Testate Amoebae as a Performance Indicator of Ecosystem Establishment in Wetlands Impacted By Oil Sands Processed Materials

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

Mining for oil sands in the Athabasca Basin in northeastern Alberta is rapidly expanding. As economics continue to drive growing mining practices, waste management, reclamation and bio-monitoring strategies are becoming increasingly important.

This project aims to determine the practicality of testate amoebae assemblages as an indicator of microbial community health and ecosystem establishment in wetlands impacted by oil sand processed materials (OSPM). Testate amoebae are unicellular, shelled protists that live in abundance in soils, leaf litter and in fresh water habitats. This group of protists forms shells (or tests) which makes them relatively easy to identify. Ecological studies have shown they occupy specific niches controlled by environmental parameters such as pH and moisture variables. These features make testate amoebae excellent bioindicators, and this project explores the potential of applying testate amoebae bioindicators in wetlands affected by OSPM.

Using compound and epifluorescent microscopy techniques, testate amoebae species assemblages were identified and tabulated from a series of wetlands with different impacts by oil sands processed materials. Bacterial and fungal proportions were characterized to compare with the testate amoebae and identify possible links within the microbial community.

A total of 44 species of testate amoebae were encountered in 24 wetlands, with *Centropyxis platystoma* and *Centropyxis aculeata* being the most common taxa. Natural peatland sites, not affected by OSPM contained the most diverse assemblage of testate amoebae containing *Arcella, Assulina, Centropyxis, Englypha, and Heleopera*. Open-water

wetlands not impacted by OSPM were less diverse than peatland sites, but maintained between two and 12 taxa per site. Open-water sites amended with OSPM contained fewer taxa (between 0 and 4 taxa at any given site) and fewer individuals than any other site type, with *Difflugia* being most common.

Bacteria contributed an average of 65% of the microbial community in open-water sites and an average of 80% in peatland sites. Peatland sites were significantly different ($P<0.05$) from all other site types in terms of testate amoebae, bacteria, and fungal biomass.

This study demonstrated that differences exist in testate amoebae assemblages between site types do exist, this study establishes the fact that testate amoebae are too few (<1% of biomass), and not in sync with the other microbial facets studied (bacteria and fungi) limiting their use as bioindicators of microbial community establishment in wetlands impacted by oil sands processed materials. However the predominance of bacteria in all site types calls attention to their vital role in these sites and their importance in further research in oil sands reclamation.

Acknowledgements

"The problem is not that there are problems. The problem is expecting otherwise and thinking that having problems is a problem." *Theodore Rubin*

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1.0 Introduction

Testate amoebae are unicellular, shelled protists that live in abundance in soils, leaf litter and near/in fresh water habitats (Charman *et al*., 2000). These protists form shells (or tests) made of smooth secreted material, pre-formed plates or cemented particles, which are gathered from the surrounding environment (Charman *et al*., 2000). These particles can include small pieces of silica, pollen grains, fungal hyphae, and other organic detritus (Charman *et al* 2000). Simple tests made by secretion are called autogenous tests, whereas tests formed by the agglutination of foreign material are called xenogenous tests; it is not uncommon for organisms to use a combination of strategies to construct their tests (Charman *et al*., 2000). The shells of testate amoebae possess an aperture for the emergence of pseudopodia (Westphal 1976). Pseudopodia are temporary cell extensions that are used for locomotion and taking in food (Woodland *et al*., 1998). Testate amoebae taxa can be differentiated by their test characteristics and the kind of pseudopodia they possess (lobose, filiform) (Westphal 1976).

Testate amoebae reproduce asexually via binary fission. Reproduction in testate amoebae begins with the growth of a second shell and can occur in three ways (Westphal 1976). In delicate organisms, shells divide longitudinally, after which mitosis begins (Westphal 1976). More robust shells (like that of *Euglypha*) begin by producing silica platelets in the cytoplasm close to the nucleus (Westphal 1976). These plates serve to strengthen the shell, eventually traveling to the periphery of the protruding cytoplasm as the new cell is being formed, after which the nucleus prepares for division (Westphal 1976). In the third case, the production of the new shell begins only after the cytoplasm has protruded to the final size of the new cell, as occurs in the *Pyxidicula* genus (Westphal 1976). A sexual component to reproduction has not been observed in these organisms (Charman *et al* 2000).

More and more is being learned about the ecology of the wide range of habitats in which these protists live. Testate amoebae have repeatedly shown to be good indicators for natural macro-environmental gradients, like pH and hydrology, as well as metal pollutants in aquatic and soil environments (Charman and Warner 1992, Gilbert *et al* 1998(1), Foissner 1999, and Mitchell *et al* 2003). In the last three decades testate amoebae have been used as indicators of sea level change (Charman *et al* 1998, Gehrels *et al* 2001, Mediolo *et al* 1990), paleohydrology and paleoclimate (Beyens and Chardez 1995, Smith 1992, 1996, Wilkinson 1994), atmospheric pollution (Lüftenegger and Foissner 1989, Balik 1991, Tolonen *et al* 1992, 1994) and limnological variables such as pH, oxygen concentration, temperature and heavy metal content (Gilbert *et al.* 1998(2)*;* Muqi and Wood 1999, Escobar *et al* 2008). Studies on the ecology of lakes and rivers indicate that testate amoebae respond to pH, and pollution of various types (Beyens *et al* 1986, Burbidge and Schroder-Adams 1998, Dalby *et al* 2000, Kumar and Patterson 2000). These studies also suggest testate amoebae may respond to changes in land use such as deforestation, watershed management and chemical use (fertilizers and pesticides) (Fry 1990, Mitchell personal communication). Studies conducted with testate amoebae are predominantly focused, but not limited to, indicators of hydrological conditions in peatland soils and primarily depth of the water table, (Lüftenegger and Foissner 1989, Balik 1991) or in paleohydrology and paleoclimate studies to reconstruct former hydrological conditions.

Given the sensitivity of testate amoebae to environmental gradients and water chemistry, it is not surprising that studies have investigated their use in soil and air pollution monitoring. According to a number of studies, diversity and density of testate amoebae were lower in polluted or “impacted” peatland (Kandeler *et al* 1992, Wanner and Dunger 2001, 2002, Balik 1991) and lake sites (Nguyen-Viet *et al* 2007(1)), though little is known of the mechanisms influencing the tolerance of some species over others.

For those living in soils, research suggests that moisture content and pH play a key role in their distribution in soils (Charman *et al*., 2000). In a study of forested peatland soils situated in the boreal region of Canada, the feasibility of testate amoebae as bio-indicators was investigated (Warner and Chmielewski 1992). In this study over twenty-six species were encountered, with single taxa or species groups showing differences between control and drained sites (Warner and Chmielewski 1992). These results indicate that testate amoebae are capable of responding immediately to changes in habitat, and identified key taxa which deserve special attention as potential bioindicators; *Cyclopyxis arcelloides* and *Trinema lineare* were two groups which were identified as indicators for changes in drainage of this peatland site (Warner and Chmielewski 1992). In another study conducted in Frache-Comte, France, Nguyen-Viet *et al.* found that lead contamination reduced density as well as the number of testate amoebae species, the effect was in proportion to the lead concentration accumulated in *Sphagnum fallax* (2007(1)). This research found *Nebela carianata*, *Euglypha strigosa*, and *H. sphagni* to be sensitive species to lead contamination while *A. discoides* and *C. aculeate* were found to be the most tolerant species.

Testate amoebae are excellent candidates for environmental indicators for a variety of reasons: they are abundant and diverse, about 100 potential species in mosses alone (Charman *et al*., 2000), most species are cosmopolitan, meaning they are not limited to one part of the world (although exceptions exist) (Bonnet 1973), their identification is relatively easy based on the morphology of their tests (shell) that remains even after the death of the organism, and lastly they are good signifiers of disturbance because of their trophic position at the top of the microbial food web, and consuming key players in the microbial loop (Gilbert *et al* 1998, 2000). Lastly, studies have shown that testate amoebae are more sensitive than other protozoa (ciliates) and responded more dramatically in a study looking at the conversion of soil to agriculture by a

reduction in richness or more than 50% compared to unaffected soils (Fry 1990, Mitchell personal communication).

1.1 Testate amoebae as Part of the Microbial Community

Microorganisms are organisms that are microscopic and are at the base of every ecosystem. They are an incredibly diverse group and include bacteria, fungi, archea and protists (Sigee 2005). These groups play a fundamental role in the nutrient cycling processes of ecosystems. Microorganisms are the primary consumers of organic carbon and they have the potential to supply carbon and energy to organisms at higher trophic levels. Although microbial communities consist of many groups, bacteria are of paramount importance, as they act as intermediaries for nutrient release from detritus and are the only organisms documented as consumers of toxic naphthenic acids (Hadwin *et al*., 2006). Bacteria are able to remobilize nutrients, making them bioavailable at higher trophic levels. When bacteria are consumed by microbial grazers, a significant portion of the nutrients in the bacterial cells will be recycled into the food web (Berman *et al* 1987). The nutrients supplied by primary producers have also been proven to pass through the "microbial loop" to higher trophic levels (Pomeroy 1974, Azam *et al*., 1983, Berman *et al*., 1987). Bacteria are consumed by other microorganisms (protists), which are then consumed by larger aquatic organisms, thus allowing carbon to be cycled through multiple trophic levels, which is the basis for the "microbial loop" (Figure 1) (Berman *et al*., 1987). Bacterial communities are of paramount importance but require more intricate sampling methods and extraction techniques to characterize than the protists.

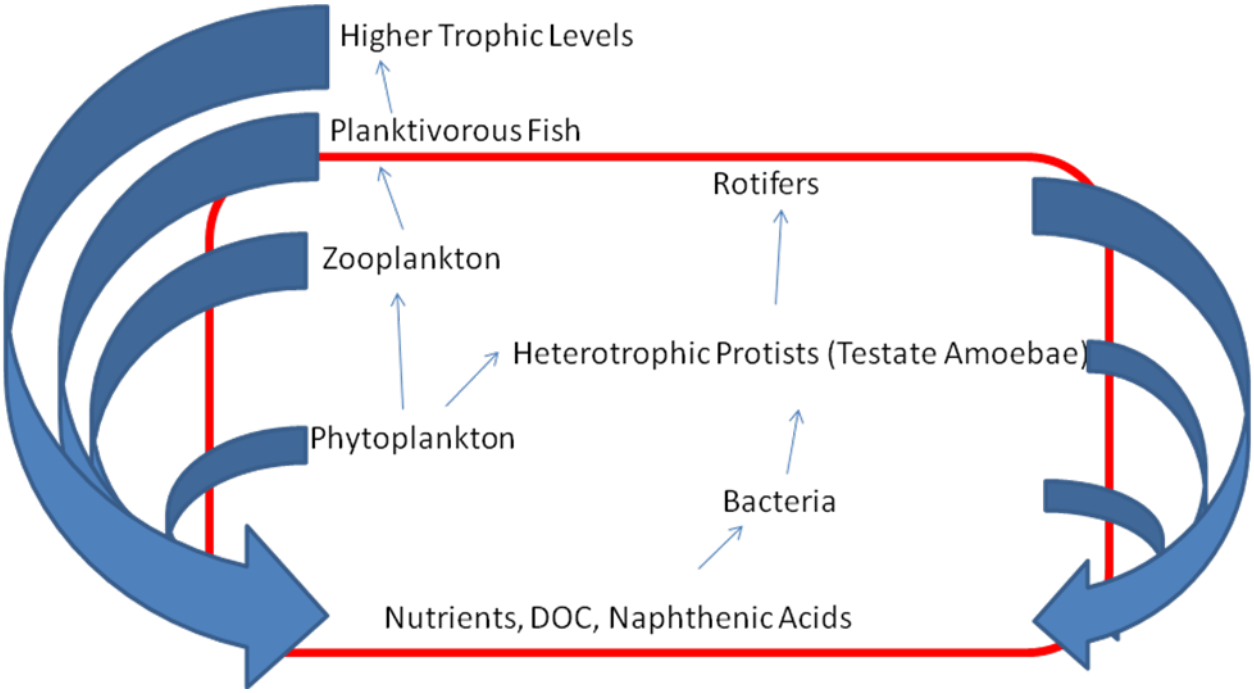


Figure 1: The Microbial Loop

This figure shows the flow of nutrients into a food chain through the microbial loop. The microbial loop is indicated by the box, DOC is representative of dissolved organic carbon. Bacteria are the only organisms that are able to remobilize dissolved organic carbon making it bioavailable at higher trophic levels. Adapted from http://www.esf.edu/.

Research investigating the composition of and representation of relative components of wetland microbial communities, including peatlands in North America, only recently received some attention (Batzer and Sharitz 2008). Most studies relate to protists (specifically the community composition and ecology of testate amoebae) to environmental variables, or pollutants, with little focus on other fractions of the microbial community (bacteria, fungi, and micrometazoa) (Warner 1987, Charman and Warner 1992).

Testate amoebae are a major part of the microbial community in peatlands ecosystems. In a study conducted by Gilbert *et al* testate amoebae were dominant, contributing 48% of the total biomass (2007). Peatland ecosystems are rich in organic matter content suggesting the microbial loop has a vital role in the operation of these ecosystems. The dominance of testate amoebae implies protozoa (including ciliates and heterotrophic flagellates) are central players to

the microbial loop in peatland systems, as they are less sensitive to the acidity of the medium than other groups (micrometazoa).

1.2 Oil Sand Mining in Northern Alberta

Open-pit mining results in the destruction of landscapes and the creation of large quantities of tailings that are produced when bitumen is separated from oil sand. The increasing scale of oil sand operations reveals the essential need for reclamation. While oil sand operators claim that reclamation efforts have reached "4357 hectares of land" and millions of dollars have been put into reclamation efforts (© 2006 Syncrude Canada Ltd.), there is no standard regarding land restoration. Under the Environmental Protection and Enhancement Act (EPEA) and supervised by the Alberta Department of Environment, oil sand operators are required by law to reclaim areas affected by mining by creating a landscape of equivalent production to those that existed prior to mining activities.

On-site tailing ponds are constructed to store noxious compounds (tailings, process water) produced by the oil sands process (MacKinnon 1989). In these holding facilities tailings become densified and thicken to become what are referred to as mature fine tailings (MFT). Over time, as MFT settles, the over-laying water is clarified and reused in the Clark caustic hot water process to separate bitumen from oil sand or in reclamation strategies, as a water-cap for reclaimed wetlands (Leung *et al* 2001, Salloum *et al* 2002).

Abiotic factors in wetlands that receive oil sands processed materials (OSPM) are expected to deviate from those of natural systems. For example, there will be higher concentrations of residual bitumen from the extraction process (MacKinnon and Sethi 1993) and elevated levels of naphthenic acids (NA's), which may be upwards of 30 times higher than natural levels (Holowenko *et al* 2002). NA levels of 0.3 – 0.5 mg L^{-1} have been reported in

natural wetlands while concentrations as high as 88mg L^{-1} have been reported in tailings ponds (Holowenko *et al* 2002).

Wet landscapes with the incorporation of oil sand extraction by-products have been recommended as sustainable ecosystems for reclamation strategies. In created wet landscapes MFT is transferred from tailings ponds into open pits created by mining and capped with water (either process-affected and/or freshwater) to produce lakes, ponds, and wetlands (Madill *et al* 2001, Leung *et al* 2001).

In recent years many wetlands have been created or restored to mitigate the damage and destruction caused by oil sand mining and a number of strategies have been tested for reclamation purposes. Creation of shallow open-water bodies and wetlands presented new challenges for aquatic scientists, to produce high-quality wetland habitats. Many types of wetlands have been created on oil sand leases with different OSPM and non-OSPM amendments in order to determine environmental sustainability. These ponds have allowed researchers the opportunity to conduct studies on the effects of different OSPM amendments on aquatic organisms and the overall health of reclaimed wetland systems.

