentrations.

2.3.6 Statistical Analysis

Periphyton dry weight, periphyton Chl *a*, TSS and phytoplankton Chl *a* data were collected at each study site (SWSD, DP and MP) at three different sampling points. All data were $\log_{10}$ transformed prior to analyses. Statistical analyses were applied to each measured parameter, at each site, at each sampling point.

To examine differences in nutrient enrichment (with enrichment, without enrichment) and substrate type (control, Sand, MFT/SAND mix, Peat+MFT/SAND mix) for the measured endpoints, a two factor ANOVA test was performed. All statistical analyses were performed at $\alpha = 0.05$ using Systat 17 (SPSS, Chicago, IL, USA). *Post hoc* multiple comparisons were made using the Bonferroni test to determine which substrate type(s) differed from the other(s) for the measured parameters.

2.4 Result

2.4.1 Study Site Characteristics

During the sampling season in 2008 (June 13th - August 16th), water temperatures increased on average from 16.2 °C in June to 23.5 °C in August for the three sites. Temperatures varied by less than 2°C between sites for any given sampling period. Water pH ranged from 6.65 to 8.76 among study sites (mean pH: SWSD, 7.6; DP, 8.1; MP, 7.6). Dissolved oxygen (DO) ranged from 79 to 160 % saturation (Appendix, Table 2.1 to 2.3). Water chemistry parameters (conductivity, DOC, DIC, TN and TP) were measured for each site (Table 2.2). SWSD had the lowest conductivity, followed by DP and MP, respectively. MP had the lowest DOC, DIC, TN and TP relative to the other study sites.

Table 2.2 Water chemistry parameters of study wetlands in June 2008.

Wetland	Conductivity (µS/cm)	DOC (mg/L)	DIC (mg/L)	TN (µg/L)	TP (µg/L)
SWSD	900	88	102	1020	17
DP	1850	87	169	1240	39
MP	4600	36	92	616	6

2.4.2 Water Chemistry and Estimates of Community Growth in Microcosms

SWSD – Water Chemistry

At SWSD, in the microcosms, the temperature ranged from 16 °C to 22 °C. DO ranged from 21.9 to 109.4 % saturation, and pH ranged from 6.3 to 7.8 (Appendix, Table 2.1). TP in SWSD microcosms ranged from 8 to 251 µg/L among substrate treatments and was elevated in nutrient-enriched microcosms (32 to 565 µg/L) (Figure 2.1 a). Following nutrient addition in mid-June, all nutrient-enriched microcosms showed elevated TP, but by early July TP concentrations were often reduced. The Peat+MFT/SAND treatment had higher TP in comparison to other substrate treatments, regardless of nutrient enrichment. Similarly, Peat+MFT/SAND treatments had the highest TN relative to the other treatments (Figure 2.1 b). Nutrient-enriched treatments with MFT/SAND and Peat+MFT/SAND had reduced TN (by early July) similar to TN levels in treatments without nutrient enrichment. DOC was highest in Peat+MFT/SAND microcosms in comparison to other substrate treatments (Figure 2.1 c). DOC concentrations ranged from 24 to 244 mg/L among all treatments. DIC concentrations ranged from 45 to 141 mg/L (Figure 2.1 d). DIC concentrations were similar among treatments relative to DOC and had similar trends with slight decreases in early July and increases in mid-August. The increase in mid-August was more evident for treatments containing MFT.

SWSD – Seasonal Estimates of Community Growth

Seasonal estimates of community growth were measured as Chl *a* for phytoplankton and periphyton samples, as well as total suspended solid (TSS) and dry weight of periphyton samples. In SWSD, material collected from artificial substrates resulted in higher periphyton dry weight for the early July deployment period compared to later periods (Figure 2.2 a). Measurements of dry weight for periphyton samples indicated that significant higher periphyton growth in Peat+MFT/SAND treatments (July 7, p=0.048; July 27, p=0.000; August 16, p=0.003) relative to other substrate treatments (water only, sand, MFT/SAND) (Appendix, Table 2.4). Similar to periphyton dry weight estimates, TSS estimates were generally highest in early July (Figure 2.2 b) however, there were no differences between substrate treatments (Appendix, Table 2.5). Measurements of Chl *a* for periphyton samples indicated higher algal growth in Peat+MFT/SAND treatments (Figure 2.2 c). Substrate treatments with Peat+MFT/SAND had significantly elevated periphyton Chl *a* growth estimates (July 7, p=0.000; July 27, p=0.000; August 16, p=0.000) relative to other substrate treatments (water only, sand, MFT/SAND) (Appendix, Table 2.4). Periphyton Chl *a* estimates were also significantly elevated in response to nutrient enrichment on July 7 (p=0.020) and August 16 (p=0.039) (Appendix, Table 2.4). Phytoplankton Chl *a* was low (0.15 µg/L to 4.43 µg/L) for the mid-June sampling period in SWSD (Figure 2.2 d). Unfortunately, samples for the July 7 period were lost. For the July and August sampling periods, maximum mean Chl *a* levels were 47.7 µg/L and 29.8 µg/L, respectively. Phytoplankton Chl *a* estimates were significantly elevated by both Peat+MFT/SAND treatments (August 16, p= 0.027) and nutrient enrichment (August 16, p=0.002).

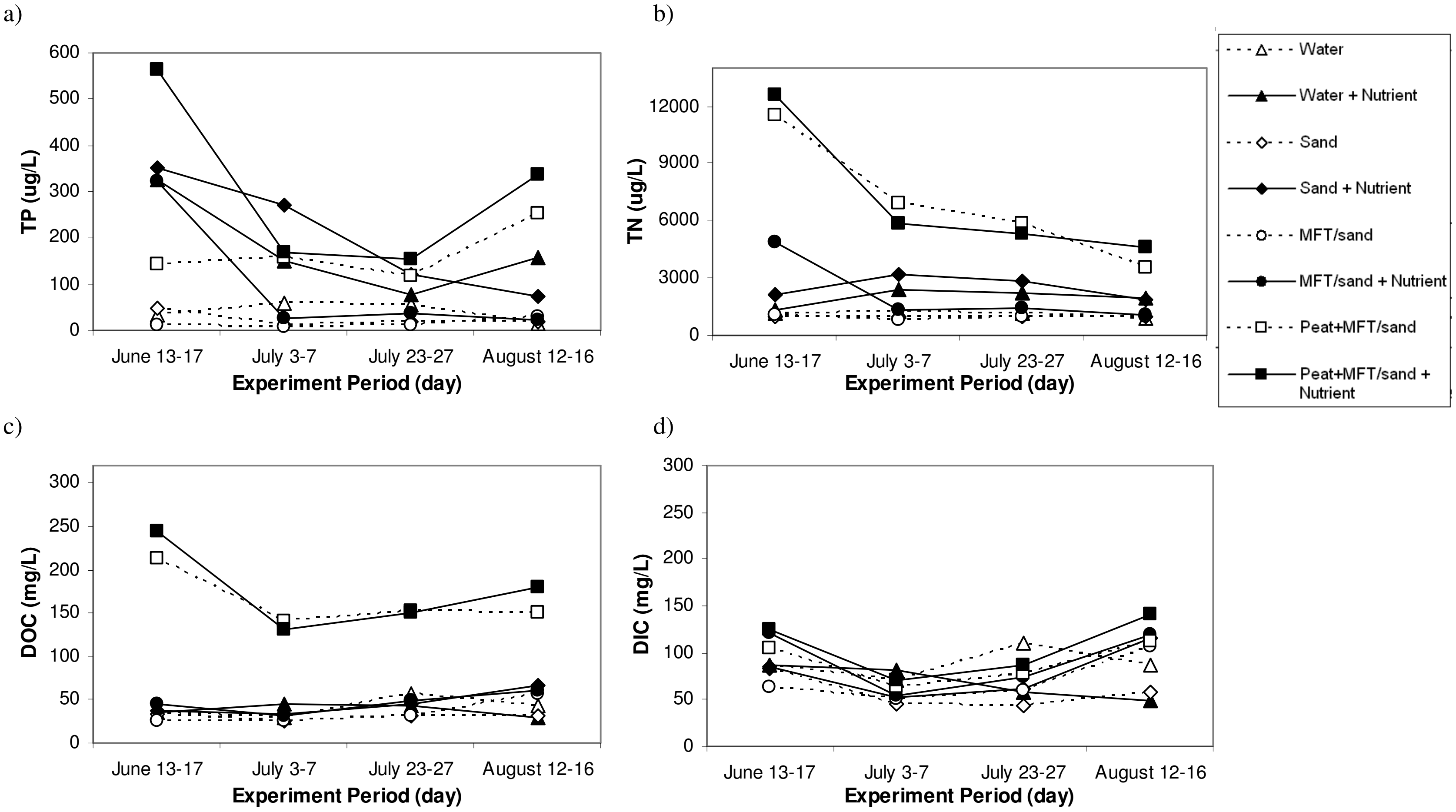


Figure 2.1: Total phosphorus (TP; a), total nitrogen (TN; b), dissolved organic carbon (DOC; c) and dissolved inorganic carbon (DIC; d) concentrations in different microcosms in Shallow Wetland South Ditch from June 13 to August 16 of 2008.

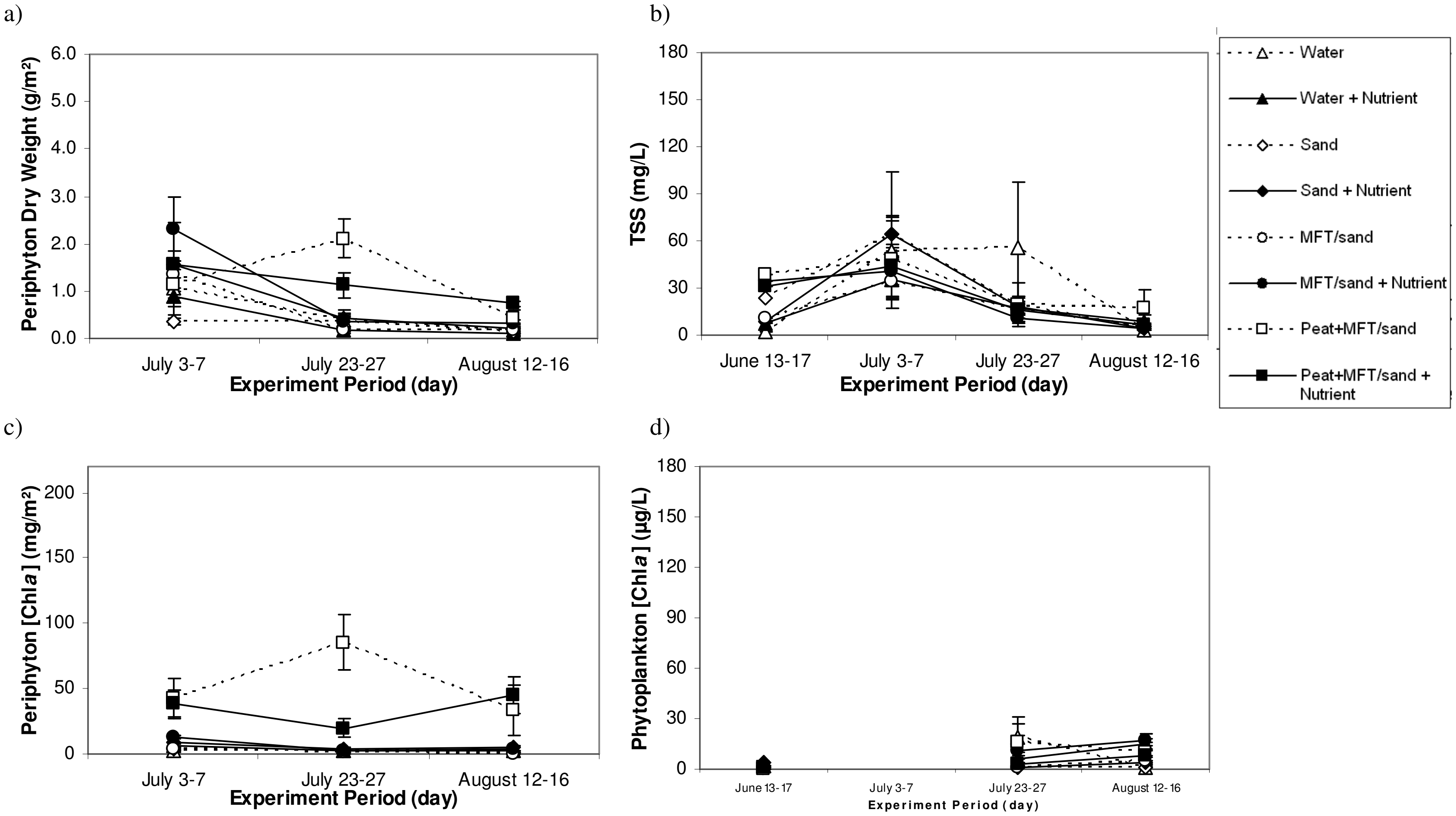


Figure 2.2: Mean ± SD of periphyton dry weight (a), total suspended solid (TSS; b), periphyton Chl *a* (c) and phytoplankton Chl *a* (d) concentrations in different microcosms in Shallow Wetland South Ditch from June 13 to August 16, 2008. Note that phytoplankton Chl *a* samples were missing for the July 3-7 sampling period.

DP – Water Chemistry

At DP, in the microcosms, the temperature ranged from 19 °C to 23 °C. DO ranged from 49.6 to 101.2 % saturation, and pH ranged from 7.0 to 8.7 (Appendix, Table 2.2). TP in microcosms without nutrient enrichment ranged from 17 to 210 µg/L while nutrient-enriched microcosms had concentrations in the range of 20 to 584 µg/L (Figure 2.3 a). All nutrient-enriched treatments had elevated TP for the mid-June sampling period. The Peat+MFT/SAND treatment had higher TP in comparison to other substrate treatments. TP levels decreased by mid-July and remained relatively constant throughout the season (with the exception of the 'water + nutrient' treatment). The concentration of TN ranged from 1240 to 7630 µg/L in microcosms without nutrients and from 1380 to 8700 µg/L in microcosms with nutrient enrichment (Figure 2.3 b). In general, TN was highest in mid-June, with the greatest decrease by early July among nutrient-enriched microcosms. In microcosms with Peat+MFT/SAND, TN was higher compared to other substrate treatments. DOC ranged from 48 to 317 mg/L among microcosms throughout the season (Figure 2.3 c). In some cases, DOC concentrations were depleted in July. Peat+MFT/SAND microcosms had higher levels of DOC in comparison to other substrate treatments. DIC concentrations ranged from 101 to 280 mg/L (Figure 2.3 d). DOC and DIC concentrations had similar seasonal trends with lower concentrations in July and the highest concentrations in August.

DP – Seasonal Estimates of Community Growth

In DP, periphyton dry weight was highest in early July compared to later in the season (Figure 2.4 a). Measurements of dry weight for periphyton samples indicated significantly higher quantitiesin Peat+MFT/SAND treatments (July 3, p=0.048; July 23,p=0.000; August 12, p=0.003) compared to the other treatments (water only, sand, MFT/SAND) (Appendix 2.4). TSS levels were more variable than periphyton dry weight estimates. There was a general trend of decreasing TSS over the season, with the exception of the 'water + nutrients' treatment (Figure 2.4 b). Similar to periphyton dry weight estimates, TSS estimates were significantly higher in Peat+MFT/SAND treatments (July 3, p=0.001; July 23, p=0.002; August 12, p=0.043). Nutrient enrichment also elevated TSS estimates on July 23 (p=0.001) (Appendix 2.5). Periphyton Chl *a* was similar among treatments with the exception of the Peat+MFT/SAND treatments which increased in August (Figure 2.4 c). Peat+MFT/SAND treatments had significantly higher periphyton growth estimates for Chl *a* (July 3, p=0.000; July 23,p=0.000; August 12, p=0.000) (Appendix 2.4). Phytoplankton Chl *a* varied among different treatments with high mean values for some treatments in July (Figure 2.4 d). Peat+MFT/SAND treatments had significantly higher phytoplankton Chl *a* estimates on July 3 (p=0.002) and July 23(p=0.016) (Appendix 2.4).