Naturally occurring microbial communities found in the sediments of open-water habitats created by oil sand operators in northern Alberta are exposed to residual levels of bitumen and the components associated with oil sands processing (Naphthenic Acids (NA), sulphates, and chlorides) (Hadwin *et al* 2006). These microbial communities have been shown to have some capacity for breaking down hydrocarbons and mixed cultures of bacteria were found to be proficient in NA degradation both in natural and in-vitro systems (Hadwin *et al* 2006). While naturally occurring microbial communities are capable of breaking down these compounds, thus reducing the toxicity of oil sands processed materials (OSPM) in impacted wetlands, little is known concerning the composition of these communities (Herman *et al* 1994, Holowenko *et al* 2002, Hadwin *et al* 2006).

Created wetlands are put in place with the goal of replacing the functionality of former systems that have been lost through disturbance. Unfortunately, not all functions of a wetland are, or can be considered during reclamation efforts and many wetland restoration projects have shown that structure does not necessarily imply function (Reinartz and Warne 1993). Therefore, as researchers we are unable to assume that these created systems are functioning properly and require biological indicators to provide this information for us. This project aims to draw on the broad knowledge of the testate amoebae community composition and ecology as an indicator of the response of the overall microbial community to stressors in the environment as a result of oil sands mining.

1.3 Objectives

Wetland creation using mining by-products (OSPM, oil sands processed water (OSPW)) relies heavily on the ability of aquatic bacteria to metabolize residual hydrocarbons and assimilate carbon making it available at higher trophic levels. Given that testate amoebae are a large component of the microbial community, and sensitive to micro-environmental gradients, we suspect that species assemblage of testate amoebae can be used as indicators of the whole microbial community. Thus, this project aims to characterize testate amoebae composition in representative OSPM wetlands and compare these communities with non-OSPM wetlands, including both open-water wetlands and peatlands. This requires the establishment of protocols for characterizing testate amoebae communities in peatland and open-water wetlands, and to identify and quantify testate amoebae in various wetland types (peatlands, control, reference, and OSPM) that are, or will be, a part of future oil sands reclamation strategies. Testate amoebae will be related to other a parts of the whole microbial community: bacteria and fungi, thus protocols must also be established for quantifying these microbial fractions. The final objective

is to assess the practicality of using testate amoebae in various wetlands as indicators of microbial community health, wetland establishment, and performance of created wetlands.

The fundamental purpose of this project is to provide a novel performance indicator to be included in the Wetlands Guideline for oil sand operators in the region of Wood Buffalo, Alberta. Ultimately, it will be a standard which oil sands operators can use efficiently and easily to determine the status of reclaimed wetlands. This performance indicator will be based on; 1)an index of microbial communities (bacteria and fungi) from varying wetlands (OSPM and non-OSPM-affected) and 2) testate amoebae assemblages related to general abundance values for bacteria and fungi. It is expected that the success of wetland restoration will be predicted based on testate amoebae indicators and biomass measurements of the rest of the microbial community (bacteria and fungi).

2.0 Materials and Methods

2.1 Study Region

2.1.1 Location

Fort McMurray (56.66° N 111.21° W), Alberta, lies approximately forty kilometres south of two of the largest oil sand mining and refinery operations in the world, Suncor Energy Inc. and Syncrude Canada Ltd. (Figure 2). Syncrude Canada Ltd. and Suncor Energy Inc. can be reached on Highway 63 north which runs parallel to the Athabasca River. On the west side of the river lies the Syncrude Canada lease site (Figure 3) occupying approximately 102,000 hectares (© 2006 Syncrude Canada Ltd.) while the main operation for Suncor Energy Inc. (Figure 4) is situated on the east side of the highway and river, and occupies approximately 990 hectares (Suncor Energy Inc, 2006). Once on the Syncrude or Suncor lease sites wetlands were accessed in pick-up trucks via mining roads, as well as dirt roads specifically created for access to research wetlands, all sites were within thirty-five to forty minutes of the main lease site access.

2.1.2 Vegetation

Fort McMurray and surrounding area are located in the Boreal Plains Ecoregion which encompasses the broadleaf forest and the mixed forest (Figure 5 and 6) (National Atlas of Canada 1993). Forestry is the primary industry in the Boreal Plains (National Atlas of Canada 1993). Key tree species include white and black spruce, balsam fir, jack pine, tamarack, and lodge-pole pines, broadleaf species which are also common within this region are aspen and poplar, and birch in some areas (National Atlas of Canada 1993). Fire has the greatest effect on distribution and growth rates of trees in this area, but native insect pests and disease are also likely to affect these forests (National Atlas of Canada 1993).

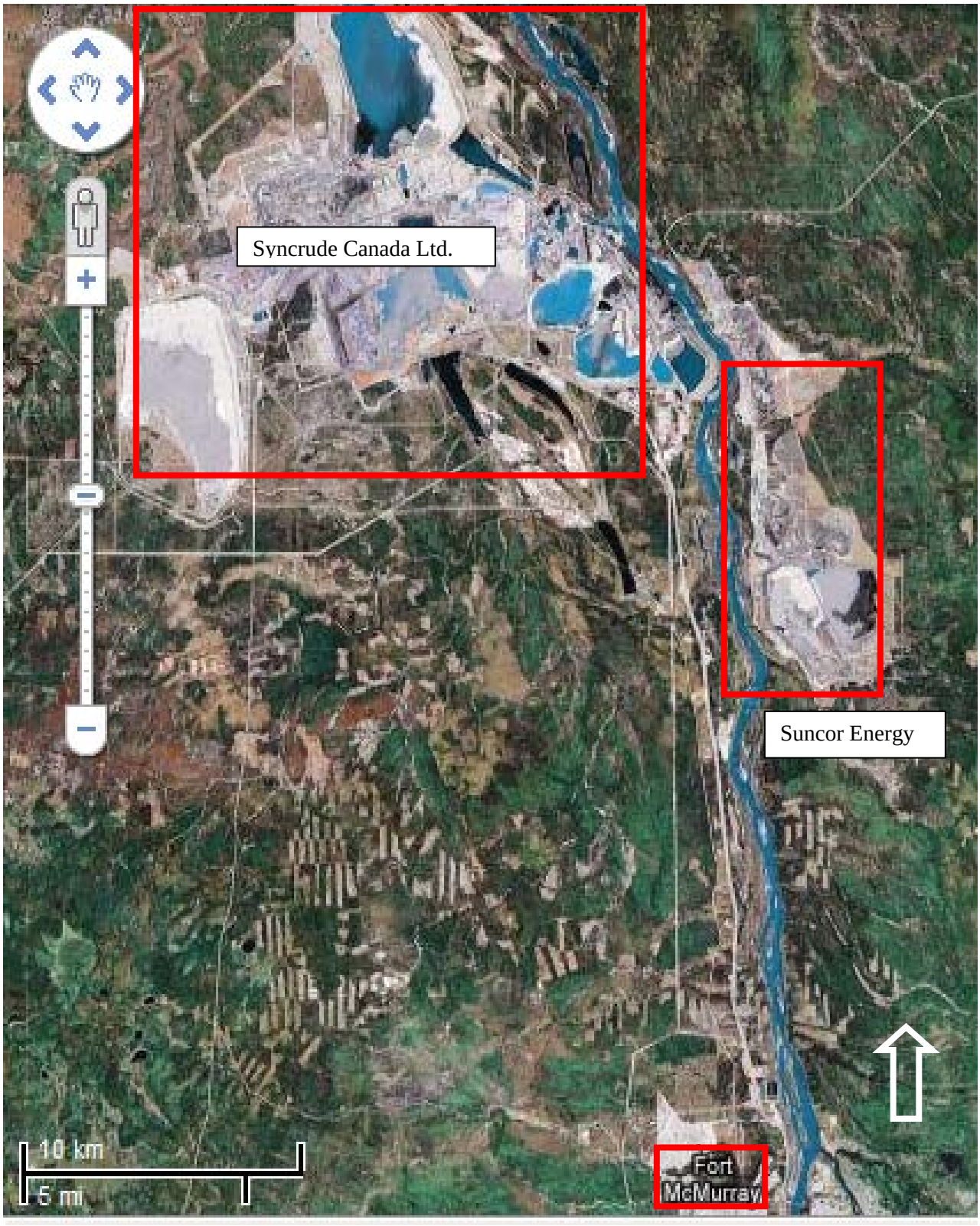


Figure 2: Aerial View of Oil Sand Mining and Refinery Operations; Suncor Energy Inc. and Syncrude Canada Ltd. Relative to the City of Fort McMurray, Alberta

(Google Maps, 2009).

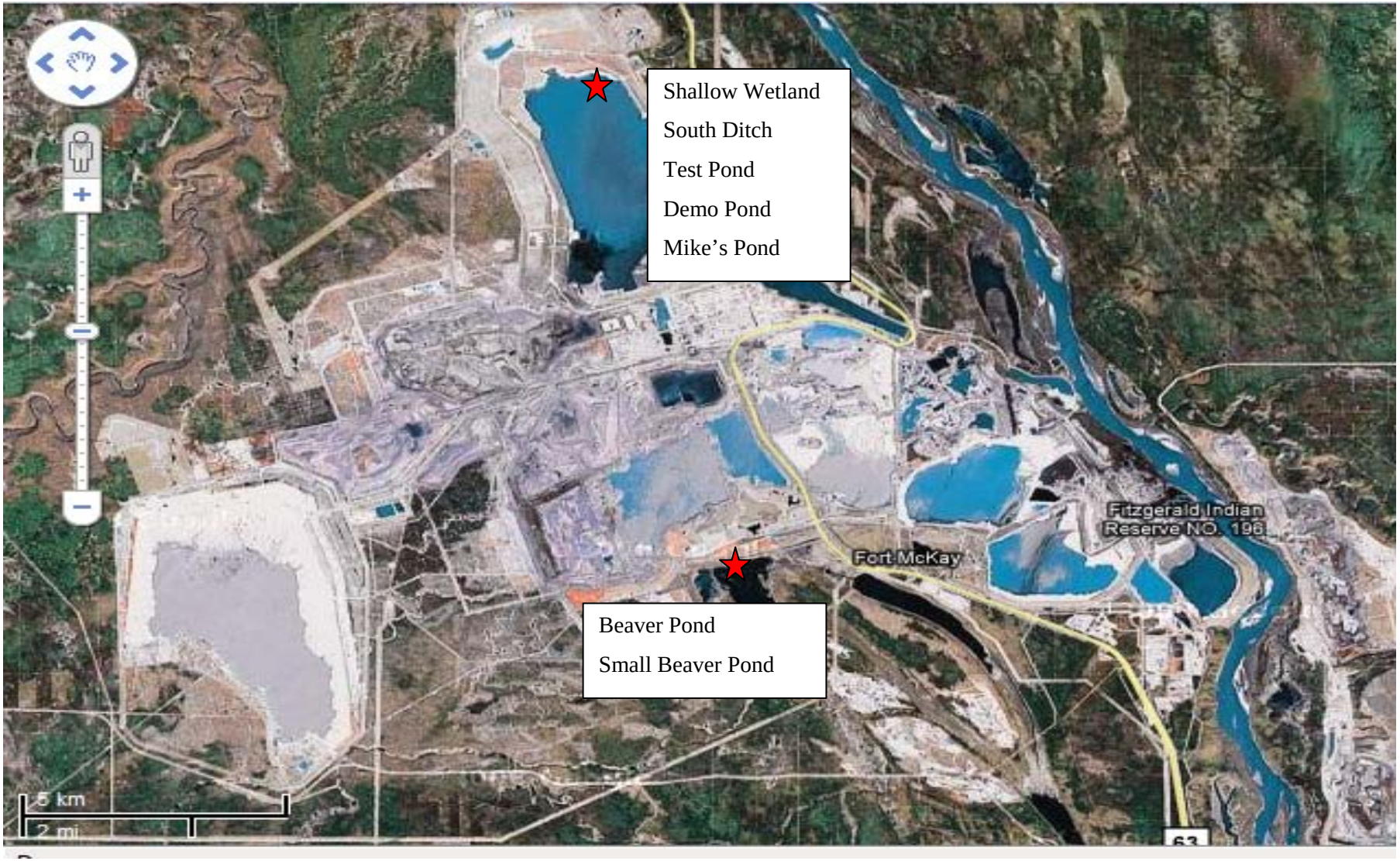


Figure 3: Aerial View of Wetland Sites on Syncrude Canada Ltd. Lease

(Google Maps 2009)

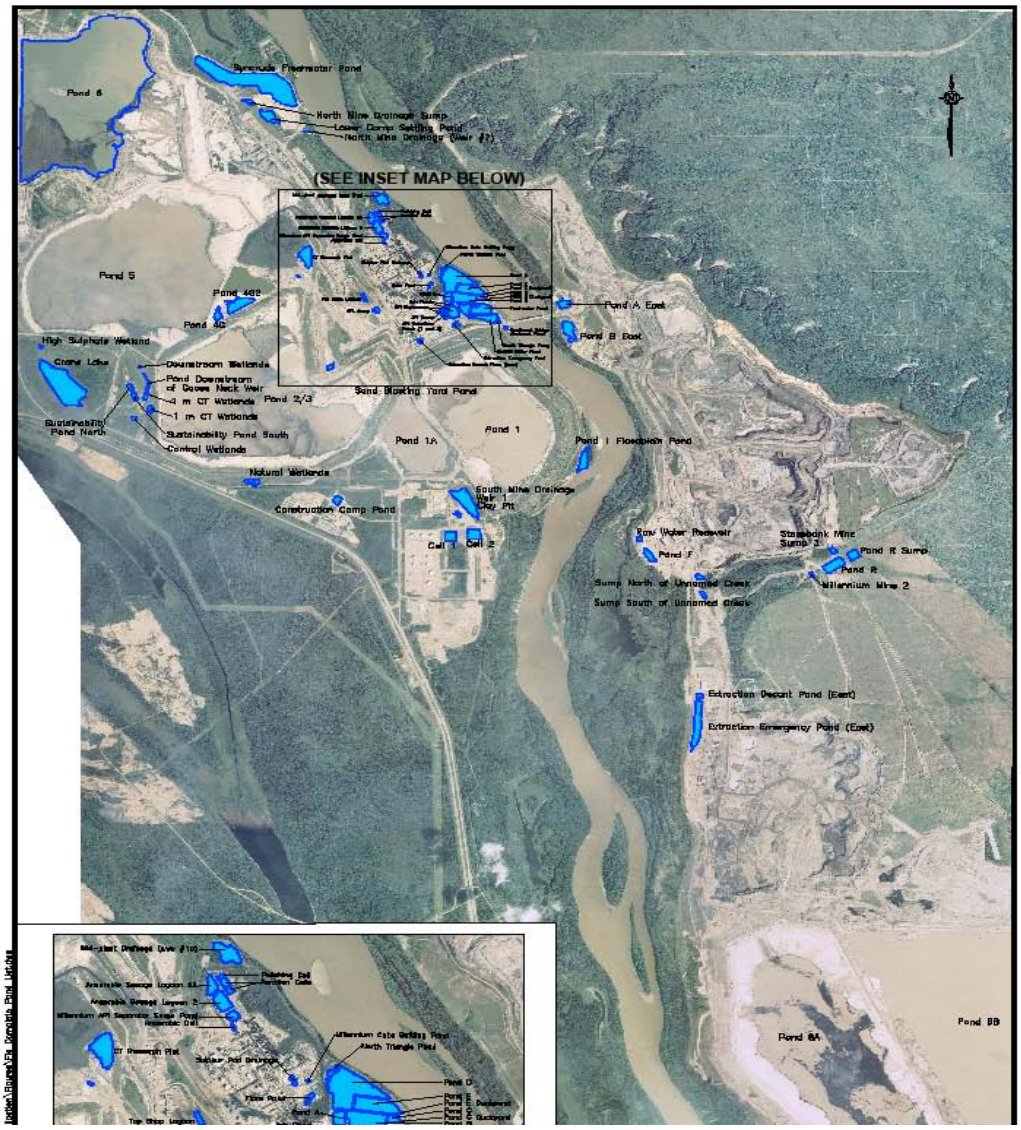


Figure 4: Aeriel View of Wetland Sites on Suncor Energy Inc. Lease

(Courtesy of Suncor Energy Inc.)

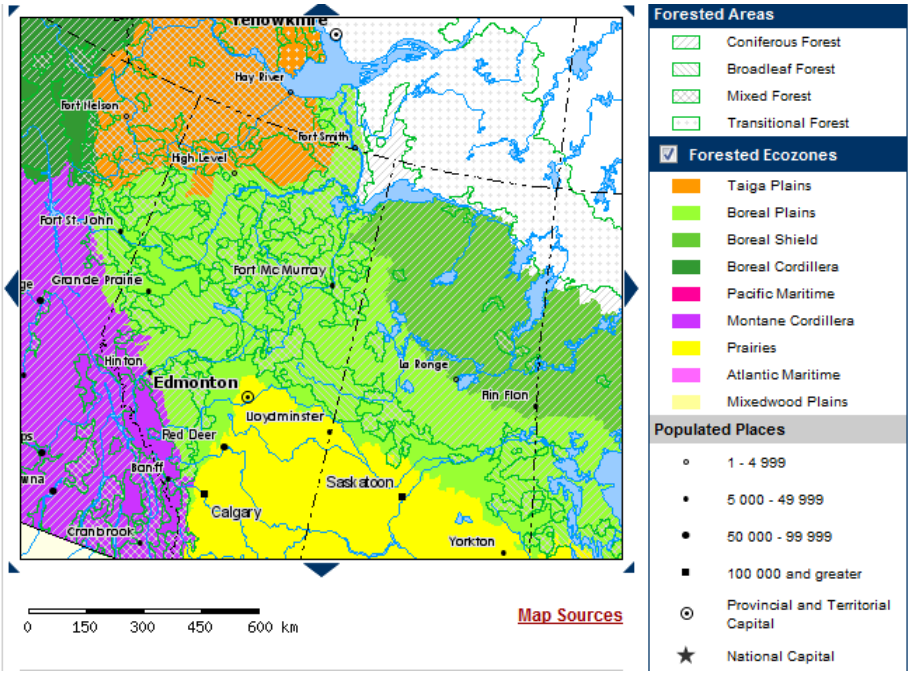


Figure 5: Forested Ecoregion of Canada

Fort McMurray and surrounding areas fall into the Boreal Plains Ecoregion (Canada-Vegetation Cover, Fifth Edition of the National Atlas of Canada. 1993).

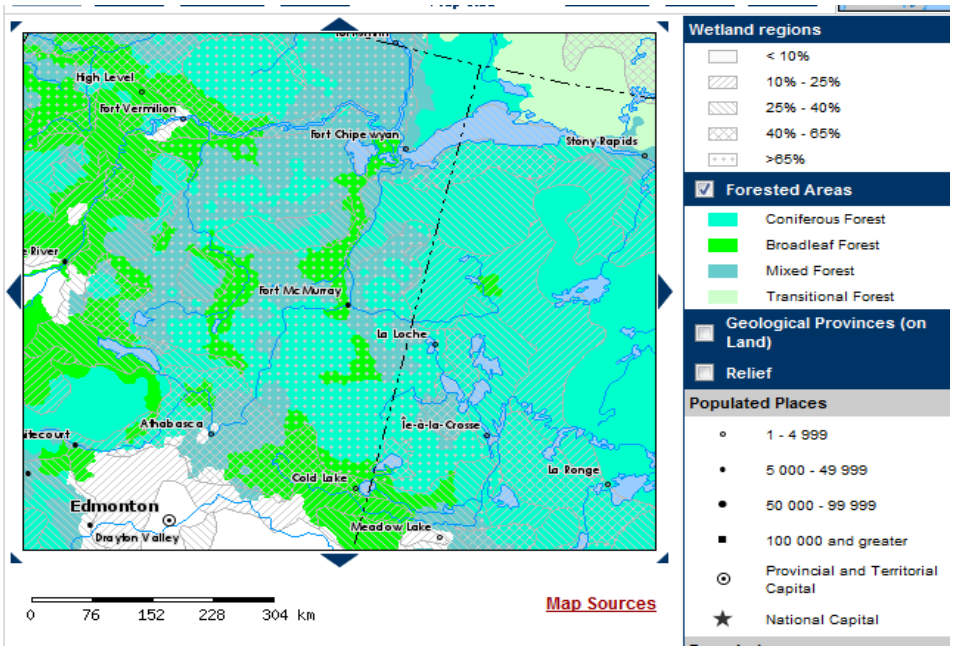


Figure 6: Forested Areas within Ecoregion

Fort McMurray and surrounding areas fall into the Broadleaf and Mixed Forest areas (Canada-Vegetation Cover, Fifth Edition of the National Atlas of Canada. 1993).

2.1.3 Wetland Region

About 20% of the land in the Boreal wetland region is covered by wetlands with bog and fens being the most common type of wetland (National Atlas of Canada 1993). Delta marshes are also common around large lakes and rivers, most notable are the Slave River delta (which is found in Great Slave Lake), and the Peace-Athabasca delta (located just west of Lake Athabasca) (National Atlas of Canada 1993). Within this region there are over 100 000 beaver ponds covering 5 to 10% of its total area (National Atlas of Canada 1993).

Fort McMurray and surrounding areas fall into the category containing 25-63 % of land coverage as wetlands (Figure 7) with marshes (bog, and fen peatlands) being the characteristic wetland type in this particular forest region (National Atlas of Canada 1993).

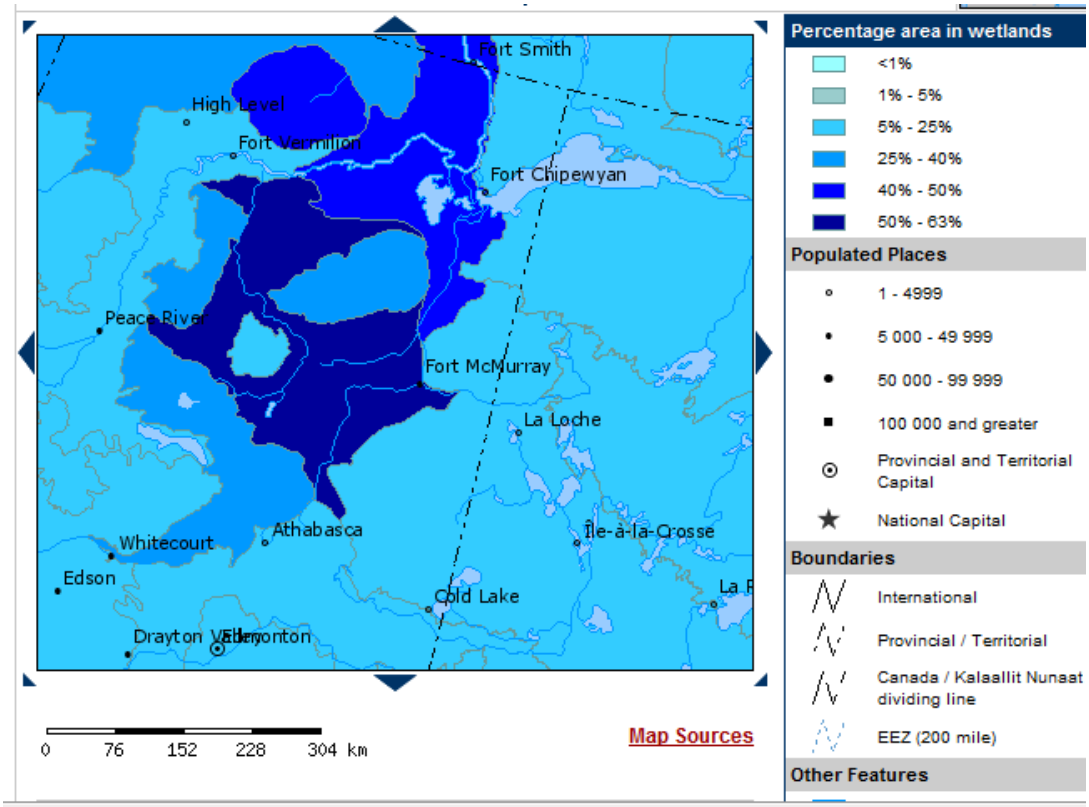


Figure 7: Percentage of Wetland Cover within Ecoregion

Fort McMurray and surrounding areas fall into categories of 25-63% of wetland coverage (Canada-Vegetation Cover, Fifth Edition of the National Atlas of Canada. 1993).

2.1.4 Climate

Fort McMurray and surrounding area experiences fairly warm summers and cold winters; with annual temperatures on average ranging from -18.8 to 16.8º Celsius (National Climate Data and Information Archive). The daily minimum ranges from -2.2º to -24º Celsius in the winter months (October to April) but have been recorded as cold as -50º Celsius (National Climate Data and Information Archive). The temperature in summer months ranges from 10º to 21.9º Celsius but has been recorded as high as 37º Celsius (National Climate Data and Information Archive).

The annual precipitation in Fort McMurray is 455 mm, with the majority (310.6 mm) falling between May and September in the form of rainfall (National Climate Data and Information Archive). Snow fall, between September and May, accounts for approximately 155.8 mm of the annual precipitation (National Climate Data and Information Archive).

2.2 Sampling Methods

2.2.1 Wetland Sites

In July of 2007 and 2008, twenty-four wetland sites were selected and sampled (Table 1). Field reconnaissance on fifteen sites in the summer of 2007 was completed in order to determine the type of wetland sites available, the accessibility to these sites and which sites were being used by other groups. Sites were chosen in roughly equal numbers for each site type (peatland, control, reference, OSPM). The same fifteen wetlands were sampled in 2008, with an additional nine wetlands for a total of twenty-four wetland sites (Table 1, and Figures 3 and 4). Wetlands were selected in consultation with scientific staff at Syncrude Canada Limited (Nadia Loubiri and Christopher Beierling) and Suncor Energy Incorporated (Wayne Tedder and Christine Daly) as well as other research groups (Waterloo Group – Dixon Laboratory, Windsor Group – Ciborowski Laboratory).

All wetland sites were categorized into one of four categories based on the degree they had been affected by oil sand mining; 1) natural peatland sites having never been affected by mining, these are Aurora peatland sites which were located on the Aurora lease north of the main mine site on the Syncrude Canada Ltd. land lease, 2) natural/control open-water marsh sites having never been affected by mining, 3) reference sites on reclaimed mine sites, but without OSPM incorporated into the wetland and, 4) OSPM wetlands which have OSPM (oil sands process material) or OSPW (oil sands process water) directly incorporated into the wetland.

Table 1: Summary of Wetlands Sampled on Suncor Energy Incorporated and Syncrude Canada Limited Lease Sites

Wetland Site	Abbrev.	Age	Amendment	Classification
Aurora Peat Site #1	AU 1.	N/A	Naturally occurring peatland - an accumulation of partially decayed vegetation matter	Peatland
Aurora Peat Site #2	AU 2.	N/A	Naturally occurring peatland - an accumulation of partially decayed vegetation matter	Peatland
Loon Lake	LL	~1970	Large pond that is surrounded by roads. Area was disturbed and possibly excavated, however the area was never mined. Roads were built around the pond in the late 60s. Filled with water from precipitation and runoff.	Control
Sand Pit Wetland	SP	2004	Opportunistic wetland, natural sand capped w/ peat-mineral mix , receives fresh water from run-off	Control
Sedimentation Pond	SED	2000	This wetland formed opportunistically in 2000 when a damn was constructed adjacent to a depression in the boreal peatland. Water levels, fed by surface runoff, rose to establish this wetland. The wetland was designed to lower Total Suspended Solids (TSS), derived from the surrounding watershed, before the water from this wetland was released into the Athabasca River.	Control
MacLean Creek Wetland	MC	2000	This wetland formed opportunistically in 2000 when a damn was constructed adjacent to a depression in the boreal peatland. Water levels, fed by surface runoff, rose to establish this wetland.	Control
Beaver Pond	BP	N/A	Natural wetland, was (temporarily) affected in 2004 by removal of other upstream beaver dams threatening nearby access road	Control
Small Beaver Pond	SBP	N/A	Natural wetland	Control
Duck Pond	DUCK	~1980	Formed opportunistically after the mid-berm road was constructed. There was water pooling in the Duck Pond in the early 1980s, although the footprint retreated over the years as a result of reclamation activities. Reclamation placed top soil and planted trees nearby in 1990.	Reference
V-Notch Weir	V-NOT	1999-2000	Natural sediments. No tailings or process water was ever added to the wetland. Elevated conductivities & pH indicates some seepage from Pond 2/3 enters the wetlands via groundwater movement.	Reference
Control Reservoir	CON-RES	1999-2000	Natural sediments. No tailings or process water was ever added to the wetland. Elevated conductivities & pH indicates some seepage from Pond 2/3 enters the wetlands via groundwater movement.	Reference
Shallow Wetland	SW	1993	One of six Large Scale Test Ponds (LSTP) established in 1993. This is a constructed wetland that ranges between 0.5 to 0.75m deep. Originally, a single wetland was constructed to store non-process-affected waters diverted from the West Interceptor Ditch. Subsequently, a dividing berm was constructed to take advantage of the very different topography. Water levels are supplemented from surface waters collect in and pumped from a sump located along the north berm.	Reference
South Ditch	SD	1993	Constructed with Shallow wetland, after a dividing berm was constructed	Reference
Bill's Lake	BL	1996	Opportunistic wetland that occurred due to the break in slope and depression between the two reclamation sites, resulted in the accumulation of water. This resulted in the formation of Bill's Lake. This lake is part of the capping depth study and was formed in 1996. There is 20 to 50 cm of saline overburden at the bottom of the watershed.	Reference
Peat Pond	PP	1999	The land was graded and contoured in 1999 and reclamation. It is constructed of 80m of saline overburden overlain with 20 cm of peat.	Reference
High Sulphate	HS	1987	reclaimed by adding 15 cm of muskeg soil. This wetland developed naturally in a depression in this reclaimed area in 1987. Water levels rose in the depression and were fed by precipitation, runoff and possibly seepage from overburden	Reference
Sustainability Ponds	SUS	1991, 1992	North SW – 54m x 124m x 8m: and South SW – 49m x 111m x 8m, contain MFT from ponds 1&3and are capped w/ tailings recycle water, phosphorus was added to the South SW in 1992	OSPM
Jan's Pond Wetland	JANS	1999	A thin layer of CT, constructed over overburden, CT process water circulates through this wetland at 75L/min	OSPM
1MCT Wetland	1MCT	1999	20cm of peat-mineral mix overlying 1MCT	OSPM
Demonstration Pond	DP	1993	Large Scale Test Pond, constructed in 1993. Nine meters of Fine Tailings overlain by 2.5 meters of diverted local surface stream flow. Constructed in Saline	OSPM
Mike's Pond	MP	1997	Surface area approximately 4ha, contents – CT Release water from 1997 CT Prototype	OSPM
Natural Wetland	NW	1986	Tailings sand w/ 15cm peat-mineral mix cap, opportunistic wetland, fed by run-off (~25%) and dyke seepage (~75%), CT process water was added to the west end in 1996 and 1997	OSPM
Test Pond 9	TP9	1993	One of six Large Scale Test Ponds (LCTP) established in 1993. This pond is defined as the Tailings Pond Water Detoxification and consists of 6000m^3 tailings pond surface water that was transferred from the Mildred Lake Settling Basin (MLSB).	OSPM
4MCT Wetland	4MCT	1999	4 m, uncapped CT, constructed over overburden, two areas capped w/ 20cm of peat-mineral reclamation mix, CT process water circulates through this wetland at 75L/min	OSPM

2.2.2 Site Characterization and Sediment Sample Collection

In each wetland field plots were identified (meter-square quadrants) in each zone of vegetation. A sediment sample was collected from each zone of vegetation, up to a maximum of three samples and designated as “vegetated” or “non-vegetated”. When two (or three, in the case of Shallow Wetland) samples were taken, the first (Loon Lake-1, for example) came from an area with the most dense vegetation thus is the “vegetated” sample while sample two (Loon Lake-2) was from a deeper area, with less vegetation consequently referred to as the “non-vegetated” sample. This technique yielded thirty-five samples from twenty-four wetland sites. Plant species were identified in each field plot, vegetation percent cover was estimated visually (vascular and non-vascular plants), multi-meter data and a visual survey of water turbidity were recorded. Water turbidity was graded on a scale of 0, which was completely clear, to 3 which was completely opaque.