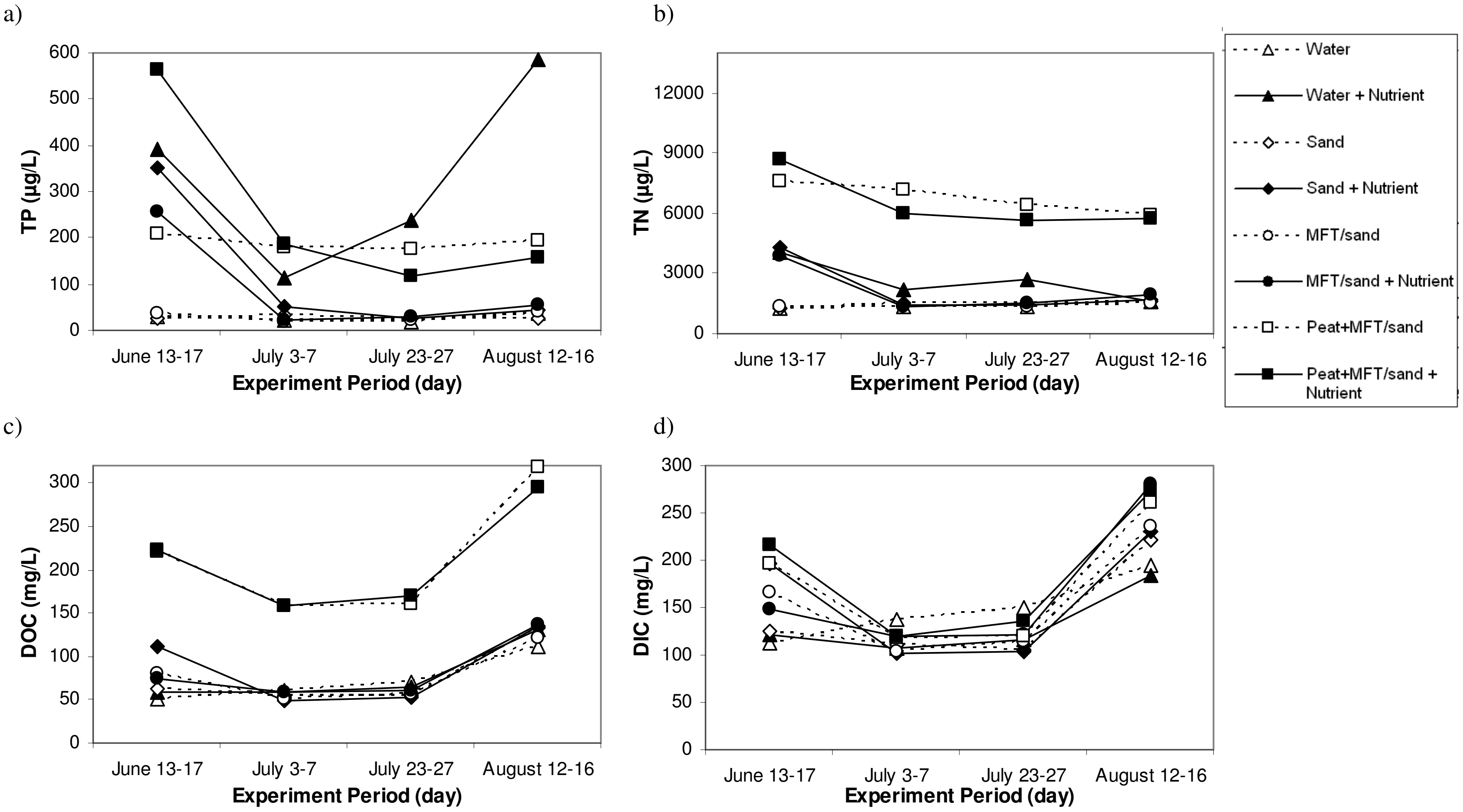


Figure 2.3: Total phosphorus (TP; a), total nitrogen (TN; b), dissolved organic carbon (DOC; c) and dissolved inorganic carbon (DIC; d) concentrations in different microcosms in Demonstration Pond from June 13 to August 16, 2008.

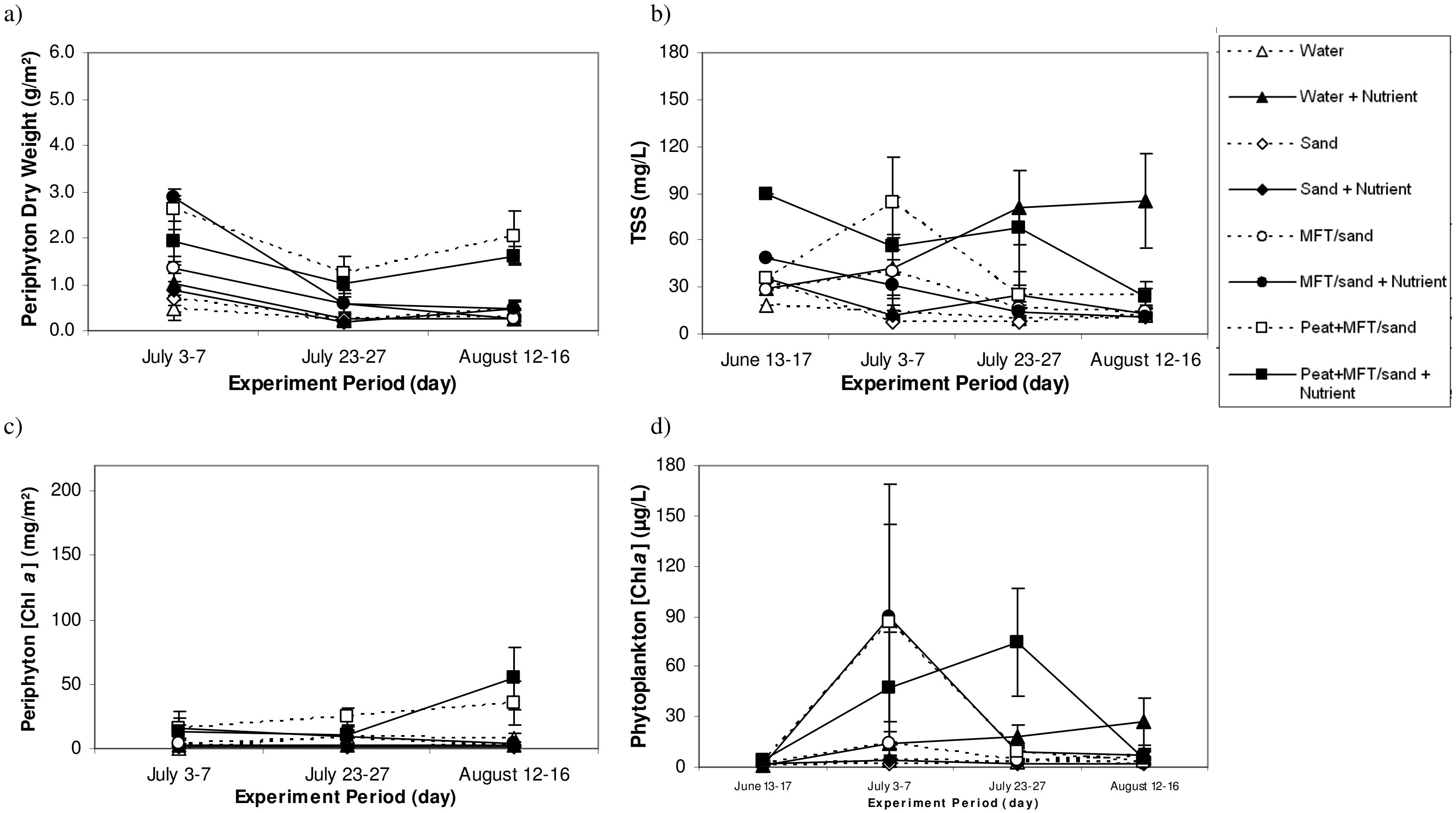


Figure 2.4: Mean ± SD of periphyton dry weight (a), total suspended solid (TSS; b), periphyton Chl *a* (c) and phytoplankton Chl *a* (d) concentrations in different microcosms in Demonstration Pond from June 13 to August 16, 2008.

MP – Water Chemistry

At MP, in the microcosms, the temperature ranged from 16 °C to 22 °C. DO ranged from 46.60 to 128.6 % saturation, and pH ranged from 5.8 to 8.3 (Appendix 2.3). In MP, TP in the microcosms without nutrient enrichment ranged from 4 to 263 µg/L while nutrient-enriched microcosms had concentrations in the range of 32 to 525 µg/L (Figure 2.5 a). All substrate treatments with nutrient enrichment had elevated TP concentrations in mid-June that decreased by early July. The concentration of TN ranged from 657 to 6740 µg/L in the microcosms without nutrient and from 1430 to 10,800 µg/L in the microcosms with nutrient-enrichment (Figure 2.5 b). TN levels in microcosms without nutrient enrichment remained relatively unchanged throughout the season. TN levels in microcosms with nutrient enrichment decreased by early July. Peat+MFT/SAND treatments had higher levels of TN in comparison to other substrate treatments. DOC ranged from 44 to 234 mg/L among all treatments at all sites throughout the season (Figure 2.5 c). In some cases, DOC concentrations were depleted in July. Peat+MFT/SAND microcosms had higher levels of DOC in comparison to other substrate treatments. DIC concentrations ranged from 32 to 137 mg/L (Figure 2.5 d). Similar to DP, the DOC and DIC concentrations in MP varied little among treatments and sampling periods with the exception of DOC for Peat+MFT/SAND treatments.

MP – Estimates of Community Growth

In MP, periphyton dry weight decreased from July to August in Peat+MFT/SAND treatments (Figure 2.6 a). Peat+MFT/SAND treatments had significantly elevated periphyton dry weight estimates (July 5, p=0.000; July 25, p=0.000; August 14, p=0.035) compared to the other substrate treatments (Appendix 2.4). Nutrient enrichment resulted in significant increases in periphyton dry weight estimates on July 5 (p=0.008) and August 14 (p=0.020) (Appendix 2.4). TSS levels remained relatively constant throughout the season; however some treatments increased in late July (Figure 2.6 b). Peat+MFT/SAND treatments had significantly increased TSS estimates on July 5 (p=0.001) (Appendix 2.5). Generally, periphyton Chl *a* decreased in late July and increased in August, with the exception of Peat+MFT/SAND treatments that increased in late July and decreased in August (Figure 2.6 c). Peat+MFT/SAND treatments had significantly elevated periphyton Chl *a* estimates (July 5, p=0.000; July 25, p=0.000; August 14, p=0.002) (Appendix 2.4). Nutrient enrichment also significantly increased periphyton Chl *a* estimates on July 5 (p=0.002) (Appendix 2.4). Phytoplankton Chl *a* varied among treatments with maximum Chl *a* concentrations in July (Figure 2.6 d). Phytoplankton Chl *a* from Peat+MFT/SAND treatments were significantly higher than other substrates treatments (July 5,

$p=0.003$; July 25, $p=0.032$; August 14, $p=0.060$) (Appendix 2.5). Nutrient enrichment also significantly increased phytoplankton Chl *a* estimates on July 5 ($p=0.019$) (Appendix 2.5).

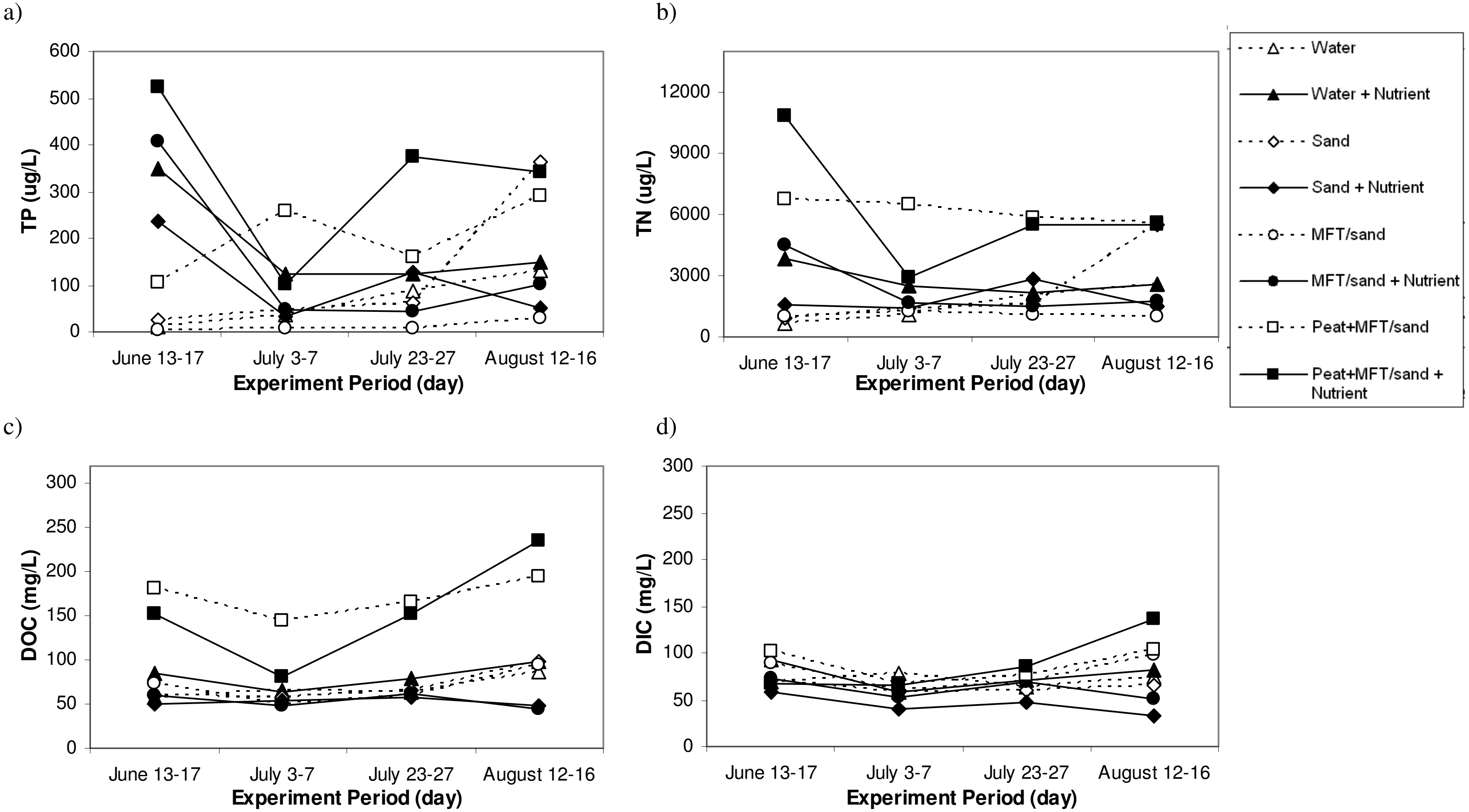


Figure 2.5: Total phosphorus (TP; a), total nitrogen (TN; b), dissolved organic carbon (DOC; c) and dissolved inorganic carbon (DIC; d) concentrations in different microcosms in Mike's Pond from June 16 to August 14, 2008.