In open-water sites data gathered included; water depth, water pH, water conductivity and dissolved oxygen (Table 3). A surface sample of two-centimeters of sediment from the sediment-water interface was collected, and put in to whirl pak ™ bags and stored in a cooler for transport to the laboratory. All aquatic sediment samples were preserved with a 2% gluteraldehyde solution and stored at 4^{o}C until processing.

Two peatland sites were sampled for this study; Aurora site 1 and Aurora site 2. Aurora site 1 was located near an open-water marsh and therefore was a “wetter” peatland site compared to Aurora site 2. Two samples were taken in Aurora site 1 and three samples were taken in Aurora site 2, each sample from a different elevation from the water table. In peatland sites data gathered included; water table depth, pH and percent vegetation cover. A surface sample of *Sphagnum*

and peat was collected. A block, 10 centimeters deep and 5 centimeters wide was cut with a serrated bread-knife and bagged. All peatland samples were transported to the laboratory in dark coolers and stored at 4ºC until processing.

2.3 Analyses

2.3.1 Testate Amoebae

Analysis of testate amoebae followed Warner (1987, 1988) and Warner and Charman (1994). For peat samples two cubic centimeters from the top two centimeters of the sample was weighed. In a separate test-tube, two lycopodium spore tablets (1 tablet = 13911 spores) were dissolved in concentrated hydrochloric acid (to release the spores) and topped with distilled water. A known number of spores were added to each sample and used to calculate the concentration of testate amoebae per gram of sample. The spore tablets were centrifuged three times at 1000 rpm for two minutes each time in an IEC CentraCL2 centrifuge, decanting off excess hydrochloric acid and water each time. After centrifugation spores were poured into a beaker with the two cubic centimeters of pre-weighed peat, adding about twenty milliliters of distilled water, this solution was boiled for five to ten minutes. The mixture was then filtered using a tea sieve into a small beaker. The *Sphagnum* moss was rinsed before being disposed of. This solution settled for one to two hours and then the top clear layer of water was decanted. The remaining solution was poured into test tubes and centrifuged in an IEC HN-SII centrifuge for two minutes three times, decanting excess water each time. The concentrate was poured into small tubes and centrifuged in an IEC HN-SII for two minutes and excess water was decanted. Two drops of a mixture of glycerol containing safranin was added and the samples were then left in an oven at very low heat overnight to evaporate remaining water.

To make slides, a drop from the safranin-glycerol concentrate was smeared on a glass slide in a drop of pure glycerol which was then covered by a cover-slip and sealed in each corner with nail polish. The slides were systematically scanned and testate amoebae were counted and identified.

Testate amoebae in sediment samples were processed in much the same way. Organic content was separated out by washing the fresh sediment samples on a coarse screen (150µm mesh) and then a fine screen (20µm mesh), lycopodium spores were added and samples were stained as described above. The slides were made, scanned, and testate amoebae were counted and identified.

2.3.2 Bacteria and Fungi

Analysis of the bacterial and fungal community components was done using epifluorescence microscopy to determine biomass. Bacterial and fungal isolation followed Fry 1990, Mitchell personal communication. Two cubic centimeters of fresh sediment (preserved in a gluteraldehyde solution) was weighed and disaggregated using a kitchen food chopper. The sample was washed on a 500µm Nitex™ mesh filter using de-ionized water to bring the total volume to 500mL. This volume was then filtered through a series of Nitex™ screens (300, 100, 50, 20 and 10µm) in order to remove larger particles from the sample and isolate the bacterial and fungal fractions of the sample. An aliquot of ten milliliters was used for bacterial analysis and another ten milliliters for fungal analysis.

Bacteria samples were filtered through a 10µm white filter to remove all contaminants. The sample was then stained with 4,6 diamidino-2-phenylindol (D.A.P.I) solution for thirty minutes in the dark. Once stained, the sample was homogenized with a Vortex Mixer (SB 223-1) before being vacuum-filtered through a 0.2µm pore size, black nucleopore™ filter mounted

over a 0.45µm pore size support filter. The filter was rinsed three times with one milliliter of de-ionized water, each time. The filter was dried with a kimwipe™ and placed on a glass slide in a drop of immersion oil. Another drop of immersion oil was added on top of the filter and covered with a cover-slip (22mmX50mm). The cover slip was gently pressed in to place and the slide was covered in aluminum foil and stored at 4ºC until analyses could be performed (two to five days).

Fungal samples were stained with Calcofluor-white M2R (also called Cellufluor) for two to four hours in the dark after disaggregation and filtration (as with testate amoebae and bacteria samples) down to 15µm. Once stained, the sample was homogenized with a Vortex Mixer (SB 223-1) before being vacuum-filtered through 0.8µm pore size, black nucleopore™ filter. The filter was rinsed three times with one milliliter of de-ionized water each time and mounted on a glass slide over a drop of immersion oil. Another drop was added over the filter and a cover-slip is laid on top (22mmX50mm). The slides are stored at 4ºC until analyses can be performed (two to five days).

All bacteria and fungi samples were viewed under epifluorescence microscopy at X1000 magnification. Ten pictures were taken of each prepared slide (one for each sample) and the images were recorded using a digital camera connected to the microscope and a computer. Using ImageJ the photos were analyzed. ImageJ is an image processing program developed by the National Institute of Health. Once photos were captured and saved they were viewed in ImageJ and number of bacteria (Beeckman *et al* 2009) in each field and length of fungal hyphae was determined (Protocol in Appendix B).

2.3.3 Biomass for Testate Amoebae, Bacteria and Fungi

Using testate amoebae counts and experimentally determined geometric shapes and sizes (Charman *et al* 2000), an average volume for each species was determined, a conversion factor given by Nguyen-Viet *et al* 2007(1) ($1\mu m^3 = 5.6 \times 10^{-7}$ µg C) permitted volume to be converted into biomass (microgram of Carbon per gram of sample (dry weight)) at each site.

For bacteria, photos were analyzed in ImageJ to yield an average (ten fields of view) number of cells per field of view at X1000. These values were recorded and substituted into the following equation (equation 1) to yield the number of cells/weight of dry sample (Fry 1990, Mitchell personal communication).

$$\textbf{\# of Bacteria/gram of Dry weight} = \textbf{(N* S/s *V/v)/DW} \qquad (eq^n\ 1)$$

Where N is the mean number of bacteria per slide (average number of bacteria per field of view multiplied by sixteen), S is the useful surface of the filter (determined by measuring discoloration on the filter), s is the surface of one field at X1000 magnification, V is the total volume of the sample, v is the volume filtered, DW is the dry weight of the sample (Fry 1990, Mitchell personal communication). From number of bacteria per g of dry weight a standard for bacterial volume of $0.125\mu m^3$ per cell was used to determine bio-volume at each site. Finally a conversion factor given by Nguyen-Viet *et al* 2006 ($1\mu m^3 = 5.6 \times 10^{-7}$ µg C) was used to convert volume into biomass.

A similar technique was used for fungi where the length and width of hyphae were measured and averaged for ten fields of view using ImageJ. Biovolume (μm^3) was determined using a derivative of the equation for volume of a cylinder (equation 2);

$$\mathbf{V = (\pi/4)W^2(L\text{-}W/3)} \qquad (eq^n\ 2)$$

This value is then multiplied by a conversion factor given by Nguyen-Viet *et al* 2006 ($1\mu m^3 = 2.5 \times 10^{-7}$ µg C) in order to give biomass, in µg of Carbon per gram of sample.

3.0 Results

3.1 Environmental Parameters and Sediment Characteristics

3.1.1 Water Characteristics

Detailed water chemistry data for each wetland site (in the 2008 field season) are provided in Table 2. The pH in twenty-four wetland sites varied between 3.7 and 8.2. The pH in control, reference, and OSPM wetlands ranged from 6.95 to 8.18. The pH in peatlands ranged from 3.7 to 6.8. The wetter peatland site (Aurora 1) was more alkaline (pH ranging from 6.76 - 6.88) when compared to the drier site (Aurora 2, pH ranging from 3.7 to 5.35).

Dissolved oxygen values for open-water sites spanned 78 and 161% for all sites. One measurement was taken in each site therefore no comparisons can be made between vegetation zones. Conductivities in all sites (open-water and peatland) ranged from 298 to 4689 μS. Conductivity values measured in OSPM wetlands ranged from 1330 to 4689 μS but on average were greater than 2000 μS. Reference and control wetlands had conductivity values between 309 and 2998μS, with the majority of values falling below 1000 μS.

3.1.2 Vegetation Communities

A survey of the vegetation composition and cover in each wetland was completed using a one meter by one meter quadrant. The dominant species are highlighted in Table 3 and a full list of species is provided in Appendix A. The plants which make up the communities in each wetland varied by wetland type; OSPM wetlands were dominated by *Typha latifolia* and *Potamogeton* with low percent vegetation cover (both submerged vegetation and emergent, Table 2). The differences between vegetation communities in reference wetlands and control wetlands were negligible. The percent cover in control and reference wetlands was substantially higher than the communities present in affected wetlands (OSPM). These communities

encompassed a higher number of taxa as well as a greater number of individuals (Table 3, full list in Appendix). Taxa that were commonly found in open-water control and reference sites were *Typha latifolia, Potamogeton, Chara ssp. Scirpus, Carex,* and *Lemna,* among others. Peatlands were completely dominated by *Sphagnum* moss with minor communities of *Carex* and *Equisetum*.

Table 2: Summary of Multi-meter Data for 36 Samples from 24 Wetland Sites

***Dissolved Oxygen was measured in terms of percent saturation (the ratio of DO to the potential capacity expressed in terms of percentage)*

Site	Type	Cond.(µs)/cm	pH	% DO	Depth(cm)	Total % Veg. Cover	% Emergent Veg. Cover	Turbidity (1-3)
AU 1.1	Peatland	712	6.76	N/A	18	100	100	N/A
AU 1.2	Peatland	407	6.88	N/A	17	100	100	N/A
AU 2.1	Peatland	303	3.7	N/A	47	100	100	N/A
AU 2.2	Peatland	350	3.85	N/A	34	100	100	N/A
AU 2.3	Peatland	1753.0	5.35	N/A	19	100	100	N/A
LL-1	Open-Water Control	835	7.64	104.8	11	40	40	1
LL-2	Open-Water Control	835	7.64	104.8	28	0	0	1
SP-1	Open-Water Control	309	8.18	109.9	35	90	90	2
SED-1	Open-Water Control	429	7.13	91.2	54	40	0	2
MC-1	Open-Water Control	433	7.05	96	9	20	0	1
BP-1	Open-Water Control	2244	7.3	97.8	32	35	0	2
SBP	Open-Water Control	298	8.02	102	40	50	40	2
DUCK	Open-Water Reference	971	7.4	97.7	39	100	90	1
V-NOT-1	Open-Water Reference	1134	7.08	116.6	12	40	40	2
CON-RES	Open-Water Reference	547	7.06	107.5	14	55	55	2
SW-1	Open-Water Reference	877	7.5	103.9	12	80	65	1
SW-2	Open-Water Reference	877	7.5	103.9	40	100	15	1
SW-3	Open-Water Reference	877	7.5	103.9	50	95	0	2
SD-1	Open-Water Reference	885	7.8	138	12	40	40	1
SD-2	Open-Water Reference	885	7.8	138	56	95	0	1
BL-1	Open-Water Reference	883	6.95	105	35	100	15	3
BL-2	Open-Water Reference	883	6.95	105	65	90	0	3
PP-1	Open-Water Reference	1765	7.35	122.3	33	85	0	2
HS-1	Open-Water Reference	2998	7.71	160.6	32	75	0	2
HS-2	Open-Water Reference	2998	7.71	160.6	7	60	60	2
SUS-N	Open-Water OSPM	2200	7.68	95.3	32	60	60	3
SUS-S	Open-Water OSPM	2093	7.48	94.6	36	45	45	3
JANS	Open-Water OSPM	2229	7.11	81.6	17	30	30	3
1MCT	Open-Water OSPM	2018	7.39	89.9	11	25	25	3
DP-1	Open-Water OSPM	2233	7.27	80.4	60	90	0	3
DP-2	Open-Water OSPM	2233	7.27	80.4	42	80	80	3
MP	Open-Water OSPM	4689	7.97	114	22	45	0	2
NW-1	Open-Water OSPM	1330	7.8	78	10	70	0	2
NW-2	Open-Water OSPM	1330	7.8	78	3	100	55	3
TP9	Open-Water OSPM	1345	6.92	94.4	60	40	40	3
4MCT-1	Open-Water OSPM	2290	7.97	123	15	0	0	3

Table 3: Summary of Vegetation Characteristics of 24 Wetlands Sampled during Field Season 2008

Dominant species only, Full version in Appendix

Wetland	Wetland Type	Identity and Percent Cover
Aurora Peatland 1	Peatland	*Carex* 45 % *Moss* 45 %
Aurora Peatland 2	Peatland	*Sphagnum* 90% *Equisetum* 20%
Sedimentation Wetland (SED)	CONTROL	*Potamogeton* 40%
MacLean Creek (MC)	CONTROL	*Lemna* 20%
Loon Lake (LL)	CONTROL	*Scirpus* 15% *Typha latifolia 25%*
Beaver Pond (BP)	CONTROL	*Chara* 35%
Small Beaver Pond (SB)	CONTROL	*Carex rostrata* 40% *Lemna* 10%
Sandpit Wetland (SP)	CONTROL	*Scirpus* 40% *Typha latifolia* 50%
Control Reservoir (CON RES)	REFERENCE	*Typha* 25% *Scirpus* 30%
V Notch Weir (V NOT)	REFERENCE	*Scirpus* 40%
Shallow Wetland (SW)	REFERENCE	*Typha latifolia* 50% *Scirpus* 15%
South Ditch (SD)	REFERENCE	*Typha* 30% *Charales* 85%
High Sulphate (HS)	REFERENCE	*Chara* 75%
Peat Pond (PP)	REFERENCE	*Chara* 85%
Bill's Lake (BL)	REFERENCE	*Ceratophyllum* 90%
Duck Pond (Duck), formerly South West Corner Waste Area 11	REFERENCE	*Utricularia* 10% *Typha latifolia 90%*
Test Pond 9 (TP9)	OSPM	*Typha latifolia 40%*
Natural Wetland (NW)	OSPM	*Potamogeton filiformis* 70%
4 Meter Consolidated Tailings (4MCT)	OSPM	*Typha* 60%
Mike's Pond (MP)	OSPM	*Potamogeton* 45%
Sustainability Pond North (SUS N)	OSPM	*Typha latifolia* 60%
Sustainability Pond South (SUS S)	OSPM	*Typha latifolia* 45%
Demo Pond (DP)	OSPM	*Potamogeton* 90%
1 Meter Consolidated Tailings (1MCT)	OSPM	*Typha* 25%
JANS Pond	OSPM	*Typha* 20% *Scirpus* 10%

3.1.3 Sediment Characteristics

Bulk density ranged from 0.02 to 1.29 g/cm^3 in all site types (Table 4). In peatland sites bulk density ranged from 0.03 to 0.08g/cm^3, while percent moisture ranged from 12 to 91%. The expectation was that aquatic sites would have higher bulk density values, and conversely, lower percent moisture content when compared to peatland values, due to the mineral substance in these sediments. The actual values for open-water control sites ranged from 0.17 and 1.29 g/cm^3, with percent moisture values of 21 to 81%, reference sites had bulk density values of 0.2 to 0.79 g/cm^3 and OSPM sites had bulk density values of 0.16 to 0.76 g/cm^3.

Percent organic content in peatland sites ranged from 74 to 99%, consistent with complete coverage of *Sphagnum* moss with minor *Carex* and *Equisetum* communities. Aurora site 1 (samples 1.1 and 1.2) was the wetter of the peatland sites with less distance to the water table, when compared to Aurora site 2, and though there was no apparent difference in bulk density, percent moisture or percent organic content, the percent carbonate content was lower (from 0 to 0.31% in Aurora site 1, compared to 0.11 to 1.15% in Aurora site 2). Aurora site 1 was also found to be more alkaline (6.76 to 6.88, when compared to 3.7 to 5.35 in Aurora site 2) when compared to Aurora site 2, the drier of the two sites.

Reference sites had organic matter content values ranging from 5.3 to 31%. In this group of sites there were a number of samples that were taken from “vegetated” areas but had organic content values less than 20%; Control Reservoir, Shallow wetland-1, South Ditch-1, and Bill’s Lake-1. Percent organic content in OSPM wetlands spanned 3.02 to 55%. The two sites with percent organic content greater than 50 % were Sustainability Pond North and Sustainability Pond South.

Table 4: Sediment Characteristics of 36 Samples from 24 wetland sites

Site	Site Type	Vegetated /Not Vegetated Sample	Dry Weight (g/cm^3)	Bulk Density (g/cm^3)	% Moisture	% Organic Matter Content	% Carbonate Content
AU 1.1	Peatland	Vegetated	0.32	0.03	67.82	84.62	0.31
AU 1.2	Peatland	Vegetated	0.40	0.05	59.77	74.29	0
AU 2.1	Peatland	Vegetated	0.16	0.03	84.27	81.13	0.64
AU 2.2	Peatland	Vegetated	0.88	0.08	12.02	99.38	0.11
AU 2.3	Peatland	Vegetated	0.09	0.02	91.29	97.50	1.15
LL-1	Control	Vegetated	0.34	0.32	65.95	9.10	33.19
LL-2	Control	Non-Vegetated	0.78	1.29	21.79	2.05	18.16
SP-1	Control	Vegetated	0.21	0.23	79.29	29.35	6.85
SED-1	Control	Vegetated	0.19	0.17	81.10	39.36	15.24
MC-1	Control	Non-Vegetated	0.52	0.51	48.41	8.73	2.65
BP-1	Control	Non-Vegetated	0.43	0.36	57.19	10.77	20.16
SBP	Control	Vegetated	0.74	0.85	26.25	41.00	3.04
DUCK	Reference	Non-Vegetated	0.22	0.20	77.76	20.36	18.71
V-NOT	Reference	Vegetated	0.26	0.28	73.71	25.77	9.89
CON-RES	Reference	Non-Vegetated	0.54	0.70	46.20	9.74	3.86
SW-1	Reference	Vegetated	0.60	0.78	40.33	5.97	5.03
SW-2	Reference	Vegetated	0.35	0.35	64.50	9.86	5.07
SW-3	Reference	Non-Vegetated	0.53	0.59	47.41	6.80	4.18
SD-1	Reference	Vegetated	0.32	0.36	67.63	13.17	4.02
SD-2	Reference	Non-Vegetated	0.44	0.44	55.67	11.66	6.54
BL-1	Reference	Vegetated	0.63	0.79	37.09	5.30	7.31
BL-2	Reference	Non-Vegetated	0.54	0.61	45.52	6.58	4.77
PP-1	Reference	Vegetated	0.27	0.28	72.52	31.71	5.46
HS-1	Reference	Vegetated	0.34	0.29	65.87	23.73	7.62
HS-2	Reference	Non-Vegetated	0.26	0.25	73.84	25.20	32.49
SUS-N	OSPM	Vegetated	0.16	0.16	83.97	55.38	72.35
SUS-S	OSPM	Vegetated	0.23	0.28	76.81	53.21	6.47
JANS	OSPM	Non-Vegetated	0.42	0.42	57.66	14.13	29.05
1MCT	OSPM	Non-Vegetated	0.25	0.29	74.64	19.05	9.47
DP-1	OSPM	Vegetated	0.51	0.56	48.69	15.30	15.01
DP-2	OSPM	Non-Vegetated	0.51	0.59	49.27	6.09	6.70
MP	OSPM	Non-Vegetated	0.56	0.70	43.67	3.02	6.92
NW-1	OSPM	Vegetated	0.38	0.41	61.72	9.75	3.40
NW-2	OSPM	Non-Vegetated	0.33	0.35	66.98	17.12	9.09
TP9	OSPM	Non-Vegetated	0.54	0.76	45.64	3.86	23.55
4MCT	OSPM	Non-Vegetated	0.27	0.34	73.07	20.71	37.13

3.2 Testate Amoebae Data

A total of forty-four species of testate amoebae were encountered in the thirty-five samples analyzed from twenty-four wetland study sites, *Centropyxis platystoma* and *Centropyxis aculeata* (shown in Figure 8 a) were the most common species among all sites (Figure 9). In unaffected open-water sites (control and reference) testate amoebae communities were rich in *Centropyxis, Cyclopyxis,* and *Difflugia* (shown in Figure 8 c). Communities present in "vegetated" samples (Loon Lake-1, South Ditch-1, Sedimentation Pond-1, High Sulphate-1, Bill's Lake-1 and Shallow Wetland-1 and 2) contained populations made up of *Amphitrema flavum, Acella, Cyclopyxis* and *Centropyxis platystoma*. "Non-vegetated" samples were found to be higher in their *Difflugia* concentration.

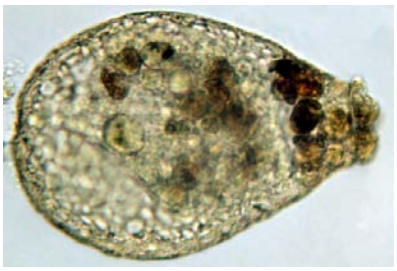
Peatland sites maintained more diverse assemblages than open-water sites, containing more taxa; *Arcella, Assulina, Centropyxis, Englypha,* and *Heleopera* (shown in Figure 8 b) but also more individuals (Figure 9). Aurora site 1 contained a large number of *Arcella*, *Centropyxis*, and *Heleopera*. Aurora site 2 (the drier site) was rich in *Assulina* (shown in Figure 8 d), *Englyphyta* and two *Heleopera* species (*sphagni* and *rosea*), this site had more individuals and taxa suggesting that drier habitats were conducive to an assortment of testate amoebae.

Open-water sites affected by OSPM contained fewer taxa and fewer individuals when compared to unaffected wetlands (Figure 9). Taxa most common in OSPM sites were *Assulina*, *Difflugia*, and *Hyalosphenia* (Figure 9).

Using CANOCO 4.5 (ter Braak & Smilauer 2002) a detrended correspondence analysis (DCA) was used to ordinate testate amoebae counts by site type (Figure 10, showing axis one, which accounted for 14.8% of variance while the second axis accounted for an additional 10.1% of variance). The greatest amount of variability was found in peatland sites (represented by red circles). Testate amoebae assemblages in the open-water control wetlands was the next most

variable (represented by yellow downward facing triangles). There was less diversity in testate amoebae communities in reference wetlands indicated by a more centered array of data points (green squares, which can be contained within an ellipse). Data points showing OSPM sites (blue diamonds, which are also contained within an ellipse) were clustered in the middle of the graph indicating the smallest range of testate amoebae species present in this type of wetland (See Figure 9). DCA analysis was also expressed in terms of testate amoebae species (Figure 10), *Difflugia* species were grouped (to the right, highlighted with a dividing line and indicated by DF label) indicating a likelihood of these species to appear together. *Nebela* were also grouped together (at the top of graph, indicated by NE label) but they existed only in low numbers in this study.

(a) Centropyxis aculeate



(c) Difflugia

(b) Heleopera petricola

(d) Assulina muscorum

Figure 8: Example photos of Testate Amoebae

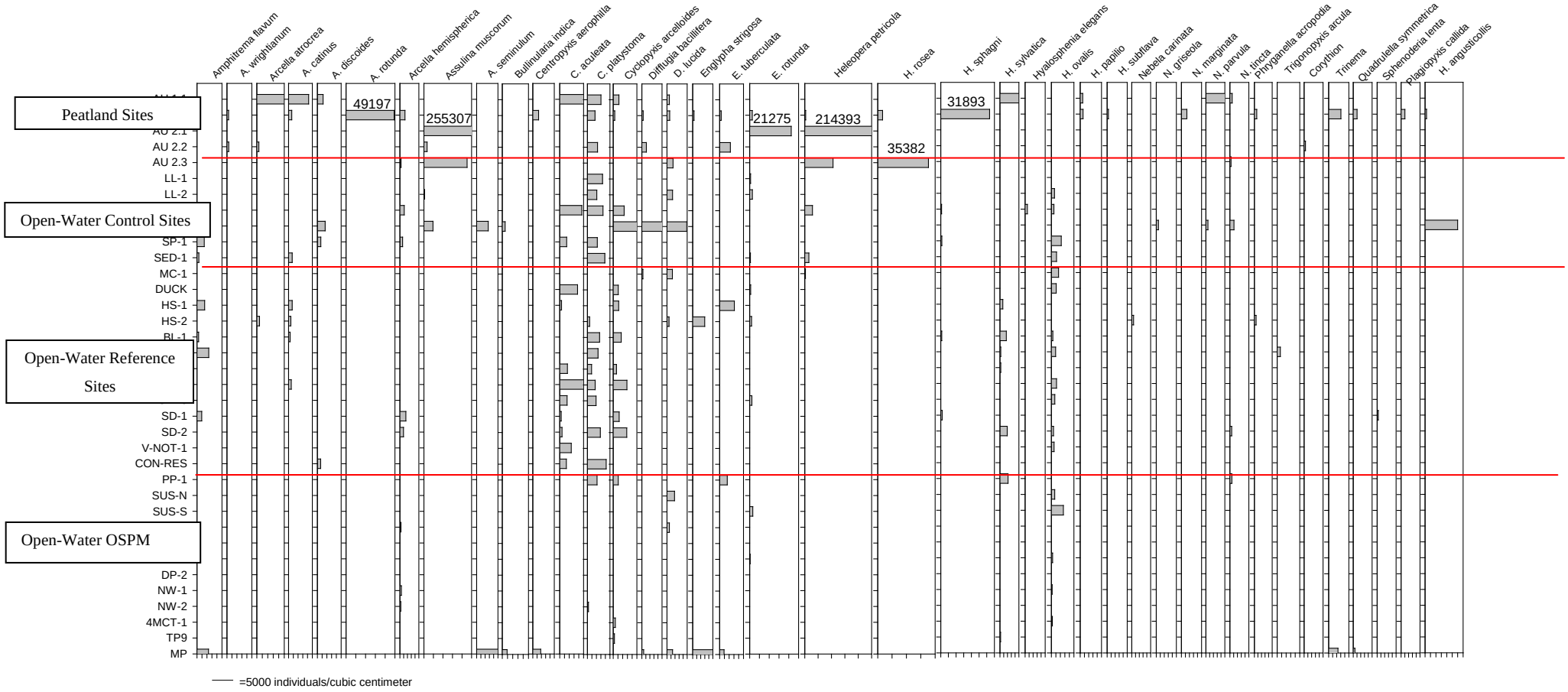


Figure 9: Concentration of Testate Amoebae Species by Site

Using lycopodium spore counts testate amoebae concentration per cubic centimeter of sample was determined. Each testate amoebae species follow the scale indicated at the bottom of the graph (5000 individuals) with the exception of Arcella rotunda, Assulina muscorum, Englypha rotunda, Heleopera petricola, Heleopera. Rosea, Heleopera. Sphagni, and Heleopera. Angusticollis; which have the number of individuals indicated directly on the graph.