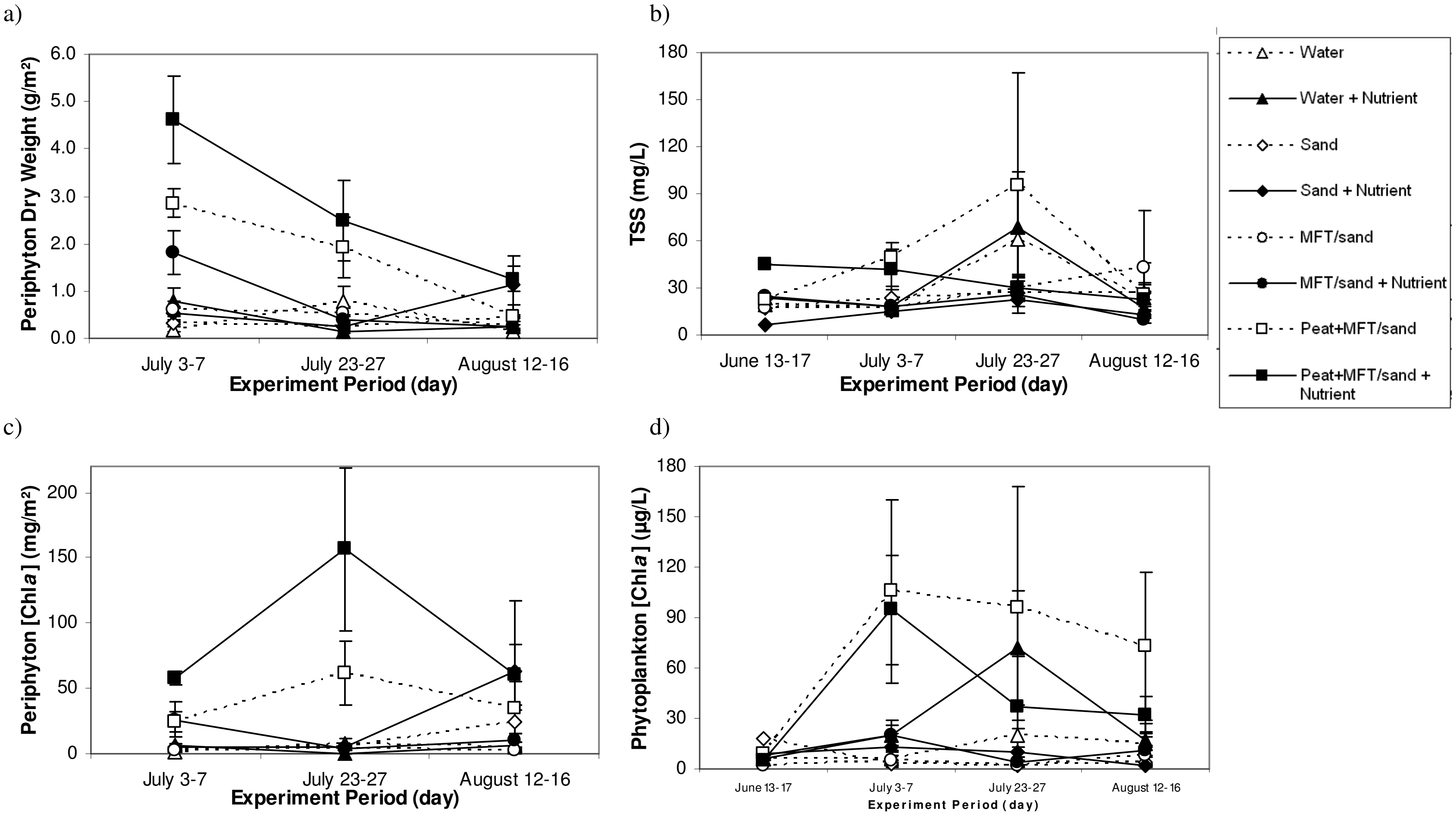


Figure 2.6: Mean ± SD of periphyton dry weight (a), total suspended solid (TSS; b), periphyton Chl *a* (c) and phytoplankton Chl *a* (d) concentrations in different microcosms in Mike's Pond from June 16 to August 14, 2008.

2.4.3 Cumulative Dry Weight and Chlorophyll *a* Estimates

Seasonal measurements for periphyton dry weight and Chl *a*, TSS and phytoplankton Chl *a* were combined per endpoint for the 3 or 4 sampling periods in 2008 to provide mean cumulative estimates per treatment. Due to the loss of SWSD phytoplankton Chl *a* samples for the July 3-7 sampling period, cumulative estimates for phytoplankton Chl *a* were calculated using 3 samples periods (mid-June, late July and mid-August) for all of the study sites.

Periphyton Dry Weight and Chl *a*

For periphyton, Peat+MFT/SAND treatments had the highest cumulative periphyton dry weight among substrate treatments for all study sites (Figure 2.7). Peat+MFT/SAND treatments had significantly increased cumulative periphyton dry weight estimates at all study sites (SWSD, p= 0.002, DP,p=0.000, MP, p=0.000) (Appendix, Table 2.10). There were no statistical differences for cumulative periphyton dry weight between treatments with and without nutrient enrichment in any of the sites. Cumulative periphyton Chl *a* was higher in Peat+MFT/SAND treatments relative to other substrate treatments for all study sites, similar to cumulative periphyton dry weight (Figure 2.8; Appendix 2.7). Peat+MFT/SAND treatments had significantly increased cumulative periphyton Chl *a* estimates at all study sites (SWSD, p= 0.000, DP,p=0.000, MP,p=0.000) (Appendix 2.10). There were no statistical differences for cumulative periphyton Chla between treatments with and without nutrient enrichment in any of the sites.

TSS and Phytoplankton Chl *a*

Trends for cumulative TSS among substrate treatments within a study site were less pronounced than cumulative periphyton dry weight (Figure 2.9). Cumulative TSS estimates were significantly elevated by nutrient enrichment (p=0.037) and elevated in Peat+MFT/SAND treatments (p=0.000) in DP, but not at the other study sites (Appendix, Table 2.10). Cumulative phytoplankton Chl *a* estimates were higher for treatments in DP and MP relative to the reference site (SWSD), particularly for Peat+MFT/SAND treatments (Figure 2.10). Peat+MFT/SAND treatments had significantly higher cumulative phytoplankton Chl *a* estimates in MP (p=0.011) (Appendix, Table 2.10). There were no statistical differences for treatments with or without nutrient enrichment for either of the endpoints measured (TSS or Chl *a*).

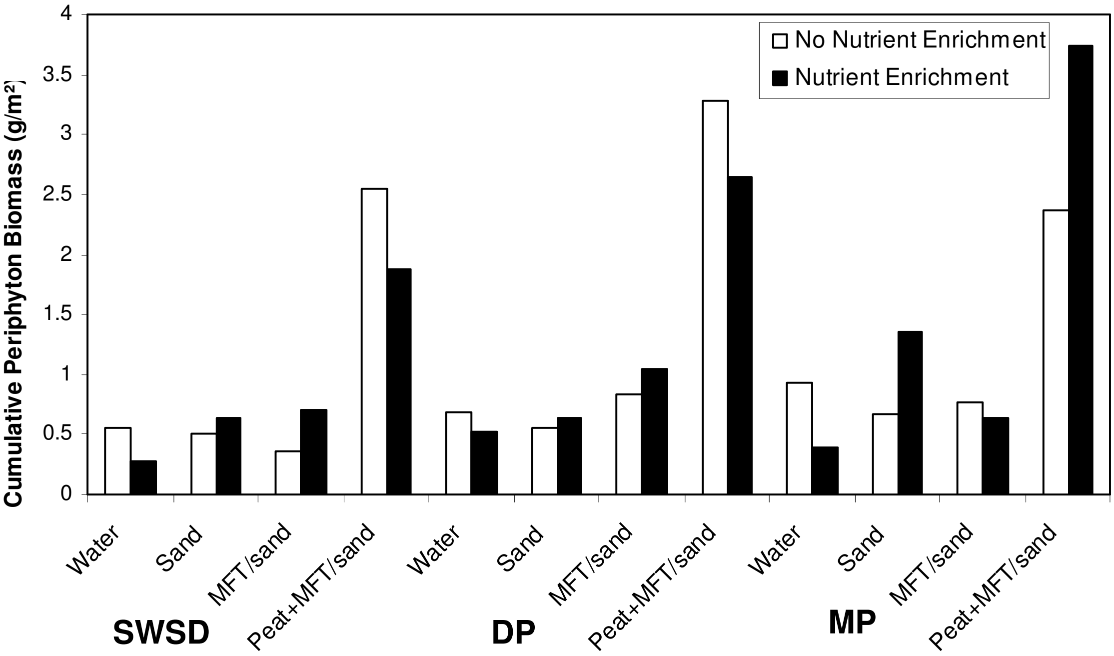


Figure 2.7: Cumulative periphyton dry weight for treatments in SWSD, DP and MP.

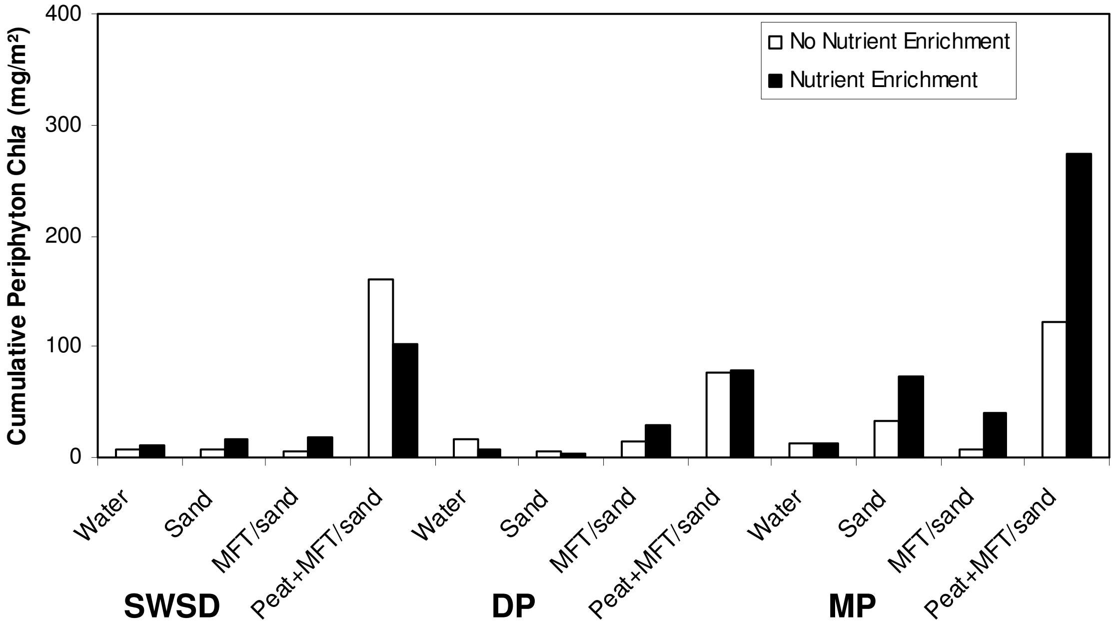


Figure 2.8: Cumulative periphyton Chl *a* concentration for treatments in SWSD, DP and MP.

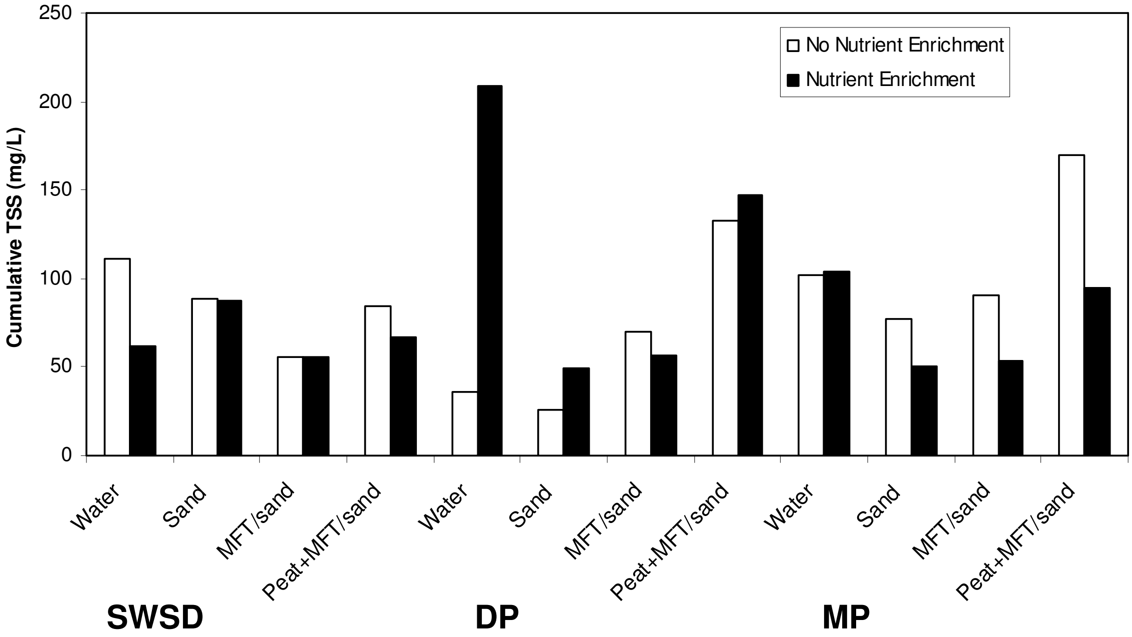


Figure 2.9: Cumulative TSS for treatments in SWSD, DP and MP.

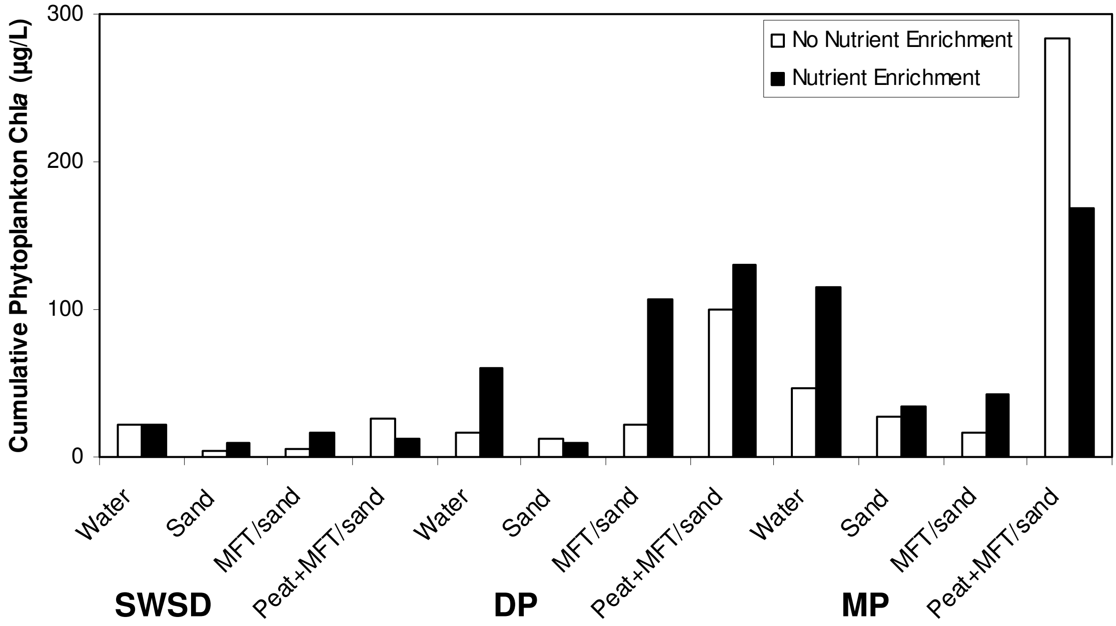


Figure 2.10: Cumulative phytoplankton Chl *a* concentration for treatments in SWSD, DP and MP.

2.4.4 Correlations between Nutrients and Community Growth Estimates

To identify the nutrients that contributed to enhancing growth estimates, statistical analyses were applied to nutrient factors (DOC, DIC, TN, TP) and measured growth estimates (Table 2.3). DOC was positively correlated to periphyton endpoints (dry weight and Chl *a*), and phytoplankton Chl *a*, while DIC was not correlated to any of the measurement endpoints. TN and TP were positively correlated to all growth endpoints with the exception of TP and periphyton dry weight.

Table 2.3 Pearson correlation analysis for nutrient factors and measurement endpoints (two-tailed significant).