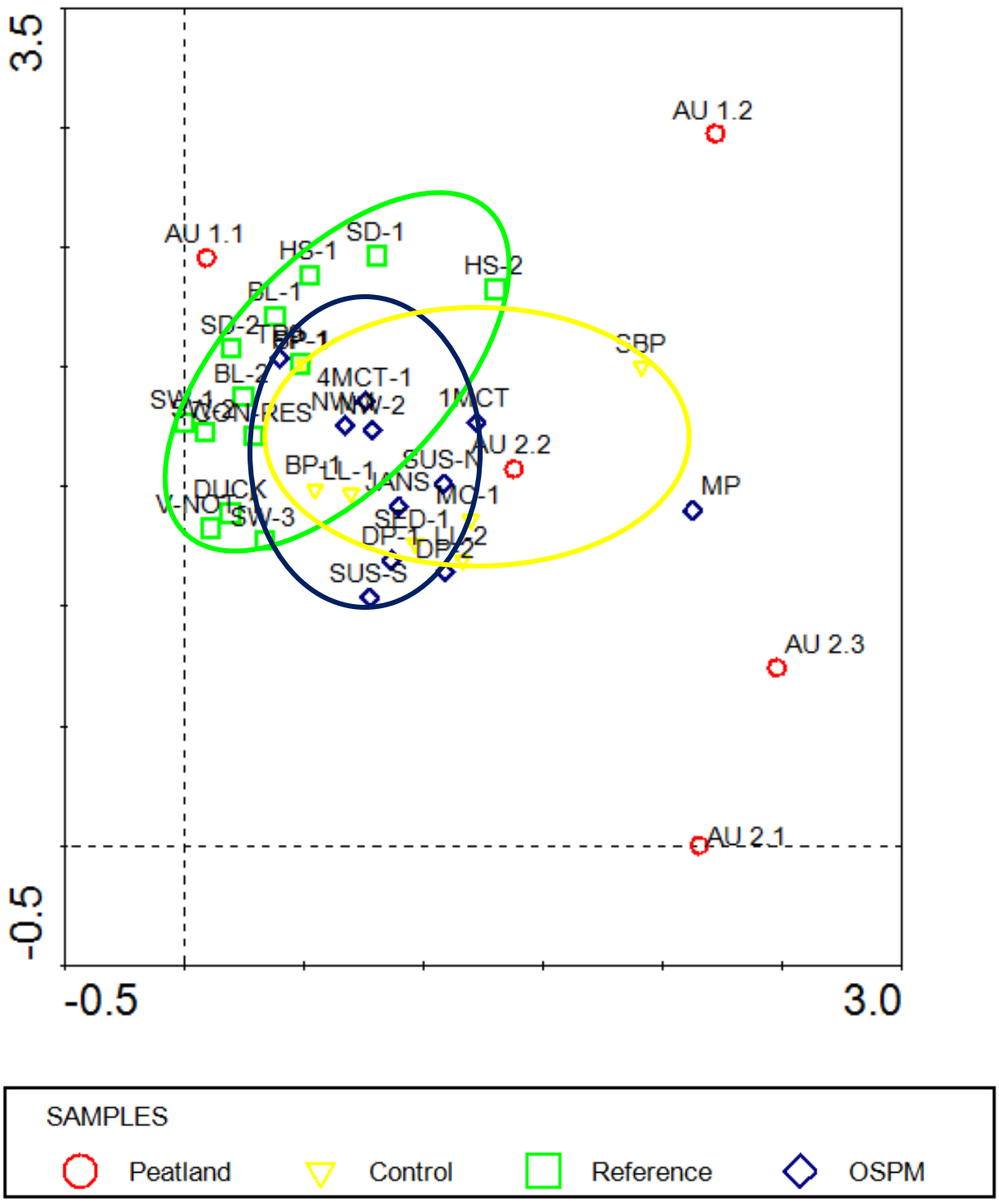


Figure 10: Detrended Correspondence Analysis of Testate Amoebae Counts: By Site

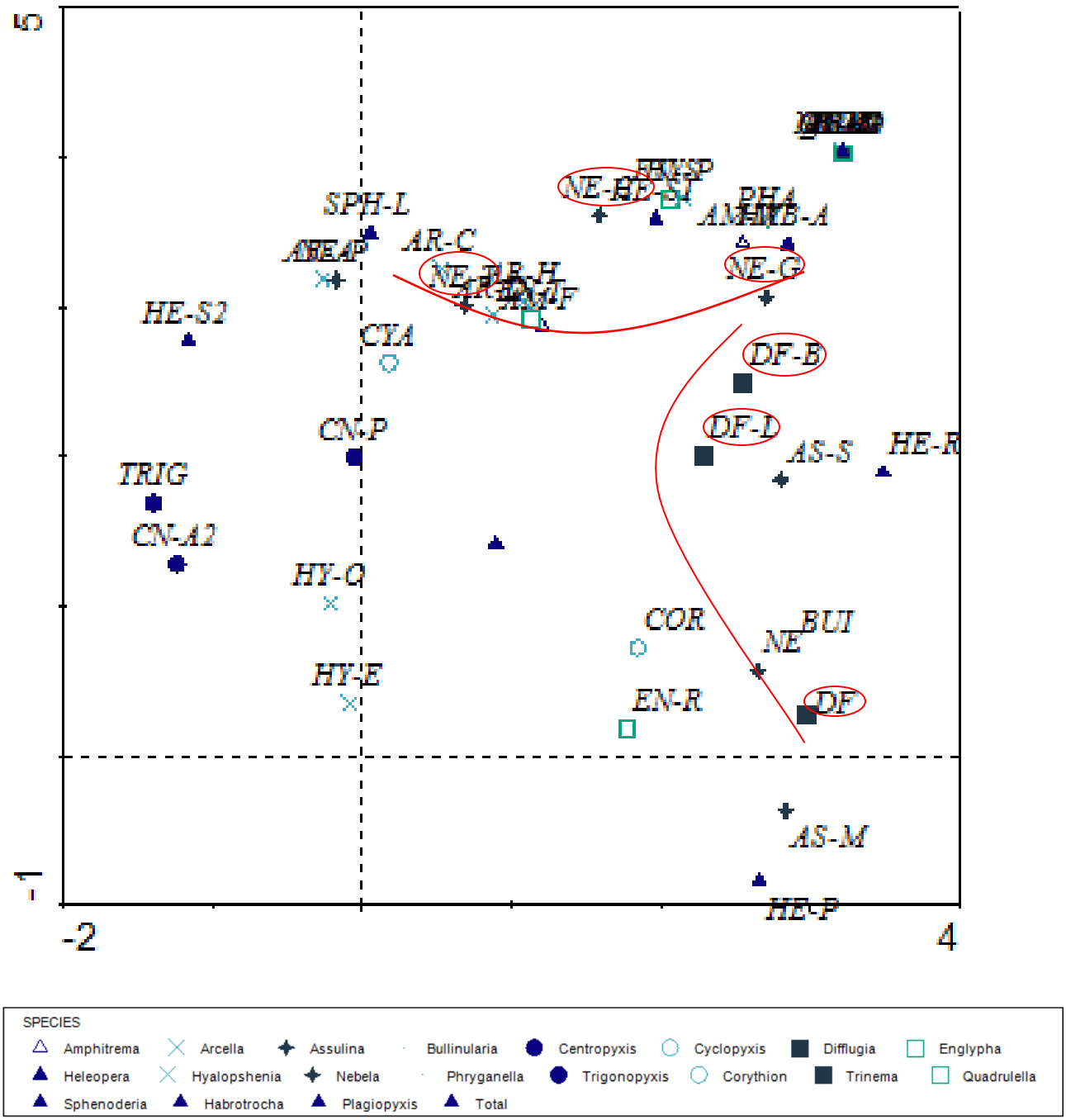


Figure 11: Detrended Correspondence Analysis of Testate Amoebae Counts: By Species

Testate amoebae data were constrained by environmental parameters, in order to determine the relationship between species distribution and environmental variables. A canonical correspondence analysis (CCA) was performed and the results were summarized in Figures 12 and 13 (the first axis of the CCA accounted for 16.7 % of variance while the second axis accounted for an additional 7.7 % of variance). Figure 12 shows the ordination of sites, as they are constrained by environmental parameters, Figure 13 shows the same ordination but represented in terms of testate amoebae species and how they are constrained by environmental parameters. In the CCA ordination of testate amoebae species (Figure 12) peatland sites were removed from the analysis. Peatland sites are very different from open-water sites in terms of environmental parameters, consequently their inclusion may have skewed the ordination of testate amoebae in these sites.

All site types are seen in Figure 13 generally fall between turbidity, DO and conductivity, indicating these may be the main players in testate amoebae establishment. Peatland sites (indicated by an AU label and red circle symbols) are clearly the outliers in this analysis solidifying the fact that these sites are completely different from open-water sites. The CCA expressed in terms of testate amoebae species (Figure 13) revealed that *Difflugia, Bullinularia* and *Nebela* were found in environments with high vegetation cover percentages (Circled in Figure 13) and *Hyalosphenia elegans* and *Nebela carinata* were found in sites with deeper water (Circled in Figure 13).

A strong parallel can be seen between Axis 1 and pH (Figure 13), and between sites and turbidity, conductivity and DO, indicating which environmental parameters could account for a proportion of the variability. To determine which parameters had the greatest impact on testate amoebae assemblages, distribution, correlation coefficients were determined (summarized in

Table 5) using SPSS and the axis scores from the output of the CCA. Conductivity was determined to have a highly significant ($P<0.01$) impact and turbidity had a significant impact ($P<0.05$). Turbidity was determined on a visual scale (from 1 to 3) in the field, based on clarity and water color. There were no significant environmental parameters for the second axis.

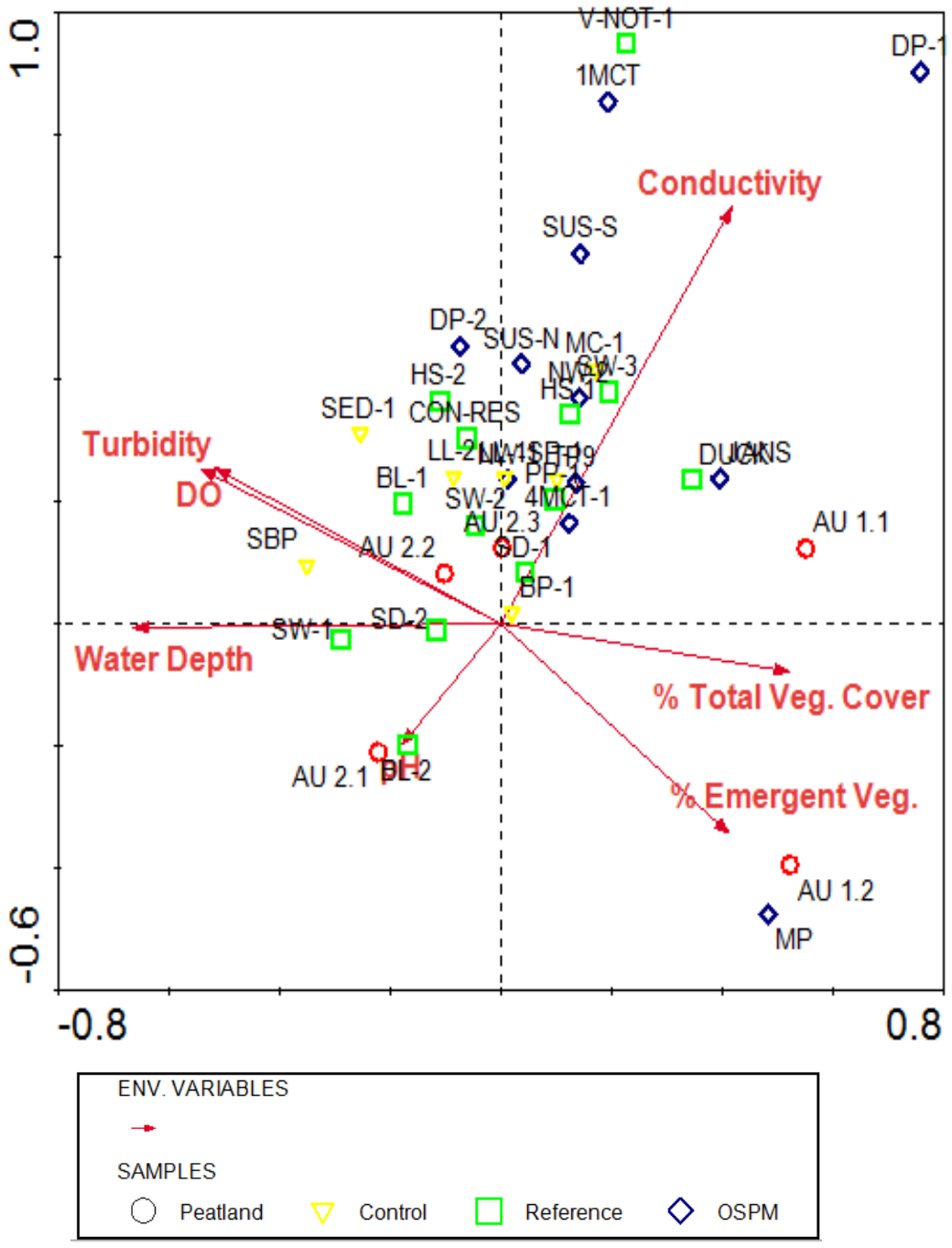


Figure 12: Canonical Correspondence Analysis of Testate amoebae Counts Constrained by Environmental Parameters: By Site

Since the first and second axis account for the greatest amount of variance, only these axes were considered for correlation coefficient, with the first axis accounting for 16.7% and the second axis adding an additional 7.7%.

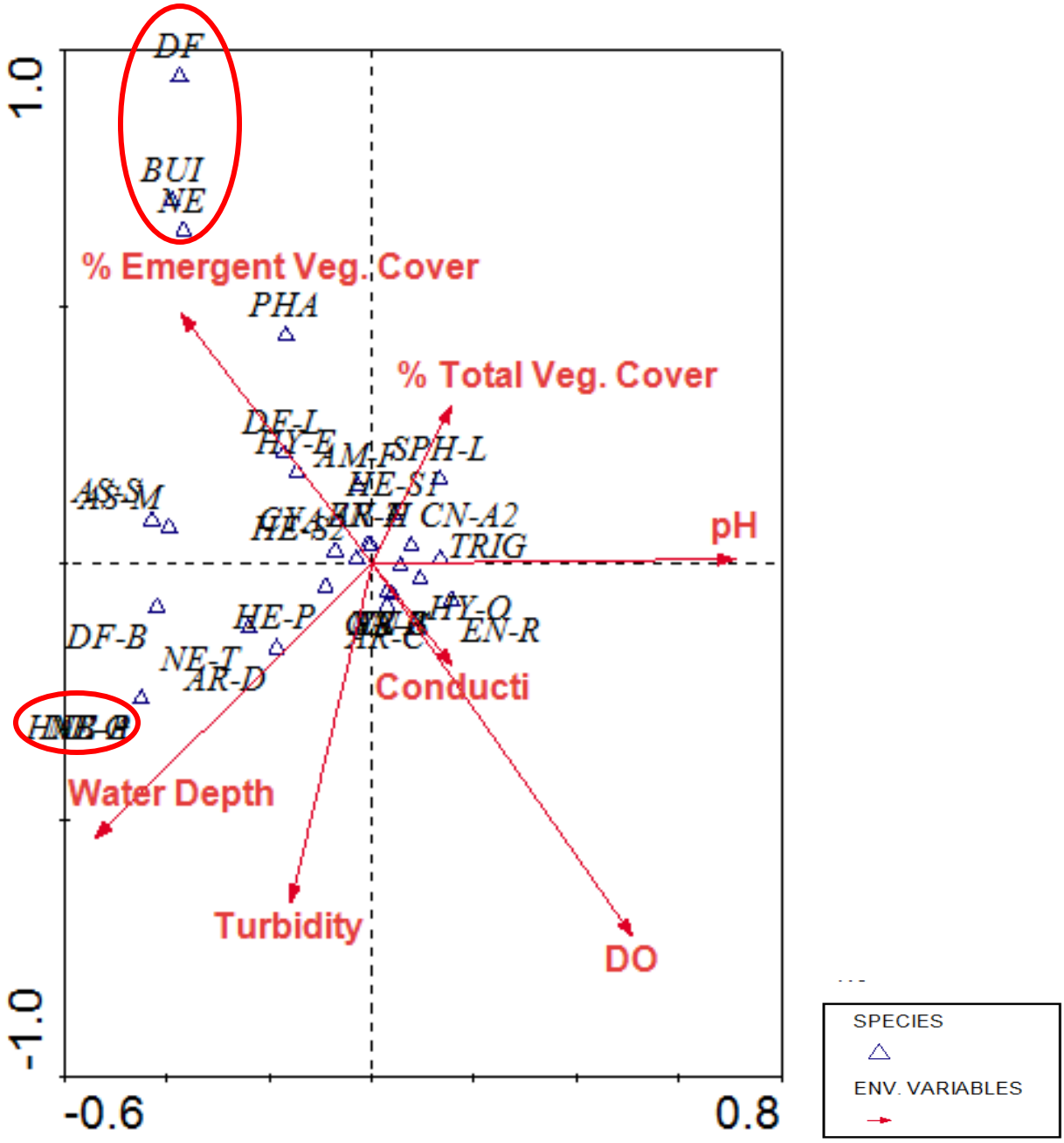


Figure 13: Canonical Correspondence Analysis of Testate Amoebae Counts Constrained by Environmental Parameters: By Species

Peatland sites were removed for this analysis. Since the first and second axis account for the greatest amount of variance, only these axes were considered for correlation coefficient, with the first axis accounting for 16.7% and the second axis adding an additional 7.7%.

Table 5: Correlation Coefficient - Spearman's Rho

Using the axis scores from the output of the CCA to determine the impact each environmental variable had on the ordination of testate amoebae.

Axis		Cond. (µs)/cm.	pH	DO	Water Depth	Total % Veg.	Total % Emergent Veg.	Turbidity (1-3)
Axis 1	Correlation Coefficient	-.431**	-.218	.288	.130	.144	.021	-.447*
	Sig. (2-tailed)	.009	.202	.116	.451	.403	.902	.012
	N	36	36	31	36	36	36	31
Axis 2	Correlation Coefficient	-.227	.023	.307	-.028	.110	.040	-.277
	Sig. (2-tailed)	.183	.896	.093	.873	.522	.816	.131
	N	36	36	31	36	36	36	31
Axis 3	Correlation Coefficient	-.233	-.088	.557**	.162	.331*	.138	-.416*
	Sig. (2-tailed)	.171	-.610	.001	.344	.049	.421	.020
	N	36	36	31	36	36	36	31
Axis 4	Correlation Coefficient	-.220	.153	.480**	.204	.281	-.129	-.259
	Sig. (2-tailed)	.197	.373	.006	.232	.097	.454	.159
	N	36	36	31	36	36	36	31
Axis 5	Correlation Coefficient	-.267	-.043	.510**	.122	.281	-.129	-.259
	Sig. (2-tailed)	.116	.805	.003	.479	.097	.454	.159
	N	36	36	31	36	36	36	31
Axis 6	Correlation Coefficient	-.286	.065	.544**	.122	.280	.083	-.342
	Sig. (2-tailed)	.090	.704	.003	.479	.098	.632	.062
	N	36	36	31	36	36	36	31

*Axis 1 and Axis 2 were considered in this analysis because they accounted for the most variance (P<0.05 indicated by *, P<0.01 indicated by **).*

3.3 Epifluorescent Photos and Analyzing

Representative photos for the bacterial preparation (one for each site type) are shown in Figure 14(a) (control site), 15(a) (OSPM site) and 15(b) (reference site). In each photo fluorescing areas indicate bacterial DNA. Figure 14(b) shows the grey-scale image produced by ImageJ of MacLean Creek (also seen in Figure 14(a), a control site) in order to count bacterial cells per gram of sample which was incorporated into biomass calculations. Figure 16 shows a representative photo for the fungal preparation for a reference site, this photo shows how ImageJ uses broken lines to measure the full length of fungal hyphae.