Variable		**Periphyton Dry Weight**	**Periphyton Chl *a***	**TSS**	**Phytoplankton Chl *a***
DOC	Correlation	**0.329	**0.521	0.139	*0.280
	Sig.	0.005	0.000	0.245	0.025
DIC	Correlation	-0.019	0.009	-0.104	-0.096
	Sig.	0.875	0.941	0.385	0.449
TN	Correlation	**0.485	**0.586	**0.395	**0.509
	Sig.	0.000	0.000	0.001	0.000
TP	Correlation	0.208	**0.464	**0.381	*0.319
	Sig.	0.80	0.000	0.001	0.010

*correlation is significant at 0.05, **correlation is significant at 0.01 (two-tailed).

2.5 Discussion

This study evaluated phytoplankton and periphyton growth in microcosms amended with nutrients (N & P) and/or peat to promote enhanced growth over MFT used as a substrate. The study indicated that peat-amended MFT/SAND treatments significantly elevated periphyton and phytoplankton growth (Appendix 2.4, 2.5). Peat-amended microcosms maintained higher levels of nutrients (DOC, TN and TP) throughout the growing season. In comparison, nutrient enrichment showed insignificant increases in periphyton and phytoplankton growth. The results suggested that peat amendment could be utilized as a valuable tool in enhancing oil sands wet-landscape reclamation.

2.5.1 Impacts of Oil Sands Process Water on Phytoplankton

Phytoplankton Species Composition

The microcosms in this study were held in three different oil sands reclamation sites that varied greatly in terms of water chemistry (ie. NA concentration and conductivity). Differences in water chemistry among these sites may also influence the abundance and composition of algal species within the microcosms. Several studies have evaluated the influence of NAs and conductivity on phytoplankton community composition (Leung et al. 2001; Leung et al. 2003; Hayes 2005). Leung et al. (2003) concluded that phytoplankton community composition would not be affected in aquatic systems with NAs and conductivity of less than 6.5 mg/L and 800 µS/cm, respectively. However, at moderately elevated NA concentrations (8-21 mg/L), characteristic of DP, Leung et al. (2001) identified six NA tolerant taxa, including *Botryococcus braunnii* and *Chlamydomonas* spp. (Chrysophyta), *Oscillatoria* spp. (Cyanophyta), and *Navicula* spp. and *Nitzschia* spp. (Bacillariophyta). Hayes (2005) also found that certain species were highly correlated to NAs including *Glenodinium* spp., *Gymnodinium* spp. (Dinoflagellate), *Peridinium cinctum* (Hydrodictyaceae) and to conductivity (*Botryococcus braunii, Chryoscoccus rufescens, Cryptomonas* spp.). Based on a survey of natural and reclaimed aquatic systems by Hayes (2005), threshold effect levels for phytoplankton community composition were 30 mg/L and 1000 µS/cm for NAs and conductivity, respectively. Although species composition of phytoplankton was not identified in the current study, there is evidence from earlier work that there are likely differences in species composition between the reference site (SWSD: low NAs and conductivity), and OSPM sites (DP: moderate NAs and conductivity; MP: high NAs and conductivity) due to differences in NA concentration and conductivity (Table 2.1). The presence of established phytoplankton communities with some species tolerant of elevated levels of NAs and conductivity may explain the higher Chl *a* estimates in MP microcosms particularly in the Peat+MFT/SAND treatment.

Phytoplankton Community Growth

NAs are considered to be a major contributor to OSPW toxicity based on toxicity tests conducted using bacteria (Clemente et al. 2004), however the effects of NAs on phytoplankton community growth are still unclear. Studies that measured phytoplankton Chl *a* in microcosms (Leung et al. 2001; Hayes 2005) or in natural and constructed ecosystems (Hayes, 2005) found no correlation between NA concentration and phytoplankton community growth estimates. Leung et al. (2001) also found no correlation between NA concentration and phytoplankton biomass (Uttermöhl method) in microcosm studies, but observed the highest phytoplankton Chl *a* (21.8 µg/L) in Mildred Lake settling basin water with elevated NA concentration ([NA] - 59.9 mg/L, Leung et al. 2001).

Measurements of phytoplankton Chl *a* were often higher in nutrient-enriched and/or peat amended microcosms with OSPW from MP and DP. The reference site, SWSD, had the lowest Chl *a* estimates among the three study sites. Water from MP had the lowest concentrations of TN, TP, DOC and DIC among the study sites (Table 2.2); concentrations of TN, TP and DOC were elevated in nutrient-enriched and/or peat amended microcosms (Figure 2.5). Numerous studies have suggested that an ecosystem which is more nutrient deprived would have a stronger short term response to nutrient addition (Levine and Schindler 1999; Roelke et al. 1999; Zimmer et al. 2003; Liboriussen and Jeppesen 2006). Nutrient deprivation in MP may explain why the highest Chl *a* levels were reported for nutrient-enriched and/or peat amended treatments in MP for both phytoplankton and periphyton.

The presence of filamentous algae in microcosms likely had an impact on phytoplankton and periphyton growth estimates. During the course of the experiment, the presence of filamentous algae was observed within several microcosm units, particularly within nutrient-enriched treatments. Filamentous algae strands can form dense floating mats on the water surface, reducing light penetration in the water column and thus limiting light for phytoplankton and periphyton photosynthesis. Similar to most algae, filamentous algae are able to uptake ammonium or nitrate, but ammonium is often preferred because it can be used in a more direct fashion than nitrate in the biosynthesis of amino acids (Andersen 2005). Utilization of dissolved nutrients by filamentous algae may also reduce nutrient availability for phytoplankton and periphyton growth. Although no studies have addressed the growth of filamentous algae in oil sands reclamation, to the best of the authors` knowledge, it is an important consideration that requires further understanding.

2.5.2 Impacts of Reclamation Substrates on Phytoplankton and Periphyton Community Growth

Aquatic reclamation options may involve the use of sand or proportions of MFT and sand to line newly created wetlands. In this study, phytoplankton and periphyton community growth estimates were evaluated in microcosms containing no substrate (Water only) as well as microcosms with substrates that are potential reclamation materials (SAND, MFT/SAND). The addition of sand did not appear to change concentrations of TN, TP, DOC or DIC in the water. In general, microcosms with SAND treatments had similar or slightly lower phytoplankton and periphyton growth estimates (ie. dry weight and Chl *a*) than controls (water only) (Appendix, Table 2.4, 2.5). In nutrient-enriched SAND treatments, community growth estimates were slightly lower in comparison to nutrient-enriched water treatments (control). A previous study, that examined the growth of macrophytes in different types of oil sands reclamation materials, reported less macrophyte growth in sand relative to other substrates (Luong, 1999). It was suggested that lower macrophyte growth may be the result of sand functioning as a nutrient sink in the aquaria, effectively reducing available nutrients in the water column (Luong 1999).

The addition of MFT/SAND did not appear to change concentration of TN, TP, DOC or DIC in the water column. In general, microcosms with a MFT/SAND treatment have similar or slightly lower phytoplankton and periphyton growth estimates compared to water treatments (control). In comparison with SAND treatment, MFT/SAND had slightly higher growth estimates in both OSPM-affected sites (DP and MP), but slightly lower growth estimates in the reference site (SWSD). In nutrient-enriched MFT/SAND treatments, community growth estimates were slightly higher in comparison to nutrient-enriched water treatments. In comparison with nutrient-enriched SAND treatments, MFT/SAND had slightly higher growth estimates in SWSD and DP, but slightly lower in MP.

In some MFT/SAND treatments, growth estimates were lower in comparison to sand treatments in SWSD and MP. This could be a function of both physical and chemical differences between the treatments. The quantity of MFT used in the microcosms could increase concentrations of toxic oil sands constituents (ie. NAs and PACs) and negatively affect growth. In addition, MFT/SAND treatments had increased turbidity due to a high proportion of fine clay particles in MFT. Increased turbidity, limiting the amount of sunlight to the system, can reduce primary production (Andersen 2005).

2.5.3 The Effects of Peat Amendment and Nutrient Enrichment

Peat+MFT/SAND treatments had significantly higher growth estimates in comparison to the other substrate treatments (Appendix, Table 2.4, 2.5). Peat-amended microcosms had significantly elevated concentrations of TN (~6 mg/L), TP (~150 µg/L) and DOC (~150 mg/L) that remained high throughout the growing season. In this study TN, TP and DOC were found significantly correlated to growth estimates (Table 2.3). The quantity and possibly the quality of nitrogen, phosphorous and DOC supplied by the peat material contributed to high growth estimates in Peat+MFT/SAND treatments. Many studies had indicated the positive correlation between nutrients and primary producers (Levine and Schindler 1999; Roelke et al. 1999; Twomney and Thompson 2001; Piehler et al. 2004; Liboriussen and Jeppesen 2006; Lagus et al. 2007; Wang et al. 2008), however few have examined nutrient sources from peat material.

Overall, estimates of community growth showed positive responses to available nutrients in the water column (Table 2.3). Seasonal trend graphs for nutrient-enriched microcosms had decreased levels of TN and TP from mid-June (day 0 of experiment) to early-July (second sampling point), suggesting the utilization of added nitrogen and phosphorous in these systems. Both TN and TP were found positively correlated to periphyton dry weight, periphyton Chl *a* and phytoplankton Chl *a*, indicating the effect of nutrients on community growth.

In this study, nutrients added as a single pulse had little effect (statistically insignificant) on promoting community growth. Some studies have shown that different nutrient treatment applications could also have effects on phytoplankton composition and total biomass (Roelke et al. 1999; Buyukates and Roelke 2005). Roelke (1999) found that pulses of nutrients caused dramatic changes in phytoplankton community composition in comparison to a continuous nutrient supply. Slower growing algae were unable to accumulate biomass in pulsed system due to nutrient competition with faster growing algae. Buyukates and Roelke (2005) showed that pulsed inflow resulted in greater zooplankton biomass, which subsequently prevented higher accumulation of phytoplankton biomass.

2.6 Conclusion

In this study, different reclamation materials were utilized as substratea (SAND, MFT/SAND). SAND treatments had slightly lower phytoplankton and periphyton growth in comparison with water-only treatments (control). This might be explained by sand acting as a nutrient sink and reducing the available nutrients in the water column. MFT/SAND treatments had slightly higher phytoplankton and periphyton growth than SAND treatments in OSPM-affected sites (DP and MP), but slightly lower growth in reference site (SWSD). Phytoplankton and periphyton from OSPM-affected sites may have more tolerant species than the reference site.

Nutrient enrichment had an insignificant effect on increasing phytoplankton and periphyton growth throughout the growing season with the exception of increased growth immediately following the nutrient addition. Peat amendments significantly increased the phytoplankton and periphyton growth in all substrate treatments. Peat amendment provided the system with high level of DOC, TN and TP to support phytoplankton and periphyton growth throughout the study period. The results suggested that both nutrient enrichment and peat amendment both had the potential to be utilized as valuable tools in enhancing growth to contribute to a biological detrital layer in oil sands wet-landscape reclamation. Although peat amendment might be a more effective option due to its ability to significantly increase phytoplankton and periphyton growth over a long period of time.

Further research is required to define the optimal quantity of peat for reclamation. Future research should focus on determining the specific amount of peat required for optimal primary growth without causing significant pH changes.

Chapter 3. Microcosm Study of Benthic Invertebrate Colonization on Oil Sands Reclamation Materials

3.1 Overview

Microcosms in three experimental reclamation wetlands with different types of OSPM as bottom substrates (sand, mature fine tailing + sand, peat amended mature fine tailing + sand) were deployed in the summer of 2008 to examine initial benthic invertebrate colonization. In the summer of 2008, closed microcosms were treated with nutrients to enhance primary production, creating a biological detrital layer over OSPM substrates to potentially improve initial benthic invertebrate colonization. In the fall of 2008, the closed microcosms were converted to open microcosms to allow benthic invertebrate colonization. In the summer of 2009, benthic invertebrate samples were collected from the microcosms, as well as from the mature sediments in each of the three wetlands. There was lower benthic invertebrate abundance and fewer numbers of families in OSPM-affected wetlands in comparison to the reference wetland. Sand as a substrate resulted in increased total abundance in the reference wetland, whereas in OSPM-affected wetlands, total abundance was lower in sand treatments compared to the mature sediments. In comparison, mature fine tailing + sand as bottom substrate had lower total abundance and fewer numbers of families than sand treatments, with the exception of OSPM-affected wetlands that received mature fine tailing as input during its construction. This study suggested that benthic invertebrates have an increased tolerance to MFT as a function of MFT exposure history based on the measurement endpoint, total abundance. Peat amendment and nutrient enrichment did not show any significant impact on benthic invertebrate abundance or composition.

3.2 Introduction

Benthic invertebrates are functionally important to the flow of nutrients and energy in aquatic ecosystems, interacting directly and/or indirectly with phytoplankton, macrophytes, zooplankton and fish (Stockley et al. 1998; Covich et al. 1999). In oil sands aquatic reclamation, the health and sustainability of any oil sands reclamation strategy depends on the capability to create both a physically and chemically favorable habitat to support an abundant and diverse benthic invertebrate community. The ultimate goal of aquatic reclamation is to incorporate waste, referred to as oil sands process material (OSPM) and oil sand process water (OSPW), into created wetland ecosystems that will be functionally equivalent to wetlands on the pre-mined oil sands landscape. Both OSPM and OSPW contain natural and process related oil sands compounds that are of environmental concern. OSPM, for example, mature fine tailings (MFT), contain high levels of salts (total ion content >2000 mg/L) and dissolved organic compounds (NAs, 70-100 mg/L) (Leung et al. 2003). Consolidated tailings (CT), which is a combination of MFT and gypsum, also contains high levels of NAs and salts (MacKinnon et al. 2001). Concentrations of PAHs found in OSPM-affected wetlands were 1.5-150 fold greater in sediments and 4-6 fold greater in water compared to natural reference wetlands (Smits et al. 2000). Shallow Wetland South Ditch, an oil sands reference wetland, had PAH concentrations of 0.33 µg/mL in the water (Ganshorn 2002) and 89.2 ng/g dry weight in the sediments (Smits et al. 2000). Tailings water with elevated levels of PAHs and NAs have been shown to be acutely toxic to aquatic organisms at various trophic levels (Lai et al. 1996). Aquatic toxicity test results have indicated that fresh waste water derived from MFT and CT processes were acutely toxic to *Selenastrum capricornutum* (Warith and Yong 1994), *Daphnia magna* (MacKinnon et al. 2001), and *Onchorhynchus mykiss* (MacKinnon et al. 2001). MFT-derived water showed chronic toxicity toward *D. magna* and *O. mykiss* after one or two years of aging (MacKinnon and Boerger 1986).

In the past 20 years, numerous experimental oil sands aquatic reclamation sites have been constructed to study the impacts of OSPW and OSPM on benthic invertebrate communities. Whelly (1999) examined wetlands in the oil sands region of northern Alberta and found OSPW-affected wetlands had reduced macroinvertebrate richness compared to reference wetlands, although not significant. In another study, benthic invertebrate abundance was significantly lower in an OSPM-affected pond (DP) compared to natural lakes in the region (Gould, 2000). Gould (2000) suggested that factors such as unsuitable substrate (impact of high clay/silt content on burrowing invertebrates), low level toxicity, high turbidity (reduced plant cover as habitat) and fish predation likely contributed to the reduction of total abundance of benthic invertebrates in DP. Ganshorn (2002) compared benthic (Chironomidae: Tanypodinae) and pelagic (Chalboridae: *Chaoborus*) dipteran populations and found greater Tanypodinae densities and PAC body burdens (indicative of PAC exposure from sediments) in OSPM-affected wetlands vs. reference wetlands. These studies indicate definite exposure to toxic constituents (i.e., PACs) and potential invertebrate species sensitivity in OSPM-affected wetlands that could be ameliorated via substrate amendments.

Few studies have examined the impacts of different amendments to OSPM on benthic invertebrate richness and abundance. Successful amendments likely play a critical role in the development of new wetland reclamation. In a study by Leonhart (2003), only younger OSPM-affected wetlands (<7 years old) had significantly fewer numbers of benthic invertebrate families compared to reference wetlands (constructed or opportunistic wetlands without OSPM input) of equivalent age, indicating reduced benthic invertebrate colonization in newer OSPM-affected wetlands. Amendments, such as the addition of petroleum coke, had no effect on benthic invertebrate richness or abundance in OSPM-affected wetlands, however in reference wetlands, fewer invertebrate species were found on coke vs. control plots which was likely due to the avoidance of petroleum coke by intolerant benthic invertebrate species (Baker, 2007). In the same study, peat amendments did not affect invertebrate richness or total abundance in either reference wetlands or OSPM-affected wetlands.

In this microcosm study, nutrients and/or peat were used as amendments to enhance the initial benthic invertebrate colonization on different reclamation substrates. Both amendments provide nutrients to stimulate phytophlankton and periphyton growth, which contributes to the biological detrital layer over unfavorable OSPM. Peat also provides additional organic matter, reducing exposure to OSPM. The objective of the current study was to evaluate benthic invertebrate richness (number of families) and total abundance in microcosms with different OSPM (sand, MFT/SAND) used as a substrate in the presence and absence of amendments (nutrients and/or peat). To accomplish this goal, closed microcosms were amended with nutrients and/or peat to optimize phytoplankton and periphyton growth in year one (summer of 2008, Chapter 2) and then converted to open microcosms (fall of 2008) to allow benthic invertebrate colonization which was assessed in year two (summer of 2009). Three constructed wetlands were selected for the microcosm study: a reference wetland with no OSPM/OSPW and two OSPM-affected wetlands influenced by either MFT or CT. Benthic invertebrate samples were collected from microcosms and the surrounding mature sediments at all three study sites. The results of this research will determine the usefulness of nutrient and/or peat amendments of OSPM to enhance benthic invertebrate colonization in new oil sands wetland reclamation.

3.3 Materials and Methods

3.3.1 Oil Sands Reclamation Study Sites

In 2009, benthic invertebrate and sediment samples were collected from the microcosms deployed in the summer of 2008, as well as from the surrounding mature sediments in one reference and two OSPM/OSPW-affected reclamation study sites. Shallow Wetland South Ditch (SWSD), a reference site, was a constructed wetland with no OSPM or OSPW. Demonstration Pond (DP; Syncrude Test Pond #11), an OSPM-affected site, received MFT and surface drainage water. Mike's Pond (MP), another OSPM-affected site, received CT released water. Further details of the study sites are provided in Chapter 2.

3.3.2 Microcosm Design and 2008 Biomonitoring

A detailed outline of the microcosm design is provided in Chapter 2. In each of the three study sites, microcosms used for benthic invertebrate assessments contained three types of substrates: process sand (Sand; control), a mixture of 50:50 volume to volume MFT/SAND (MFT/SAND) and the addition of 2 cm of peat on top of the MFT/SAND mixture (Peat+MFT/SAND). On June 13 of 2008, half of the 78 microcosms were treated with nutrients (2 treatments: control - no nutrients; and nutrient-enrichment - 426 mg of ammonia nitrate (NH_4NO_3; N, 3731µg/L) and 68 mg of potassium phosphate (KH_2PO_4; P, 385µg/L) per 40L of water) and sampled throughout the growing season (mid-June to mid-August) of 2008 for estimates of phytoplankton and periphyton community growth (dry weight and Chl *a*; see Chapter 2). On September 10, 2008, the top bin of each microcosm was removed for all three substrate treatments (Sand, MFT/SAND, Peat+MFT/SAND) to allow benthic invertebrate colonization.

3.3.3 2009 Microcosm and Field Sample Collection

Benthic Invertebrate Sampling using Artificial Substrates

Artificial substrates were used to collect epibenthic (at and above the sediment surface) and epiphytic (living on plants) invertebrates (Leonhardt 2003). The use of artificial substrates are advantageous for collecting a wider range of taxa compared to the core sampling methods and are more quantitative than the D-net sweep sampling methods (Leonhardt 2003).

Artificial substrates were constructed using ceramic tile (17.7 x 17.7 cm) as a base to mount five 10-cm long sections of plastic aquarium plants (mimicking *Elodea*) as described in Leonhardt (2003). The plastic plants were glued to the unglazed side of the ceramic tile at equal distances from each other using waterproof silicon caulking (Leonhardt 2003). Red and white plastic bobbers were attached to each artificial substrate using monofilament fishing line to serve as a surface marker.

At each study site, the post-winter condition of the microcosms were assessed and one artificial substrate was placed in the centre of each intact microcosm. In addition, five artificial substrates were placed on mature sediments (outside of the microcosms) within each study site to determine benthic invertebrate composition and total abundance for each site. Artificial substrates were deployed for eight days (SWSD, July 14-21; DP, July 15-22; MP, July 16-23), which allowed sufficient time for colonization based on the research by Leonhardt (2003). After eight days, the tiles were retrieved using a 250 µm square sieve net (18 x 18 cm) and a collection bucket. The sieve net was gently placed on top of the tile to seal all contents on top of the tile. The tile was then lifted out of the water slowly with the sieve net on top. A rinse bottle containing pre-sieved study site water was used to wash the contents of the tile into the sieve net. The sample in the 250 µm sieve net was then rinsed several times to remove fine particles and retain organisms larger than 250 µm. After rinsing, the sample was transferred into a labelled 4-L polyethylene soil bag and preserved with a formalin-ethanol solution (10:5:2 ratio of water: 95% ethanol: 100% formalin). All samples were shipped to the University of Waterloo for processing.

Sediment Samples

Sediment samples were collected from each microcosm, as well as from the study sites, immediately after the retrieval of the artificial substrates. One sediment sample was collected from each microcosm unit, and five samples from each study site. Grab samples (50 g) of surface (~5 cm depth) sediments were transferred into small polyethylene soil bags. The samples were kept cool on ice and later frozen at -20°C prior to analysis.

3.3.4 Laboratory Methods

Benthic Invertebrate Assessment

For taxonomic resolution, samples were identified to family (Clifford 1991). Each sample was sorted under the dissecting microscope and stored in labelled vials for possible further identification in the future. Total abundance was calculated as numbers per m^2.

Sediment Analysis

Sediment samples were analyzed in the laboratory for organic content following the methods used by Leonhardt (2003). Approximately 50 g of sediment was used for the loss on ignition method to determine organic content.

3.3.5 Statistical Analysis

All statistical analyses were done using SPSS 17 statistical program (Conover 1980). Due to the relatively small sample size, alpha level was set to 0.05 to detect any significant relationship among variables. Measured variables underwent analyses including number of families and total abundance of benthic invertebrates and sediment organic content. To examine differences in nutrient enrichment (with enrichment, without enriched) and substrate type (Sand, MFT/Sand mix, Peat+MFT/Sand mix) for the measured endpoints, a two factor ANOVA test was performed. *Post hoc* multiple comparisons were made using the Bonferroni test to determine which substrate type(s) differed from the other(s) for the measured parameters.

3.4 Result

3.4.1 Water and Sediment Characteristics of Study Sites

Basic physical and chemical characteristics of the study sites were measured in July 2009 at the time of benthic invertebrate collections. MP had the highest conductivity and salinity among the three study sites, followed by DP and SWSD (Table 3.1). SWSD had lower dissolved oxygen (DO) level in comparison with DP and MP (Table 3.1).

Table 3.1 Water parameters for three oil sands reclamation sites sampled for benthic invertebrate assessments (July, 2009).

Wetland	**Age in 2009 (year)**	**Temp. (°C)**	**pH**	**DO* (mg/L)**	**Salinity**	**Conductivity (µS/cm)**
SWSD	16	18.2	6.69	5.07	0.22	453
DP	16	20.7	7.38	11.88	0.88	1732
MP	11	19.7	7.72	9.93	2.36	4403

*DO – dissolved oxygen

Sediment samples from each microcosm were analyzed for organic content (Table 3.2). Although there was high variability among replicates, MFT/SAND and Peat+MFT/SAND had higher organic content than sand for all three sites, as predicted. Organic content varied among non nutrient-enriched and nutrient-enriched treatments.

Table 3.2 Sediment organic content among different microcosm treatments and mature sediments from SWSD, DP and MP.

	Treatment		Organic Content	
Site	**Sediment**	**Nutrient Enrichment**	**% (n)**	**SD**
SWSD	Mature Sediment	-	1.68 (3)	0.37
	Sand	No	2.28 (4)	2.61
		Yes	0.40 (4)	0.29
	MFT/SAND	No	1.71 (4)	0.82
		Yes	2.57 (4)	1.04
	Peat+MFT/SAND	No	3.19 (3)	3.13
		Yes	4.49 (4)	3.34
DP	Mature Sediment	-	0.88 (5)	1.02
	Sand	No	1.42 (4)	0.90
		Yes	0.39 (5)	0.27
	MFT/SAND	No	2.91 (3)	1.15
		Yes	2.85 (3)	1.28
	Peat+MFT/SAND	No	2.97 (3)	1.23
		Yes	2.35 (3)	0.22
MP	Mature Sediment	-	-	-
	Sand	No	1.57 (2)	1.81
		Yes	0.35 (3)	0.11
	MFT/SAND	No	1.50 (3)	1.21
		Yes	2.72 (4)	1.72
	Peat+MFT/SAND	No	2.17 (3)	0.43
		Yes	2.02 (4)	0.60

3.4.2 Benthic Invertebrate Assessment

Benthic Invertebrate Assessment of Mature Sediment

Benthic invertebrate samples were collected from mature sediments at each test site to qualify and quantify the benthic invertebrate communities in ecosystems with different exposure histories. Samples collected from the mature sediments of the reference wetland (SWSD) had the highest total abundance of 2651 per m^2 (Table 3.3). In comparison, OSPM sites had lower total abundance ((DP 602; MP 1396 per m^2). For SWSD, a total of 10 families were represented, which is higher in comparison to the OSPM sites (DP, 3 families; MP, 4 families). At all sites, Chironomidae and

Amphipoda were the most common families, representing greater than 80 % of the total numbers of benthic invertebrates (Table 3.4). Gastropoda accounted for 4.1 % of the benthic invertebrates collected in SWSD but was not found in DP and MP.

Table 3.3 Total abundance of benthic invertebrates collected from artificial substrates on the mature sediments of each study site (n=5).

Site	Total Abundance (per m^2)	
	Mean (n)	**SD**
SWSD	2651 (5)	2317
DP	602 (5)	592
MP	1396 (3)	831

Table 3.4 Percentage of benthic invertebrates collected from artificial substrates on the mature sediments of each test site (n=5).

	Site		
Families	**SWSD**	**DP**	**MP**
Oligochaeta	0.4%	-	2.4%
Amphipoda	48.7%	48.0%	35.5%
Ephemeroptera	3.2%	-	-
Anisoptera	2.0%	19.6%	1.3%
Plecoptera	0.1%	-	-
Hemiptera	0.1%	-	-
Megaloptera	0.1%	-	-
Lepidoptera	0.1%	-	-
Gastropoda	4.1%	-	-
Chironomidae	41.2%	32.4%	60.8%

Microcosm Benthic Invertebrate Assessment

SWSD

Mean benthic invertebrate total abundance ranged from 1055 to 4109 individuals per m² among the different treatments in comparison to the mean total abundance of 2651 individuals per m² in mature sediments at SWSD (Figure 3.1a). The control treatments (sand) had significantly higher total abundance than the MFT treatments (MFT/SAND – p=0.009, Peat+MFT/SAND – p=0.000, Appendix, Table 3.1). MFT/SAND and Peat+MFT/SAND treatments had similar or lower total abundances than the SWSD mature sediments. Total abundance was higher in the nutrient-enriched MFT/SAND treatment only (Figure 3.1a).

Samples collected from the artificial substrates in the microcosms had similar numbers of benthic invertebrate families as the mature sediments (Figure 3.1b). Amphipoda (48.7%) and Chironomidae (41.2%) accounted for the majority of the benthic invertebrates colonizing the substrates with lesser quantities of Anisoptera (2.0%) and Gastropoda (4.1%) (Table 3.5). These four families accounted for 97% of the total counts. There was no statistical difference in community composition among different treatments based on this level of taxonomic resolution (Appendix, Table 3.1).

DP

Mean total abundance in microcosms ranged from 363 to 1260 individuals per m² among the different treatments in comparison to the mean total abundance of 602 individuals per m² in mature sediments at DP (Figure 3.2a). All treatments (Sand (control), MFT/SAND and Peat+MFT/SAND) had similar total abundance as the mature sediments (Figure 3.2a). The addition of peat on MFT/SAND did not have any significant effects on total abundance. Nutrient-enrich treatments also had no significant effects on total abundance (Appendix, Table 3.1).

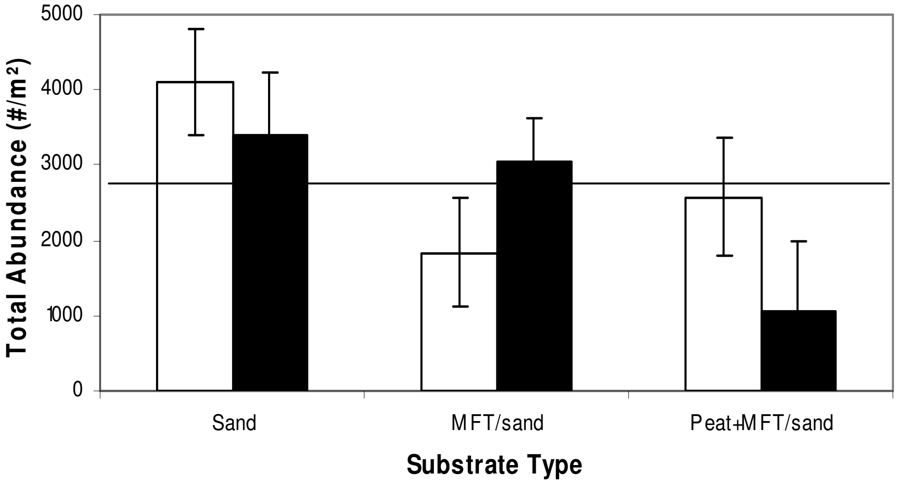
Similar numbers of families were represented in samples from the mature sediments and the different microcosm treatments (Figure 3.2b). The addition of nutrients had no effect on the total number of families represented. Amphipoda (56%), Chironomidae (33%) and Anisoptera (11%) accounted for the all of the benthic invertebrates colonizing the microcosms (Table 3.6). Sand treatments had a lower percentage of Amphipoda and a higher percentage of Chironomidae in comparison to MFT/SAND treatments, which were similar to mature sediments (Table 3.6). The addition of peat to MFT+SAND resulted in slightly higher numbers of Amphipoda. Nutrient enrichment had no impact on benthic invertebrate composition (Table 3.6).

MP

Mean total abundance in microcosms ranged from 692 to 1791 individuals per m² among the different treatments in comparison to the mean total abundance of 1396 individuals per m² in mature sediments at MP (Figure 3.3a). Sand and MFT/SAND treatments had similar total abundance, which was lower than the total abundance for mature sediment samples (Figure 3.3a). The addition of peat and nutrient enrichment had no significant impact on benthic invertebrate composition (Appendix, Table 3.1).