3.3.1 Bacteria

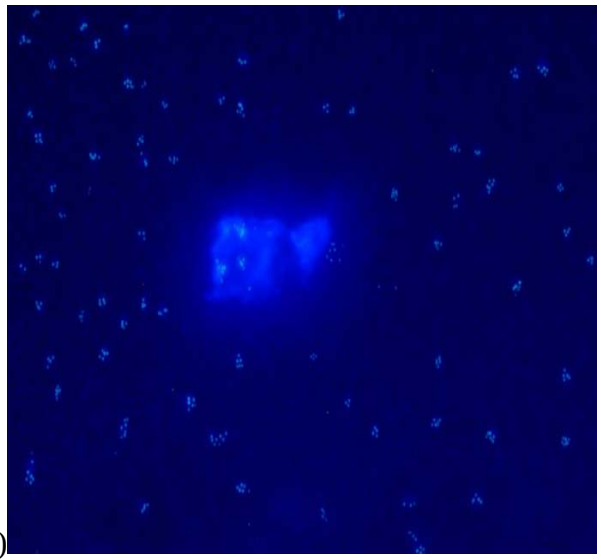
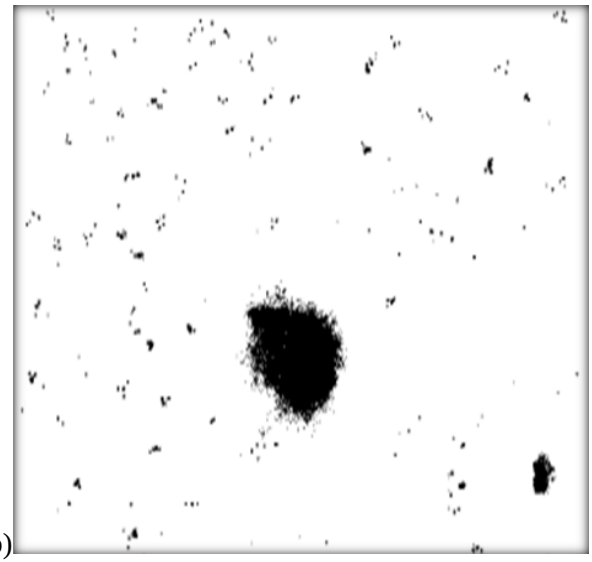
(a)  (b) 

Figure 14: Epifluorescent Photo of Bacterial Sample at 1000X of MacLean Creek (Control site) (a), and Converted, grey-scale image of MacLean Creek (b).

(a) 

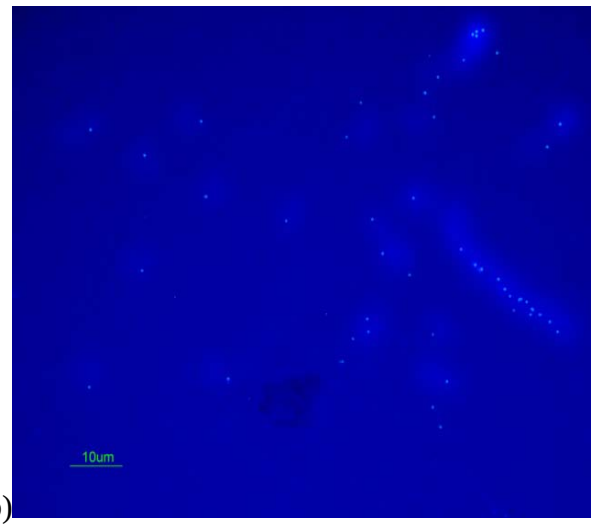
(b) 

Figure 15: Epifluorescent Photo of Bacterial Sample at 1000X of 4MCT (OSPM site) (a) and Shallow-Wetland-2 (Reference site) (b).

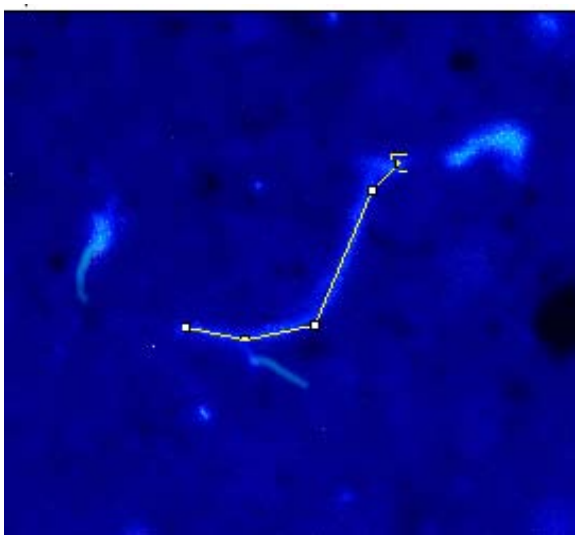


Figure 16:Epifluorescent Photo of Fungal Sample at 1000X of Duck Pond (Formerly South West Corner Waste Area 11).

Showing the line tool used to follow along fungal hyphae and measure the total length to be used in biomass calculations.

3.4 Biomass: Testate amoebae, Bacteria and Fungi

All biomass data (Table 6) were analyzed using analysis of variance (ANOVA) and summarized in Figure 17, in terms of percentage of biomass as well as raw biomass data for; testate amoebae, bacteria, and fungi. Average biomass values for each site type are summarized in pie-charts in Figures 18 through 21.

Biomass contributed by testate amoebae made up less than 1% in all open-water sites (with the exception of control sites; Small Beaver Pond at 2.4%). Peatland sites contained an average of 2% biomass contribution from testate amoebae.

Biomass contributed to the community by bacteria fell between 62 and 98% in peatland sites, 25 and 94% in control sites, between 38 and 92% in reference wetlands and between 41 and 99% in OSPM sites. Fungal biomass made up a major portion of the community in control sites contributing between 4.34 and 74% and between 7 and 62% in reference sites. Overall biomass

(including testate amoebae, bacteria and fungi) was drastically higher in peatland sites than any other site type.

Bacterial biomass was found to be higher in peatland Aurora site 2, which was drier and more acidic than Aurora site 1. In open-water control sites bacterial biomass was typically higher in samples taken from "vegetated" areas (with ≤20% of organic matter content: Beaver Pond and MacLean Creek) with biomass values ranging from 81 to 324 µg of C/g of sample, compared to 752 and 997 µg of C/g of sample in "non-vegetated" control samples.

Bacteria biomass values in reference sites were larger in "non-vegetated" samples: Shallow Wetland-2, Bill's Lake-2, and High Sulphate-2. Bacterial biomass in "vegetated" samples ranged from 29 to 428 µg of C/g of sample. Not all "non-vegetated" had values greater than 428 µg of C/g of sample; but the pair-wise trend (Shallow Wetland-1: Shallow Wetland-2, Bill's Lake-1: Bill's Lake-2 etc.) agrees greater bacterial biomass in "non-vegetated" samples. This is contrary to accepted trends where bacteria are found most abundantly in rhizopheres and in the presence of plant communities.

In OSPM sites "non-vegetated" samples typically had a smaller amount of bacterial biomass. Values ranged from 34 to 191 µg of C/g of "non-vegetated" sample and 33 to745 µg of C/g of sample in "vegetated" samples. In "vegetated" samples 4MCT was the anomaly at 33 µg of C/g of sample.

As with reference sites, no clear trend was seen with relationship to fungal biomass, sample vegetation, and organic matter content. Fungal biomass in control sites was typically higher in "non-vegetated" sites except in Beaver Pond, with a fungal biomass value of only 39 µg of C/g of sample, compared to 260 and 307 µg of C/g of sample in other "non-vegetated" samples (Loon Lake-2 and MacLean Creek, respectively).

Total biomass values reflected the trends in bacterial and fungal biomass in “vegetated” versus “non-vegetated” samples (increase bacterial and fungal biomass in “non-vegetated” samples) with Loon Lake-2, Beaver Pond and MacLean Creek having the highest values; 402, 792, and 1304 µg of C/g of sample, respectively.

A non-parametric Kruskal-Wallis one-way analysis of variance on ranks was used to analyze testate amoebae biomass, bacteria biomass and overall biomass per site type (Table 7). Dunn’s Method was used to determine all pair-wise comparison. All ANOVA analyses were performed using Sigma Plot Version 11.0.

The results of a Kruskal-Wallis one way ANOVA indicate the testate amoebae biomass is significantly different between peatlands and all other site types, and between control and OSPM sites, and between reference and OSPM sites but there is no significant difference between control sites and reference sites. Peatland sites were found to be significantly different from all open-water sites, with elevated absolute biomass of testate amoebae, bacteria, fungi, and overall biomass. No differences were detected between open-water sites in terms of bacteria, fungi or overall biomass. These results are summarized in Table 7. The mean and standard deviation for biomass values for each site type are shown in Table 8.

Table 6: Biomass Data: Testate Amoebae, Bacteria, Fungi and Total Biomass

All measurements are represented in micrograms of Carbon per gram (dry weight) of sample

Site	Site Type	Vegetated/ Not Vegetated Sample	Age	Testate Amoebae (µg of C/g)	Bacteria (µg of C/g)	Fungi (µg of C/g)	Total Biomass (µg of C/g)
AU 1.1	Peatland	Vegetated	>8yrs	38.45	5543.98	4177.54	9759.97
AU 1.2	Peatland	Vegetated	>8yrs	57.12	4993.03	22.51	5072.66
AU 2.1	Peatland	Vegetated	>8yrs	362.47	16061.74	746.98	17171.19
AU 2.2	Peatland	Vegetated	>8yrs	5.63	1455.19	44.19	1505.01
AU 2.3	Peatland	Vegetated	>8yrs	747.44	9649.71	2956.03	13353.18
LL-1	Control	Vegetated	>8yrs	0.20	204.82	105.23	310.25
LL-2	Control	Non-Vegetated	>8yrs	0.15	141.56	260.6575	402.37
BP-1	Control	Non-Vegetated	>8yrs	1.21	752.15	39.07	792.43
SBP	Control	Vegetated	>8yrs	3.14	81.45	45.74	130.33
SP-1	Control	Vegetated	≤8yrs	1.74	184.37	120.62	306.73
SED-1	Control	Vegetated	≤8yrs	1.11	324.86	132.56	458.53
MC-1	Control	Non-Vegetated	≤8yrs	0.20	997.17	307.1	1304.47
DUCK	Reference	Non-Vegetated	>8yrs	0.88	692.61	230.2688	923.76
SW-1	Reference	Vegetated	>8yrs	0.28	29.5	46.41	76.1879
SW-2	Reference	Vegetated	>8yrs	0.87	138.96	113.34	253.17
SW-3	Reference	Non-Vegetated	>8yrs	0.23	38	32.86	71.09
SD-1	Reference	Vegetated	>8yrs	0.91	212.49	224.6	438.00
SD-2	Reference	Non-Vegetated	>8yrs	0.50	143.36	74.23	218.09
BL-1	Reference	Vegetated	>8yrs	0.27	86.21	6.55	93.03
BL-2	Reference	Non-Vegetated	>8yrs	0.87	194.64	64.5	260.01
HS-1	Reference	Vegetated	>8yrs	1.20	428.43	707.92	1137.54
HS-2	Reference	Non-Vegetated	>8yrs	1.74	543.5	53.45	598.69
V-NOT	Reference	Vegetated	≤8yrs	0.32	77.75	225.71	303.78
CON-RES	Reference	Non-Vegetated	≤8yrs	0.21	243.19	13.79	257.19
PP-1	Reference	Vegetated	New	0.73	310.2	41.86	352.79
SUS-N	OSPM	Vegetated	>8yrs	0.22	745.74	17.7	763.66
SUS-S	OSPM	Vegetated	>8yrs	0.04	300.01	97.09	397.14
JANS	OSPM	Non-Vegetated	>8yrs	0	492.01	15.91	507.92
1MCT	OSPM	Non-Vegetated	>8yrs	0.14	555.86	256.61	812.61
DP-1	OSPM	Vegetated	>8yrs	0.004	405.36	2.34	407.70
DP-2	OSPM	Non-Vegetated	>8yrs	0.003	191.43	26.33	217.76
NW-1	OSPM	Vegetated	>8yrs	0.54	703.51	124.32	828.37
NW-2	OSPM	Non-Vegetated	>8yrs	0.11	34.52	48.33	82.96
TP9	OSPM	Non-Vegetated	>8yrs	0.09	138.55	19.14	157.78
4MCT	OSPM	Non-Vegetated	>8yrs	0.15	33.89	12.57	46.61
MP	OSPM	Non-Vegetated	>8yrs	1.74	282.64	10.45	294.83

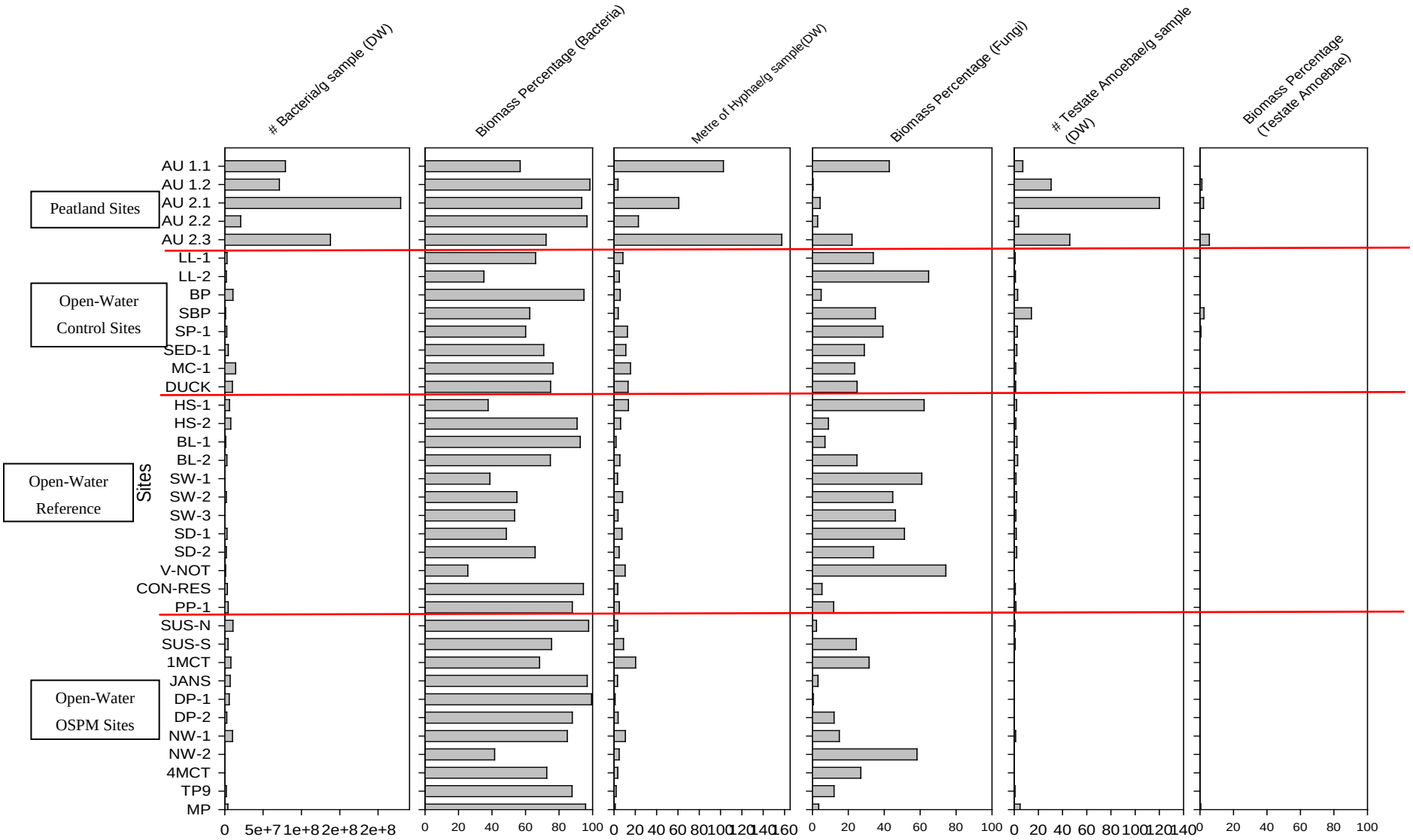


Figure 17: Biomass Percentage and Absolute Biomass Values

(Testate Amoebae and Fungi values have been reduced by a factor of 10, Bacteria values are reduced by a factor of 1000)

Table 7: Summary of Kruskal-Wallis Test Results Comparing Site Types

Comparison	P<0.05 for Testate Amoebae	P<0.05 for Bacteria	P<0.05 for Fungi	P<0.05 for Overall Biomass
Peatland VS Control	Yes	Yes	Yes	Yes
Peatland VS Reference	Yes	Yes	Yes	Yes
Peatland VS OSPM	Yes	Yes	Yes	Yes
Control VS OSPM	Yes	No	No	No
Control VS Reference	No	No	No	No
Reference VS OSPM	Yes	No	No	No

Table 8: Descriptive statistics of Kruskal-Wallis Test

Measured in µg of Carbon per gram (dry weight) of sample.