The benthic invertebrate assemblages collected from artificial substrates had similar # of families represented for both mature sediments and microcosm samples (Figure 3.3b). The addition of nutrients had no significant effect on the total number of families. Amphipoda (38%), Chironomidae (56%) and Anisoptera (1%) accounted for 95% of the benthic invertebrates in the microcosms. Sand treatments were similar to samples collected from mature sediments, which had slightly lower numbers of Amphipoda and slightly higher numbers of Chironomidae in comparison to MFT/SAND treatments (Table 3.7). The addition of peat to MFT+SAND resulted in slightly lower numbers of Amphipoda and higher numbers of Chironomidae. Nutrient enrichment had no significant effect on benthic invertebrate composition (Table 3.7).

a)



b)

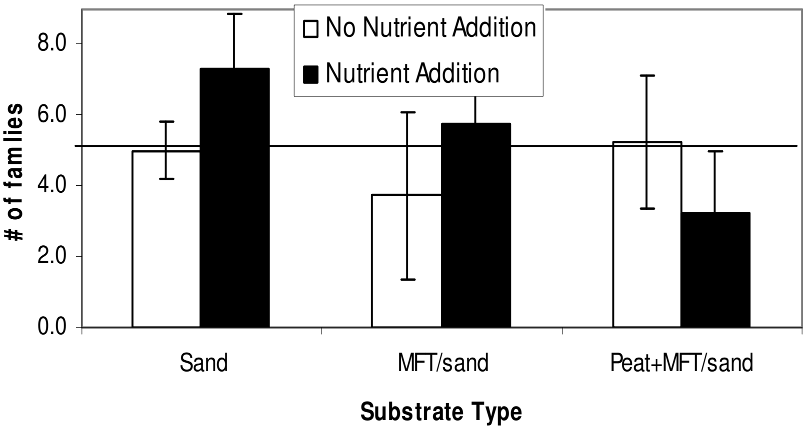
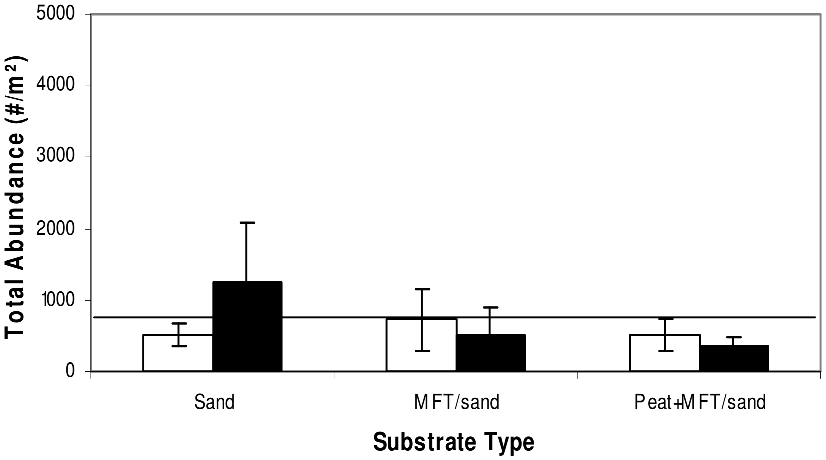


Figure 3.1: Mean ± standard deviation for (a) benthic invertebrate total abundance and (b) number of families for different treatments in SWSD (July 21, 2009). The solid horizontal lines represent mean total abundance and mean number of families for SWSD mature sediments (n=5) for (a) and (b), respectively.

a)



b)

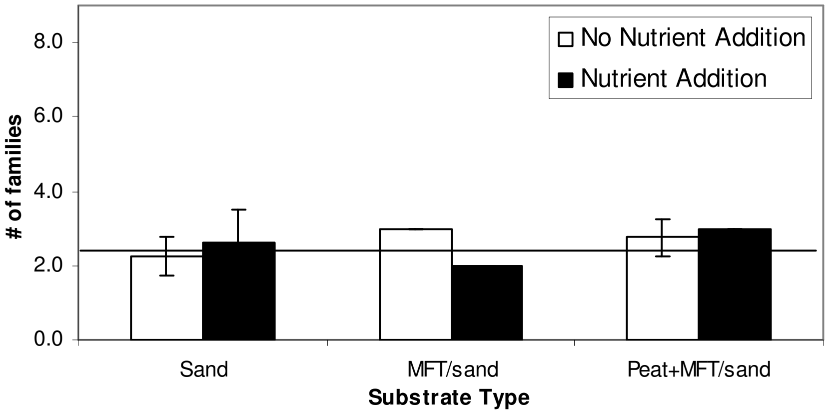


Figure 3.2: Mean ± standard deviation for (a) benthic invertebrate total abundance and (b) number of families for different treatments in DP (July 22, 2009). The solid horizontal lines represent mean total abundance and mean number of families for DP mature sediments (n=5) for (a) and (b), respectively.

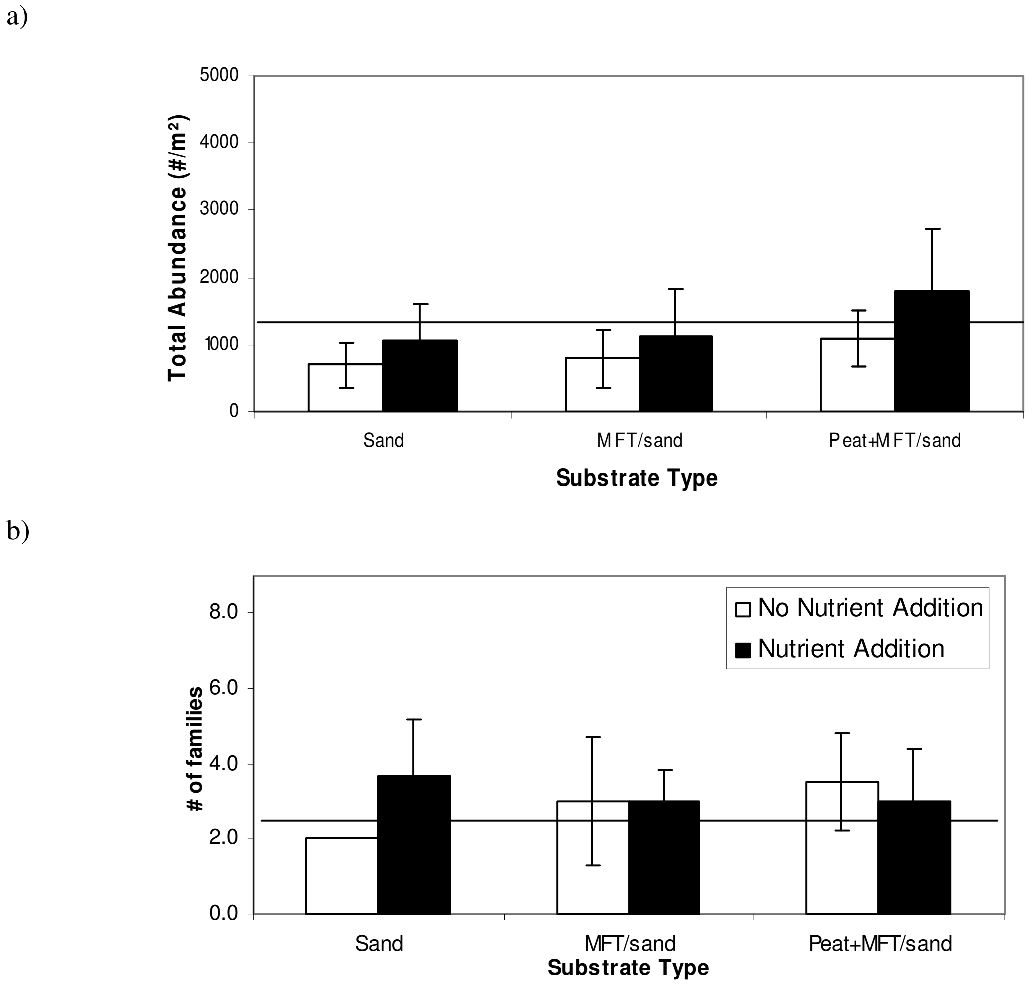


Figure 3.3: Mean ± standard deviation for (a) benthic invertebrate total abundance and (b) number of families for different treatments in MP (July 23, 2009). The solid horizontal lines represent mean total abundance and mean number of families for MP mature sediments (n=5) for (a) and (b), respectively. m^2

Table 3.5 Percentage of benthic invertebrates from mature sediments and microcosms in SWSD.

	Nutrient	**Amphipoda**		**Chironomidae**		**Anisoptera**	
Substrate Type	**Enrichment**	Mean	SD	Mean	SD	Mean	SD
Mature Sediment	- (n=5)	49%	24%	41%	23%	2%	2%
Sand	No (n=4)	58%	12%	36%	11%	1%	2%
	Yes (n=3)	59%	8%	32%	7%	1%	2%
MFT/SAND	No (n=4)	54%	14%	38%	14%	2%	2%
	Yes (n=4)	40%	28%	56%	30%	2%	2%
Peat+MFT/SAND	No (n=4)	66%	16%	27%	16%	3%	1%
	Yes (n=4)	59%	31%	32%	31%	2%	1%

Table 3.6 Percentage of benthic invertebrates from mature sediments and microcosms in DP.

	Nutrient	**Amphipoda**		**Chironomidae**		**Anisoptera**	
Substrate Type	**Enrichment**	Mean	SD	Mean	SD	Mean	SD
Mature Sediment	- (n=5)	48%	22%	32%	25%	20%	20%
Sand	No (n=4)	38%	7%	28%	4%	34%	11%
	Yes (n=5)	41%	26%	59%	26%	0%	0%
MFT/SAND	No (n=2)	55%	37%	31%	27%	14%	18%
	Yes (n=3)	50%	17%	37%	19%	14%	2%
Peat+MFT/SAND	No (n=3)	69%	15%	31%	15%	0%	0%
	Yes (n=3)	64%	30%	22%	12%	13%	25%

Table 3.7 Percentage of benthic invertebrates from mature sediments and microcosms in MP.

	Nutrient	**Amphipoda**		**Chironomidae**		**Anisoptera**	
Substrate Type	**Enrichment**	Mean	SD	Mean	SD	Mean	SD
Mature Sediment	- (n=3)	36%	7%	61%	6%	1%	2%
Sand	No (n=2)	27%	17%	65%	31%	2%	3%
	Yes (n=3)	38%	27%	56%	24%	1%	2%
MFT/SAND	No (n=3)	44%	22%	48%	18%	1%	2%
	Yes (n=4)	43%	11%	54%	11%	2%	2%
Peat+MFT/SAND	No (n=3)	36%	7%	64%	7%	0%	0%
	Yes (n=4)	36%	8%	51%	20%	0%	0%

3.5 Discussion

Benthic invertebrate colonization in experimental microcosms was assessed approximately one year after the microcosms were deployed and treated with nutrient and peat amendments. In the fall of 2008, the closed microcosms were converted to open microcosms to allow benthic invertebrate colonization. In the summer 2009, artificial substrate collections were utilized to characterize colonization on sand and MFT/SAND substrates (± nutrients and/or peat) within three aquatic reclamation sites that differed in exposure history. Differences in exposure history had a greater influence on total abundance and composition than substrate treatments or amendments. The non-OSPM reference site (SWSD) had higher total abundance and number of benthic invertebrate families than OSPM sites (DP and MP).

3.5.1 General Comparison between Study Wetlands

SWSD had the highest number of benthic invertebrate families and total abundance among the three study sites. SWSD had a well established submerged macrophyte community and high percentage area cover. Macrophyte community development in DP and MP was limited in both abundance and richness. Studies indicate that the presence of aquatic macrophytes would increase the abundance and density of most benthic macroinvertebrates (Baker 2007). A study of western boreal wetlands suggested that the volume and architecture complexity of macrophytes were significant and positively correlated with the abundance and diversity of invertebrates (Hornung and Foote 2006). These findings suggested that the limited macrophyte community in DP and MP possibly, in part, contributed to the lower richness and abundance of benthic invertebrates in these systems.

In this study, DP had the lowest number of benthic invertebrate families and total abundance. This observation may be a function of several factors including high turbidity, reducing macrophyte development, higher conductivity/salinity and the presence of fish. Water in DP had a high level of turbidity in comparison to SWSD and MP. High turbidity limits the amount of light penetration, which influences the growth of primary producers in the ecosystem. Primary producers such as macrophytes and phytoplankton were significantly correlated with benthic invertebrate richness and abundance (Baker 2007). An earlier study of benthic invertebrates in DP also suggested turbidity as a factor influencing the observed lower total abundance in DP relative to reference sites (Gould 2000). Higher conductivity and salinity in DP (conductivity - 1732 µS/cm, salinity 0.88 ‰) may also influence total abundance. Whelly (1999) suggested that in OSMP-affected wetlands, high conductivity (>1600 µS/cm) may reduce the diversity and abundance of Chironomidae. However, in DP, the most important factor influencing total abundance is likely fish predation. Over the past years, DP has been stocked with various fish species including yellow perch (*Perca flavescens*) and fathead minnows (*Pimephales promelas*) (Gould 2000).

Both OSPM-affected sites (DP and MP) had lower numbers of benthic invertebrate families and total abundance than the non OSPM-affected reference site. While MP and DP had similar total abundance, the factors influencing total abundance may be very different. In MP, higher NA and conductivity may have contributed to lower benthic invertebrate abundance, either directly via invertebrate toxicity or indirectly via reduced macrophyte colonization.

3.5.2 Effects of OSPM on Benthic Invertebrate Colonization

In this microcosm study, Sand and MFT/SAND treatments were assessed for initial benthic invertebrate colonization. In SWSD, MFT/SAND treatments had lower benthic invertebrate total abundance than Sand treatments. Sand treatments consisted of fine sand and contained little organic content. MFT/SAND treatments had a fine sandy loam texture due to the silty clay loam texture of MFT. MFT consists of clay less than 22 µm in size (Boerger et al. 1992). Microcosms with MFT/SAND substrate increased the turbidity due to unsettled fine particles from MFT. The increased turbidity could indirectly influence benthic invertebrate colonization by limiting colonization of macrophytes and phytoplankton (Gould 2000). Reduced colonization of macrophytes and growth of phytoplankton could limit organic accumulation, and therefore limiting the habitat and food sources of benthic invertebrates. MFT/SAND substrates could also pose potential problems for burrowing animals, such as Chironomidae sp. and Oligochaeta (Gould 2000). The fine particles of MFT (loose gel like) could make it difficult for burrowing animals to maintain tubes (Gould 2000).

Another potential factor influencing lower benthic invertebrate colonization on MFT treatments is the chemical characteristics of the MFT. MFT is characterized by high levels of salts (total ion content >2000 mg/L, Leung et al. 2001), NAs (, 70-100 mg/L, Leung et al. 2001), PACs (140.1 µg/g, Ganshorn 2002) and unrecovered bitumen (Leung et al. 2001). In a microcosm study, concentrations of NAs higher than 5 mg/L caused changes in zooplankton community composition (McCormick 2000). PACs are relatively insoluble in water and generally are at higher concentration in sediments. The nature of PACs could affect the "collector-gatherers" feeding types of organisms. For example, Chironomini and Orthocladiinae had lower densities, lower annual productions values, and longer turn-over times in OSPM-affected wetlands with elevated level of PACs (1.5-150 times greater than reference sediments, Gould 2000).

In the reference wetland, SWSD, the Sand treatments had a higher number of families and significantly higher total abundance in comparison to the MFT/SAND treatments. In OSPM-affected wetlands (DP and MP), Sand treatments had slightly fewer numbers of benthic families and slightly lower total abundance than MFT/SAND treatments. The reason for the fewer numbers of families and lower total abundance in MFT/SAND vs. Sand treatments in the reference wetland was likely due to reduced tolerance to MFT material. In the reference wetland, benthic invertebrates are not exposed to MFT however in both DP and MP, benthic invertebrates have a long exposure history to MFT-related sediments. Other studies examining oil sands derived petroleum coke found that sensitive taxa likely avoided colonizing coke substrates (Baker 2007). A study using the artificial substrate method to compare zoobenthic among wetlands in the oil sands region, found that Gastropoda, Chironomini midgets, caenid and baetid mayflies and amphipoda characterized mature reference wetlands (Leonhardt 2003). Similarly, in the present study, the majority of benthic invertebrates belong to the same four families (Table 3.4).

In OSPM-affected wetlands, Sand and MFT/SAND sediments had similar numbers of benthic invertebrate families and similar total abundance compared to the mature sediments suggested that benthic invertebrates in OSPM-affected wetlands were likely tolerant of MFT. Similar trends of increased tolerance of benthic invertebrates from reference sites were also observed in another microcosm study (Baker 2007). In the study, benthic invertebrate abundance decreased in response to coke amendment in the reference wetlands whereas in the OSPM-affected wetlands, benthic invertebrate abundance remained relatively unchanged with coke amendment (Baker 2007).

Effects of Amendments

In this study, the effect of nutrient enrichment on benthic invertebrate colonization was not significant based on total benthic invertebrate abundance and numbers of families. The only trend observed was in MP, where all nutrient-enriched treatments had slightly elevated total abundance. Nutrient enrichment was a one-time treatment in June 2008 that resulted in short term growth of phytoplankton and periphyton (chapter 2), and later, contributed to a biological detrital layer . Microcosms were then changed from closed systems (June to Sept. 2008) to open systems to allow benthic invertebrate colonization from the fall of 2008 to the summer of 2009, prior to sampling in July of 2009. During the course of over-wintering, physical disturbances could impact the quality of the microcosm substrates. Increased sedimentation may reduce the significance of various types of substrate treatments, as well as the significance of nutrient enrichment (to produce a thicker biological layer). Visual assessment of the biological layer within microcosms could not be carried out at either of the OSPM study sites (DP and MP), due to the high turbidity. At the reference site (SWSD), the biological layer within microcosms had similar appearances among different treatments following over-wintering. Under these circumstances, it was difficult to determine the impact of nutrient enrichment on benthic invertebrate colonization. Further research, using larger treatment systems, is needed to fully understand the influence of nutrient enrichment on benthic invertebrate colonization.

Peat amendment was utilized on MFT/SAND substrates to confine the unfavourable materials within the MFT and to improve initial benthic invertebrate colonization. The results of this study showed no significant difference in benthic invertebrate colonization between MFT/SAND treatments with and without peat amendment. Studies had showed that OSPM reduced benthic invertebrate richness and abundance, possibly due to high salinity and NAs (Leonhardt 2003). Although peat may provide an effective barrier to MFT, it may also change other water-sediment interface parameters which could influence benthic invertebrate colonization. For example, the decay of organic matter such as peat and MFT could create a chemical oxygen demand, reducing oxygen levels in the sediments and the surrounding water (Nelson et al. 2000). The reduced oxygen environment could cause the absence of oxygen-sensitive zoobenthic taxa (Nelson et al. 2000). Baker (2007) conducted a microcosm study on macrophyte and benthic invertebrate colonization utilizing peat as an amendment on coke substrate. The study observed that the addition of peat reduced *Chara* cover and biomass in the reference wetland, but had no impact on plants and invertebrates in the wetlands with little organic content (Baker 2007).

3.6 Conclusion

OSPM-affected wetlands had fewer numbers of families and lower total benthic invertebrate abundance in comparison to the reference wetland. This could be due to the higher conductivity (>1600 µS/cm) in OSPM-affected wetlands. Wetlands with high percent cover of aquatic macrophytes had higher benthic macroinvertebrate abundance. At the reference wetland, the Sand treatment had significantly higher total abundance than the MFT/SAND treatments, whereas at the OSPM-affected wetlands, both treatments had similar total abundance. Benthic invertebrates in OSPM-affected wetlands were likely more tolerant to general stress, including stress from the unfavourable materials within MFT. MFT may influence the benthic invertebrate colonization due to the bitumen and PAHs within MFT. In MP, benthic invertebrate abundance and community composition were not affected by the different types of OSPM substrates. It is likely that benthic invertebrates in MP have a higher tolerance to OSPM due to quality of the water and sediments and the long exposure history.

Nutrient and peat amendments had no impact on benthic invertebrate total abundance and community composition in this study. Both amendments provided nutrients to stimulate phytoplankton and periphyton growth (Chapter 2), which contributes to the biological detrital layer over unfavorable OSPM. The fact that there were no differences in benthic invertebrate endpoints between the mature sediments and treatments (substrates with and without amendments), but there were differences between study sites, indicates that substrate quality is likely not a critical factor influencing total abundance and composition. Water quality and habitat characteristics such as macrophyte development are likely more important variables.

Chapter 4. General Discussion

4.1 Contribution to the knowledge of nutrient enrichment and peat amendment as wet landscape reclamation strategies

The objectives of this microcosm study were to assess the use of OSPM as part of a wet landscape reclamation strategy and to determine the impact of the addition of nutrients and/or peat on phytoplankton and periphyton growth and benthic invertebrate colonization. The following are the key findings of this study that will contribute to future oil sands reclamation strategies.

1) Effects of OSPW on phytoplankton and periphyton community

- MP had the highest level of NAs and conductivity among all study sites, yet it had the highest Chl *a* growth estimates (for both phytoplankton and periphyton) for the different treatments.

- Early studies indicated that high level of NAs and conductivity would have effects on phytoplankton community composition (Leung at a., 2003; Hayes 2005). There are likely differences in species composition between reference site (SWSD: low NAs and conductivity), and OSPM sites (DP: moderate NAs and conductivity; MP: high NAs and conductivity) due to differences in NA concentration and conductivity (Chapter 2).

2) Effects of OSPM on phytoplankton and periphyton community

- Process sand as a substrate had little effect on phytoplankton and periphyton community growth. Sand may act as nutrient sink and reduce nutrient availability in the water column when inorganic nutrient (nitrogen and phosphorus) are added (Chapter 2).

- MFT/SAND as a substrate reduces phytoplankton and periphyton community growth likely due to a combination of factors including physical characteristics (increased water turbidity, presence of un-recovered bitumen) and chemical characteristics (elevated salt, NA and PAC concentrations). This observation was found in the non OSPM-affected wetland (SWSD). In OSPM-affected wetlands, no reduction was observed in systems that received MFT inputs during its construction, which suggests that phytoplankton and periphyton communities may have developed some tolerance to MFT constituents (Chapter 2).

3) Effects of amendments on phytoplankton and periphyton community

- Peat-amended MFT/SAND substrates had significantly elevated phytoplankton and periphyton community growth. With the peat addition, levels of nutrients (DOC, TN, and TP) were elevated and maintained throughout the experimental period.

- Phytoplankton and periphyton communities initially responded to nutrient addition with increased community growth. The added nutrients quickly became depleted and the growth of phytoplankton and periphyton was reduced to levels similar to the growth in non nutrient-enriched microcosms (Chapter 2).

4) Effects of OSPW on benthic invertebrate colonization

- The reference wetland had higher numbers of benthic invertebrate families and total abundance in comparison to OSPM-affected wetlands.

- Studies have suggested that the volume and structural complexity of macrophyte communities were positively correlated with the abundance and diversity of invertebrates (Homung and Foote, 2006, Baker 2007). In this study, the reference site had well established submerged macrophyte community and high percentage area cover in comparison to OSPM-affected wetlands (Chapter 3).

5) Effects of OSPM on benthic invertebrate colonization

- In the reference wetland, benthic invertebrate total abundance for sand treatments was higher than for mature sediments. In OSPM-affected wetlands, benthic invertebrate total abundance for sand treatments was slightly lower than for mature sediments (Chapter 3).

- In DP and MP (OSPM-affected sites), the benthic invertebrate total abundance for MFT/SAND treatments was slightly higher than sand treatments. In SWSD (reference site), the total abundance of MFT/SAND treatments was slightly lower than sand treatments.

SWSD, the reference site, has no OSPM which suggests that the benthic community in DP and MP may have developed tolerance to MFT (Chapter 3).

6) Effects of amendments on benthic invertebrate colonization

- Neither peat and/or nutrient amendments had a significant influence on benthic invertebrate abundance or composition.

4.2 Recommendations for Future Research

The goal of this project was to assess the impact of an enhanced biological detrital layer on the rate of colonization of benthic invertebrates and macrophytes. Contributions to the detrital layer were quantified via measures of phytoplankton and periphyton growth. Later, benthic invertebrate colonization was assessed. Recommendations for expanded research include:

1) Investigating the effects of OSPM and OSPW on primary producers

- Community growth was estimated by measuring phytoplankton Chl *a*, periphyton Chl *a*, periphyton biomass and TSS. This eventually contributes to the detrital layer. Rates and quantities of detrital material could also be quantified using sedimentation chambers. Also, filamentous algae should be quantified.

2) Investigating the effects of amendments on primary producers

- In this study peat amendment had a significant effect on promoting algal growth. Measurements indicated high levels of TN, TP and DOC were maintained in the water throughout the experimental period. Additional detailed chemical analysis for C, N, and P species and trace elements would be useful for determining growth promoting factors.

- Nutrient enrichment had no effect on promoting community algal growth based on the parameters measured. Filamentous algae should also be quantified.

- In this study, nutrient enrichment was delivered at the beginning of the experiment as a single-dose treatment. Further investigation should consider nutrient delivery styles (i.e. pulse delivery vs. single-batch) and different quantities of nutrients (i.e. different TN:TP ratio).

3) Investigating the effects of OSPM and OSPW on benthic invertebrate colonization

- In this study, there was high variability between replicates of the same treatment in terms of benthic invertebrate total abundance. A larger scale mesocosm system would allow more samples to be collected from each unit, thus reducing variability. In addition, a larger surface area would allow for use of multiple sampling techniques (artificial substrate and core sampling).

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Appendix

Appendix 2.1 – Water quality parameters measured in microcosm treatments in Shallow Wetland-South Ditch from June – August, 2008.

Date	Temp. (°C)	D.O. (%)	pH	Cond. (µs/cm)	DOC (ppm)	DIC (ppm)	TN (µg/L)	TP (µg/L)
Water – No nutrients								
June 17	-	-	-	-	35.7	87.1	1180	31
July 7	-	-	-	-	28.6	69.8	1240	60
July 27	22.0	93.0	7.0	930	56.9	111.0	1170	54
August 16	21.6	71.4	6.6	753	43.3	87.0	843	16
Water – Nutrient enriched								
June 17	-	-	-	-	35.6	86.7	1370	324
July 7	-	-	-	-	45.5	80.9	2430	151
July 27	22.7	79.0	7.0	1012	42.7	57.3	2190	78
August 16	22.1	79.0	7.2	741	29.0	49.1	1930	158
Sand – No nutrient								
June 17	-	-	-	-	32.8	83.4	1000	47
July 7	-	-	-	-	24.8	45.7	1010	10
July 27	22.7	92.0	7.1	972	30.9	43.1	1010	18
August 16	21.4	78.2	7.0	807	31.2	58.0	965	20
Sand – Nutrient enriched								
June 17	16.9	84.9	7.6	888	37.8	85.5	2170	353
July 7	19.5	105.0	7.7	755	33.4	51.9	3160	271
July 27	22.5	63.0	6.5	1130	44.2	61.6	2830	120
August 16	21.9	65.3	6.9	925	65.7	116.2	1900	75
MFT/SAND – No nutrient								
June 17	16.6	68.3	7.8	993	24.4	63.1	1090	12
July 7	19.5	109.4	7.3	1054	25.4	51.4	793	8
July 27	22.2	87.0	7.0	1169	32.1	59.0	975	10
August 16	21.9	79.0	7.0	945	56.6	106.5	968	28
MFT/SAND – Nutrient enriched								
June 17	-	-	-	-	44.2	121.4	4830	321

July 7	-	-	-	-	32.1	54.7	1310	25
July 27	23.0	94.0	7.2	1300	49.1	73.7	1430	36
August 16	21.9	76.8	6.8	958	60.7	119.7	1080	22
Peat+MFT/SAND – No nutrient								
June 17	16.8	21.9	7.6	1055	212.5	104.9	11500	142
July 7	19.6	24.2	6.5	1185	140.0	64.1	6890	159
July 27	22.2	70.0	6.5	1114	152.2	77.2	5810	117
August 16	21.9	73.0	6.9	830	150.1	111.7	3560	251
Peat+MFT/SAND – Nutrient enriched								
June 17	-	-	-	-	244.5	125.1	12600	565
July 7	-	-	-	-	131.3	70.4	5840	168
July 27	22.3	55.0	6.3	1291	150.8	87.3	5350	155
August 16	21.8	61.8	6.7	1023	179.2	141.8	4650	335

Appendix 2.2 – Water quality parameters measured in microcosm treatments in DP from June – August, 2008.

Date	Temp. (ºC)	D.O. (%)	pH	Cond. (µs/cm)	DOC (ppm)	DIC (ppm)	TN (µg/L)	TP (µg/L)
Water – No nutrients								
June 13	-	-	-	-	50.4	112.1	1250	28
July 3	-	-	-	-	61.1	137.7	1340	22
July 23	21.8	94.2	7.5	1890	69.4	150.2	1390	19
August 12	19.1	77.3	7.9	1865	110.8	194.5	1590	35
Water – Nutrient enriched								
June 13	-	-	-	-	57.6	120.9	4010	393
July 3	-	-	-	-	58.9	106.9	2180	114
July 23	21.9	95.7	7.9	1794	64.2	116.8	2740	239
August 12	19.1	64.0	7.8	1674	131.0	183.8	4600	584
Sand – No nutrient								
June 13	20.9	98.5	8.7	1700	61.5	125.6	1240	26
July 3	23.4	92.4	7.8	1913	55.0	110.0	1530	32
July 23	22.0	101.2	7.5	2034	54.4	105.8	1530	25
August 12	18.9	85.0	8.0	2099	132.5	221.0	1590	24
Sand – Nutrient enriched								
June 13	-	-	-	-	111.4	196.3	4310	351
July 3	-	-	-	-	48.6	101.6	1410	51
July 23	22.3	97.1	7.4	1939	52.4	103.3	1440	25
August 12	19.2	82.0	7.9	1977	133.7	230.8	1680	43
MFT/SAND – No nutrient								
June 13	21.1	101.0	8.7	1711	80.0	166.5	1310	35
July 3	22.2	96.0	7.7	1994	50.3	103.7	1330	17
July 23	21.9	79.9	7.1	1971	57.4	114.4	1330	22
August 12	19.1	75.0	7.6	2043	120.4	235.7	1560	40
MFT/SAND – Nutrient enriched								
June 13	-	-	-	-	74.4	147.6	3870	255
July 3	-	-	-	-	58.5	119.3	1380	20
July 23	22.6	93.4	7.4	1517	60.6	122.1	1540	28
August 12	19.4	69.1	7.5	2281	136.4	280.2	1910	54

Peat+MFT/SAND – No nutrient								
June 13	-	-	-	-	220.9	197.3	7630	210
July 3	-	-	-	-	157.2	117.9	7170	180
July 23	21.2	69.3	7.0	2116	160.5	119.3	6410	174
August 12	19.0	54.0	7.4	1962	318.0	261.1	5930	195
Peat+MFT/SAND – Nutrient enriched								
June 13	21.2	58.5	8.2	1720	222.0	215.2	8700	565
July 3	22.1	73.8	7.8	2032	157.3	120.2	5960	185
July 23	21.6	80.4	7.3	2233	170.3	135.3	5690	118
August 12	19.0	49.6	7.3	2115	294.2	273.6	5760	156

Appendix 2.3 – Water quality parameters measured in microcosm treatments in MP from June – August, 2008.

Date	Temp. (ºC)	D.O. (%)	pH	Cond. (µs/cm)	DOC (ppm)	DIC (ppm)	TN (µg/L)	TP (µg/L)
Water – No nutrients								
June 16	-	-	-	-	58.3	68.3	657	10
July 5	-	-	-	-	66.0	78.7	1070	38
July 25	21.8	128.6	7.1	4968	63.5	62.9	2090	88
August 14	19.0	72.7	8.0	3711	86.4	74.2	2560	132
Water – Nutrient enriched								
June 16	-	-	-	-	85.7	93.2	3860	350
July 5	-	-	-	-	62.8	58.8	2500	124
July 25	21.9	96.3	6.9	5834	78.5	71.2	2200	122
August 14	19.0	66.6	7.6	3734	98.2	82.3	2550	149
Sand – No nutrient								
June 16	16.2	105.6	8.3	3900	60.3	70.1	952	24
July 5	18.4	87.4	7.6	4539	58.1	57.8	1440	46
July 25	21.9	116.2	6.7	5787	66.0	59.5	1550	60
August 14	19.0	99.1	8.1	4481	98.9	65.0	5480	363
Sand – Nutrient enriched								
June 16	-	-	-	-	50.0	59.1	1580	236
July 5	-	-	-	-	54.6	39.5	1430	32
July 25	22.2	109.1	6.9	4912	58.7	47.1	2800	129
August 14	18.9	55.0	7.9	3779	48.5	31.8	1540	49
MFT/SAND – No nutrient								
June 16	16.2	66.3	8.3	3927	74.2	89.9	1010	4
July 5	18.3	85.3	7.7	4420	49.3	60.3	1290	8
July 25	21.7	98.5	6.7	5111	59.2	68.8	1050	9
August 14	18.8	77.5	7.6	3654	93.7	98.5	992	28
MFT/SAND – Nutrient enriched								
June 16	-	-	-	-	60.0	72.4	4470	409
July 5	-	-	-	-	48.1	52.0	1650	47
July 25	21.4	85.1	6.5	4360	62.3	68.8	1460	42
August 14	18.7	79.4	7.9	3059	43.7	51.1	1730	100

Peat+MFT/SAND – No nutrient								
June 16	16.2	50.5	8.0	4093	180.9	102.7	6740	104
July 5	18.3	55.3	7.1	5227	144.3	66.9	6500	260
July 25	21.8	75.8	6.8	6084	165.9	76.8	5820	159
August 14	19.0	49.5	7.5	5138	194.7	102.8	5600	291
Peat+MFT/SAND – Nutrient enriched								
June 16	-	-	-	-	152.5	67.9	10800	525
July 5	-	-	-	-	80.1	65.4	2950	101
July 25	21.8	60.5	5.8	5781	152.5	85.7	5470	374
August 14	19.1	46.0	7.1	4304	234.4	136.8	5500	342

Appendix 2.4 – Result of a parametric two factor ANOVA method to examine the effects of Nutrient Enrichment (with or without) and Substrate Types (control, Sand, MFT/SAND mix, Peat +MFT/SAND mix) on periphyton productivities in both dry weight and Chl *a* during mid-June to mid-August 2008 (data had undergone $\log_{10}$ transformation prior to analysis). Bolded effects are significant at $\alpha = 0.05$.

Periphyton Dry Weight

		Substrate		Nutrient		Interaction	
Site	Date	F	P	F	P	F	P
SWSD	July 7	3.059	**0.048**	4.078	0.055	0.187	0.904
	July 27	13.112	**0.000**	0.279	0.602	1.814	0.172
	Aug 16	6.313	**0.003**	0.710	0.408	0.762	0.562
DP	July 3	6.819	**0.001**	2.112	0.156	1.065	0.378
	July 23	6.608	**0.001**	0.022	0.883	0.444	0.723
	Aug 12	8.959	**0.000**	0.085	0.773	0.662	0.582
MP	July 5	20.920	**0.000**	8.534	**0.008**	0.583	0.632
	July 25	8.808	**0.000**	1.344	0.258	1.664	0.201
	Aug 14	3.361	**0.035**	6.229	**0.020**	0.816	0.498
Periphyton Chl *a*							
SWSD	July 7	17.533	**0.000**	6.266	**0.020**	1.164	0.344
	July 27	18.448	**0.000**	0.004	0.948	3.322	**0.038**
	Aug 16	12.327	**0.000**	4.785	**0.039**	0.522	0.671
DP	July 3	8.289	**0.000**	0.988	0.328	0.344	0.794
	July 23	6.151	**0.002**	0.075	0.786	0.513	0.676
	Aug 12	16.254	**0.000**	0.289	0.594	0.507	0.681
MP	July 5	14.321	**0.000**	13.745	**0.002**	1.794	0.184
	July 25	19.876	**0.000**	0.994	0.331	3.390	**0.038**
	Aug 14	7.219	**0.002**	0.003	0.956	4.348	**0.015**

Appendix 2.5 – Result of a parametric two factor ANOVA method to examine the effects of Nutrient Enrichment (with or without) and Substrate Types (control, Sand, MFT/SAND mix, Peat +MFT/SAND mix) on TSS and phytoplankton Chl *a* during mid-June to mid-August 2008 (data had undergone $\log_{10}$ transformation prior to analysis). Bolded effects are significant at $\alpha = 0.05$.

TSS

		Substrate		Nutrient		Interaction	
Site	Date	F	P	F	P	F	P
SWSD	July 7	0.381	0.768	0.460	0.504	0.265	0.850
	July 27	0.529	0.667	0.026	0.874	0.258	0.855
	Aug 16	2.654	0.071	0.001	0.981	1.822	0.170
DP	July 3	7.060	**0.001**	0.000	0.993	1.699	0.187
	July 23	6.019	**0.002**	13.489	**0.001**	4.155	**0.014**
	Aug 12	3.036	**0.043**	3.666	0.065	3.716	**0.021**
MP	July 5	8.154	**0.001**	0.710	0.408	0.135	0.938
	July 25	1.198	0.332	0.190	0.667	0.336	0.799
	Aug 14	0.448	0.721	1.489	0.234	0.487	0.695
Phytoplankton Chl *a*							
SWSD	July 7	-	-	-	-	-	-
	July 27	2.836	0.063	0.386	0.541	0.736	0.542
	Aug 16	3.671	**0.027**	11.544	**0.002**	4.553	**0.012**
DP	July 3	6.236	**0.002**	1.420	0.242	1.449	0.248
	July 23	4.033	**0.016**	3.951	0.056	0.876	0.464
	Aug 12	1.948	0.148	0.783	0.385	1.647	0.204
MP	July 5	6.249	**0.003**	6.305	**0.019**	0.422	0.739
	July 25	3.497	**0.032**	1.286	0.268	0.924	0.445
	Aug 14	2.847	**0.060**	0.003	0.958	0.146	0.931

Appendix 2.6 – Cumulative periphyton dry weight estimates for OSPM substrates at three oil sands reclamation sites, organized in ranking form (a), and organized by experimental sites (b). Two sampling periods from July 23 – Aug.12, 2008.

A)

Treatments			**Periphyton Dry Weight (g/m²)**
SWSD	**DP**	**MP**	
		Peat+MFT/SAND + Nutrient	***3.74***
	Peat+MFT/SAND		3.28
	Peat+MFT/SAND + Nutrient		2.64
Peat+MFT/SAND			2.54
		Peat+MFT/SAND	2.37
Peat+MFT/SAND + Nutrient			1.87
		Sand +Nutrient	1.36
	MFT/SAND + Nutrient		1.04
	MFT/SAND		0.84
		MFT/SAND	0.77
MFT/SAND + Nutrient			0.70
		Sand	0.67
Sand +Nutrient			0.64
		MFT/SAND + Nutrient	0.64
	Sand +Nutrient		0.63
	Sand		0.56
Sand			0.50
MFT/SAND			0.36

[a] There was a total of 3 sampling periods however the first sampling period in July, 2008) was excluded from this summary table due to the high volume of unsettled particulates in the water column following microcosm construction which may adhere to the artificial substrates.

B)

Treatments	**Sites**		
	SWSD	**DP**	**MP**
Peat+MFT/SAND + Nutrient	1.87	2.64	3.74
MFT/SAND + Nutrient	0.70	1.04	0.64
Sand +Nutrient	0.64	0.63	1.36
Water +Nutrient	0.27	0.52	0.39
Peat+MFT/SAND	2.54	3.28	2.37
MFT/SAND	0.36	0.84	0.77
Sand	0.50	0.56	0.67
Water	0.55	0.69	0.93

Appendix 2.7 – Cumulative periphyton Chl *a* estimates for OSPM substrates at three oil sands reclamation sites organized in ranking form (a), and organized by experimental sites (b). Three sampling periods from July 3rd – Aug.16th 2008.

A)

Treatments			**Periphyton Chl *a***
SWSD	**DP**	**MP**	**(mg/m²)**
		Peat+MFT/SAND + Nutrient	***274.73***
Peat+MFT/SAND			***161.26***
		Peat+MFT/SAND	***121.63***
Peat+MFT/SAND + Nutrient			***103.08***
	Peat+MFT/SAND + Nutrient		***78.36***
	Peat+MFT/SAND		***76.33***
		Sand +Nutrient	***73.50***
		MFT/SAND + Nutrient	***40.35***
		Sand	33.07
	MFT/SAND + Nutrient		29.35
MFT/SAND + Nutrient			18.95
Sand +Nutrient			17.14
	MFT/SAND		14.86
		MFT/SAND	8.18
Sand			7.85
	Sand		6.34
MFT/SAND			5.21
	Sand +Nutrient		4.34

B)

Treatments	**Sites**		
	SWSD	**DP**	**MP**
Peat+MFT/SAND + Nutrient	103.08	78.36	274.73
MFT/SAND + Nutrient	18.95	29.35	40.35
Sand +Nutrient	17.14	4.34	73.5
Water +Nutrient	10.64	7.59	13.39
Peat+MFT/SAND	161.26	76.33	121.63
MFT/SAND	5.21	14.86	8.18
Sand	7.85	6.34	33.07
Water	8.20	16.66	13.29

Appendix 2.8 – Cumulative phytoplankton dry weight estimates for OSPM substrates at three oil sands reclamation sites organized in ranking form (a), and organized by experimental sites (b). Three sampling periods from July 3rd – Aug.16th 2008.[a]

A)

Treatments			Total Suspended Solid (mg/L)
SWSD	**DP**	**MP**	
		Peat+MFT/SAND	170.25
	Peat+MFT/SAND + Nutrient		147.28
	Peat+MFT/SAND		133.15
		Peat+MFT/SAND + Nutrient	94.15
		MFT/SAND	90.16
Sand			88.21
Sand +Nutrient			86.94
Peat+MFT/SAND			84.87
		Sand	77.05
	MFT/SAND		69.72
Peat+MFT/SAND + Nutrient			66.39
	MFT/SAND + Nutrient		56.31
MFT/SAND			55.77
MFT/SAND + Nutrient			55.22
		MFT/SAND + Nutrient	53.05
		Sand +Nutrient	50.65
	Sand +Nutrient		49.71
	Sand		25.96

[a] There was a total of 4 sampling periods however the first sampling period (June 13th, 2008) was excluded from this summary table due to the high volume of unsettled particulates in the water column following microcosm construction.

B)

Treatments	**Sites**		
	SWSD	**DP**	**MP**
Peat+MFT/SAND + Nutrient	66.39	147.28	94.15
MFT/SAND + Nutrient	55.22	56.31	53.05
Sand +Nutrient	86.94	49.71	50.65
Water +Nutrient	61.41	208.60	103.95
Peat+MFT/SAND	84.87	133.15	170.25
MFT/SAND	55.77	69.72	90.16
Sand	88.21	25.96	77.05
Water	111.45	35.72	102.25

Appendix 2.9 – Cumulative phytoplankton Chl *a* estimates for OSPM substrates at three oil sands reclamation sites organized in ranking form (a), and organized by experimental sites (b). Three sampling periods from July 3rd – Aug.16th 2008.

A)

Treatments			**Phytoplankton Chl *a* (µg/L)**
SWSD	**DP**	**MP**	
		Peat+MFT/SAND	***178.15***
	Peat+MFT/SAND + Nutrient		***83.25***
		Peat+MFT/SAND + Nutrient	***74.11***
Peat+MFT/SAND			26.14
		Sand	23.75
		MFT/SAND + Nutrient	22.28
		Sand +Nutrient	21.94
MFT/SAND + Nutrient			16.93
	MFT/SAND + Nutrient		16.73
	Peat+MFT/SAND		14.15
Peat+MFT/SAND + Nutrient			11.73
		MFT/SAND	11.40
	Sand		10.23
Sand +Nutrient			9.14
	MFT/SAND		7.41
	Sand +Nutrient		6.41
MFT/SAND			5.92
Sand			4.20

[a] There was a total of 4 sampling periods however the second sampling period (July 3, 2008) was excluded from this summary table for all reclamation sites due to a missing data set for SWSD.

B)

Treatments	**Sites**		
	SWSD	**DP**	**MP**
Peat+MFT/SAND + Nutrient	11.73	83.25	74.11
MFT/SAND + Nutrient	16.93	16.73	22.28
Sand +Nutrient	9.14	6.41	21.94
Water +Nutrient	21.87	46.07	94.82
Peat+MFT/SAND	26.14	14.15	178.15
MFT/SAND	5.92	7.41	11.40
Sand	4.20	10.23	23.75
Water	21.30	12.27	40.03

Appendix 2.10 – Result of a parametric two factor ANOVA method to examine the effects of Nutrient Enrichment (with or without) and Substrate Types (control, Sand, MFT/SAND mix, Peat +MFT/SAND mix) cumulative productivity estimates (periphyton dry weight, periphyton Chl *a*, TSS and Phytoplankton Chl *a*) during mid-June to mid-August 2008 (data had undergone $\log_{10}$ transformation prior to analysis). Bolded effects are significant at α = 0.05.

Growth Estimates	Substrate		Nutrient		Interaction	
	F	P	F	P	F	P
SWSD						
Periphyton Dry Weight	6.987	**0.002**	1.251	0.274	0.839	0.486
Periphyton Chl *a*	40.458	**0.000**	2.814	0.106	3.211	**0.041**
TSS	0.400	0.754	0.321	0.576	0.020	0.996
Phytoplankton Chl *a*	3.067	0.051	1.607	0.219	1.074	0.383
DP						
Periphyton Dry Weight	10.543	**0.000**	0.651	0.426	0.455	0.716
Periphyton Chl *a*	12.270	**0.000**	0.039	0.846	0.162	0.921
TSS	7.841	**0.000**	4.755	**0.037**	5.335	**0.004**
Phytoplankton Chl *a*	5.709	**0.004**	2.742	0.111	0.866	0.472
MP						
Periphyton Dry Weight	16.735	**0.000**	3.398	0.078	0.179	0.909
Periphyton Chl *a*	15.621	**0.000**	1.604	0.221	2.062	0.139
TSS	1.776	0.179	1.639	0.213	0.310	0.818
Phytoplankton Chl *a*	4.718	**0.011**	2.467	0.131	0.325	0.807

Appendix 3.1 – Result of a parametric two factor ANOVA method to examine the effects of Nutrient Enrichment (with or without) and Substrate Types (control, Sand, MFT/Sand mix, Peat +MFT/Sand mix) on total abundance and number of families of benthic invertebrate, sediment organic content and the percentage of three most abundance invertebrate families collected during July 14 – 26, 2009. Bolded effects are significant at α = 0.05.

SWSD

Test Parameters	Substrate		Nutrient		Interaction	
	F	P	F	P	F	P
Total Abundance	12.504	**0.000**	1.183	0.292	6.937	0.006
# of Family	2.688	0.098	1.280	0.274	4.194	0.033
Organic Content	2.560	0.107	0.011	0.918	1.236	0.315
% of Amphipoda	1.368	0.281	0.711	0.411	0.339	0.717
% of Chironomidae	1.679	0.216	0.546	0.470	0.692	0.514
% of Anisoptera	1.297	0.299	0.336	0.570	0.445	0.648

DP

Test Parameters	Substrate		Nutrient		Interaction	
	F	P	F	P	F	P
Total Abundance	1.462	0.265	0.347	0.565	2.198	0.148
# of Family	1.078	0.361	0.240	0.630	2.370	0.122
Organic Content	11.341	**0.001**	2.067	0.171	0.518	0.606
% of Amphipoda	1.671	0.223	0.042	0.841	0.033	0.967
% of Chironomidae	1.207	0.328	1.125	0.307	1.757	0.209
% of Anisoptera	0.814	0.463	0.912	0.356	3.374	0.064

MP

Test Parameters	Substrate		Nutrient		Interaction	
	F	P	F	P	F	P
Total Abundance	1.589	0.239	2.419	0.142	0.180	0.837
# of Family	0.166	0.849	0.420	0.527	1.074	0.368
Organic Content	1.847	0.197	0.009	0.925	1.712	0.219
% of Amphipoda	0.764	0.484	0.170	0.687	0.224	0.802

% of Chironomidae	0.403	0.676	0.287	0.601	0.395	0.681
% of Anisoptera	0.743	0.493	0.028	0.869	0.183	0.835