Site Type	Testate Amoebae	Bacteria	Fungi	Overall Biomass
Peatland	242.2±316.8	7540.7±5580.5	1589.5±1878.7	9372.4±6269
Control	93.7 ±220	3117.4 ±4867.8	5.1 ±1.8	3909.3 ±5778.6
Reference	0.9 ±0.5	250.2 ±206.9	4.361 ±1.2	394.1 ±337.3
OSPM	0.1 ±0.2	360.1 ±262.0	3.407 ±1.4	422.3 ±300.0

Values are means ± Std. Dev. P<0.05.

Mann-Whitney Rank Sum Tests (non-parametric data) and T-tests (parametric data) were performed in order to determine if a difference in biomass measurements exists between Young (≤8years) and Old (>8years) wetlands within a site type. Each site type was analyzed separately (sub-sections of data) in order to take advantage of parametric tests. No significant differences were found between Young and Old sites in control wetlands (9 Old sites, 4 Young) or in reference wetlands (10 Old sites, 2 Young). All OSPM, and peatland sites sampled were older than eight years and therefore this analysis did not apply to these site types. Mean and standard deviation for these analyses as well as P-values are summarized in Tables 9 and 10.

Table 9: Descriptive Statistics of Mann-Whitney Rank Sum Test/T-tests for Biomass Measurements for Testate Amoebae, Bacteria, Fungi and Overall Biomass, by Site Type, and Age (Young ≤8yrs, Old >8yrs)

Type	Age	Testate Amoebae	Bacteria	Fungi	Overall Biomass
Peatland	Young	N/A	N/A	N/A	N/A
	Old	242.2±316.8	7540.7±5580.5	1589.5±1878.7	9372.4±6269
Control	Young	0.5 ±0.4	410.7 ±404.2	169.8 ±126.1	581.0 ±490.0
	Old	135.1 ±257.5	4320.4 ±5494.6	933.1 ±1541.0	5388.6 ±6480.6
Reference	Young	1.2 ±0.7	247.3 ±89.0	81.2 ±55.7	329.8 ±32.6
	Old	0.8 ±0.5	250.8 ±226.7	155.4 ±208.6	407.0 ±371.2
OSPM	Young	N/A	N/A	N/A	N/A
	Old	0.1 ±0.2	360.1 ±262.0	62.0 ±79.1	422.3 ±300.0

Values are means ± Std. Dev. No results were found to be statistically significant. P>0.05.

Table 10: P-Values for Mann-Whitney Rank Sum Tests/T-tests to determine difference between Young and Old wetland sites within site types

Site Type	Testate Amoebae	Bacteria	Fungi	Overall Biomass
Peatland	N/A	N/A	N/A	N/A
Control	0.1	0.2	0.6	0.2
Reference	0.3	1.0	0.9	0.8
OSPM	N/A	N/A	N/A	N/A

No results were found to be statistically significant. P>0.05, indicating no differences found between Young sites and Old Sites

Table 11: Descriptive Statistics of Mann-Whitney Rank Sum Test/T-tests for Biomass Measurements for Testate Amoebae, Bacteria, Fungi and Overall Biomass, by Site Type, and Age (Young <12yrs, Old ≥12yrs)

Type	Age	Testate Amoebae	Bacteria	Fungi	Overall Biomass
Peatland	Young	N/A	N/A	N/A	N/A
	Old	57.1±30.2	5544±4109	22.5±747	9759±4181
Control	Young	1.18±1.4	173.2±111.5	112.7±103	408.8±279.6
	Old	0.46±0.44	284±160.5	169.8±126.1	581±490
Reference	Young	0.82±0.5	278.4±245.7	93.8±50	464.6±395.2
	Old	0.9±0.6	194±92	53±24.2	253.1±113.3
OSPM	Young	0.0921±0.01	359.8±275.6	47.9±45.7	407.9±291
	Old	0.144±0.04	360.6±284.7	95±139.9	385.7±222.7

No results were found to be statistically significant. P>0.05, indicating no differences found between Young sites and Old Sites

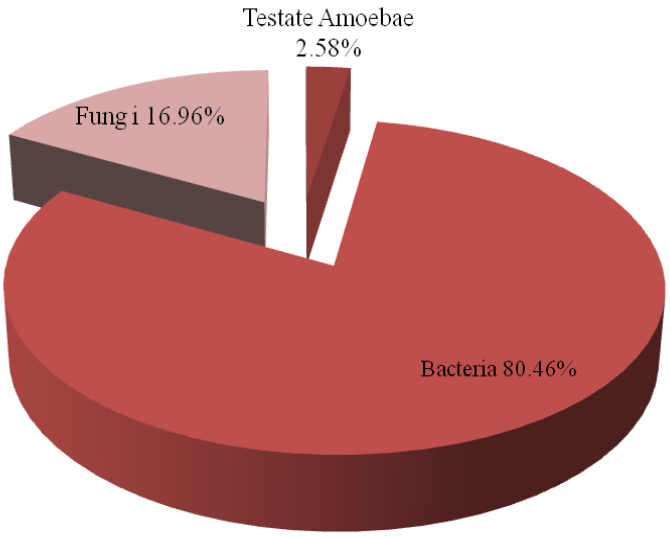


Figure 18: Average Biomass Percentages measured in Peatland Sites.

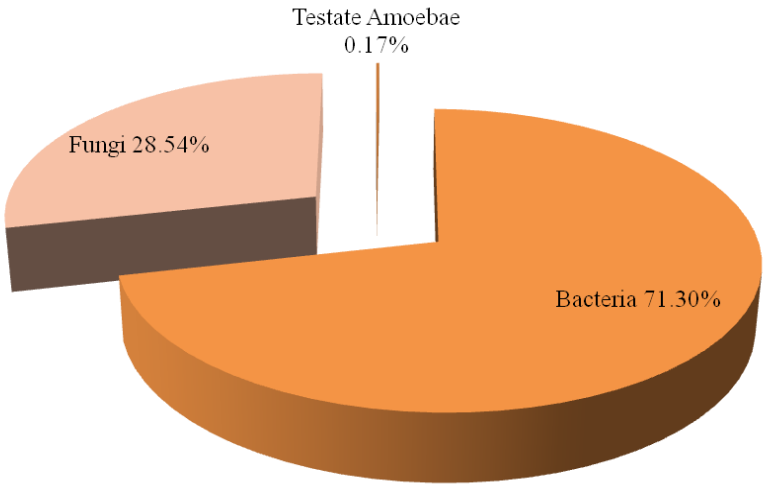


Figure 19: Average Biomass Percentages measured in Control Sites.

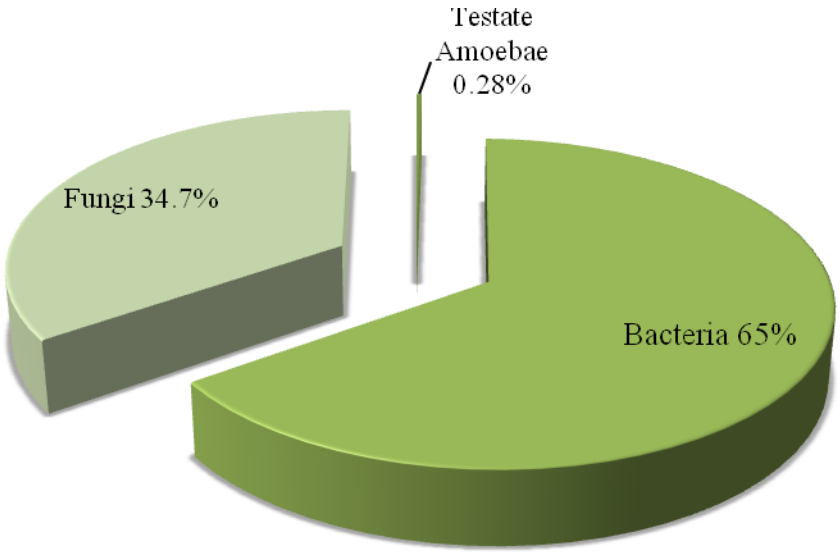


Figure 20: Average Biomass percentages measured in Reference Sites.

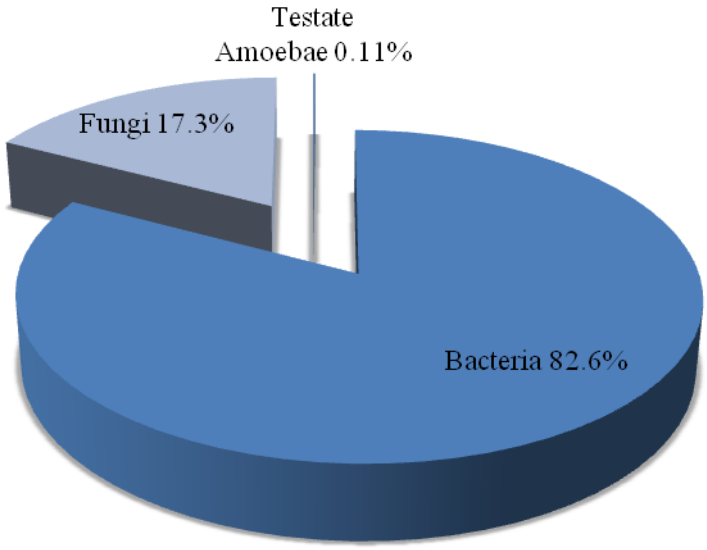


Figure 21: Average Biomass percentages measured in OSPM Sites.

4.0 Discussion

Primary immigration of microbial and plant communities to heavily disturbed oil sands mine sites and their successful colonization is of paramount importance to the restoration of biological activity and productivity at these sites (Hadwin *et al* 2006). The goal of this research project was to characterize the microbial composition in a suite of different oil sands wetlands and assess the practicality of using testate amoebae as an indicator of ecosystem establishment and microbial community health. The hypothesis was that microbial communities would be poorly represented, testate amoebae abundance and diversity would be low, and bacterial and fungal biomass would be minimal in wetlands impacted by oil sands processed materials, in contrast to natural, unaffected control type wetlands where one would expect well established testate amoebae communities with high abundance, richness and diversity and elevated biomass of bacteria and fungi.

4.1 Testate Amoebae Community Composition

Multivariate analysis (DCA, without constraints, Figure 10) revealed greater diversity in testate amoebae species composition in peatland sites than the other site types: data points for peatlands were wide spread across the primary and secondary axes (Figure 10) indicating a community with a broad assortment of testate amoebae species likely to colonize peatlands compared to open-water aquatic sites (Figure 22). Peatlands form naturally and have different soil and ecology characteristics, with considerably lower pH values (between 3.7 and 6.8) than open-water sites, which were typically neutral in this study (pH from 6.95 to 8.18). The organic content percentages in the peatland sites ranged from 81.13 to 99.38% which was much higher than the open-water sites sampled in this study (from 2 to 41%). The

peatland communities were found to contain vastly different vegetation communities than the open-water sites and were dominated by *Sphagnum* moss with minor communities of *Carex* and *Equisetum*. Aurora sites 1.1 and 1.2 were the wettest peatland sites in this study and contained a total of thirty-five taxa of testate amoebae. Aurora sites 2.1, 2.2 and 2.3 cumulatively contained only eleven taxa and were comparatively drier peatland sites. Studies have shown testate amoebae diversity in peatlands to be highest in wet sites and declines with increasing water content as in aquatic sites (Meisterfeld 1979), which is supported by this study, with greater diversity in Aurora sites 1.1 and 1.2, and lower diversity in Aurora sites 2.1, 2.2, 2.3 (drier sites), and open-water (aquatic) sites.

Prominent taxa in the Aurora peatland sites included: *Arcella, Assulina, Centropyxis, Englypha,* and *Heleopera* (Figure 9). Among the species reported: *Assulina muscorum* was present in both samples collected from Aurora site 2 (drier site) and *Centropyxis plastystoma* and *Cyclopixis arcelloides* were present in all samples collected from Aurora site 1. *Assulina muscorum* is a common species found in *Sphagnum* and terrestrial mosses (Beyens and Chardez 1984, Van Kerckvoorde *et al* 2000) and was the most abundant species in this study: with 625 individuals occurring in a single sample. *Centropyxids* have been reported as occurring more abundantly in the absence of pollutants (Kauppila *et al* 2006) which is consistent with their predominance in the peatland and control type sites. Gilbert *et al* (1997(1)) also noted that testate amoebae biomass correlated with the biomass of other groups (cyanobacteria, bacillariophyceae, heterotrophic bacteria, flagellates, and ciliates) suggesting their preferred food source in a peatland, unfortunately that type of association was beyond the scope of this study. Considering more groups in this study would have

linked preferred food sources to testate amoebae and potentially changed biomass proportions contributed by testate amoebae, bacteria, and fungi based on their relationship to other groups. Changes in biomass proportion could potentially clarify the dynamic between testate amoebae, bacterial, and fungal communities suggesting a trend or relationship that could be built upon in order to ultimately use testate amoebae as bioindicators in oil sands affected wetlands.

Below (Figure 22) is representation of a characteristic testate amoebae community present in a peatland site. In this site 6 genus and a total of 17 species were represented. This is highly diverse when compared to any open-water sites. Figure 23 is showing the biomass composition of the same peatland site (Aurora 1.2) which is dominated by bacteria (98%) with Fungi making up the smallest proportion. Peatlands were the sites in which testate amoebae contributed their largest amount of biomass and proportion, as well as the greatest number of species.

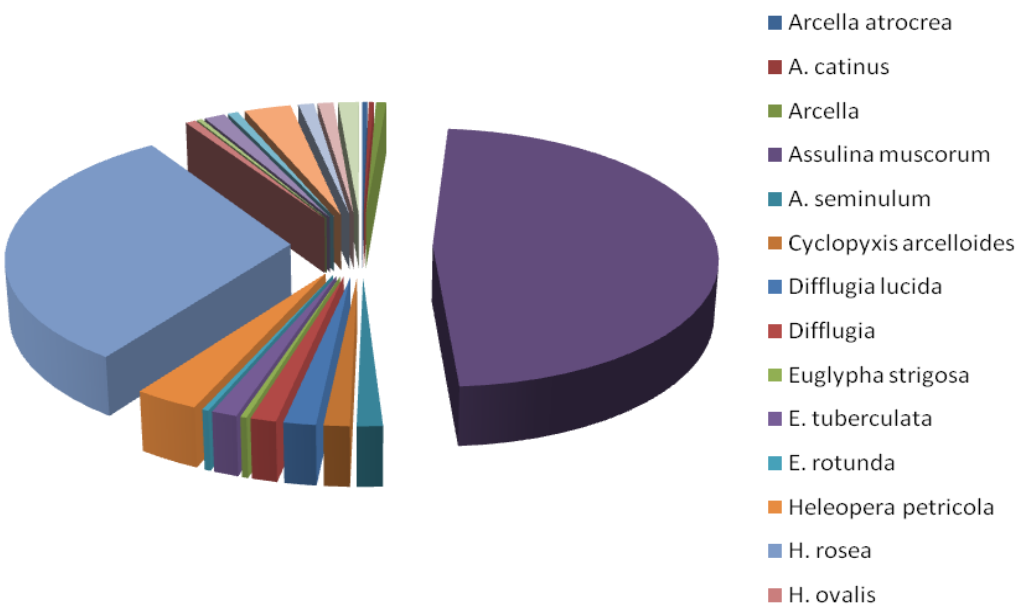


Figure 22: Testate amoebae community in a typical Peatland Site Aurora 1.2

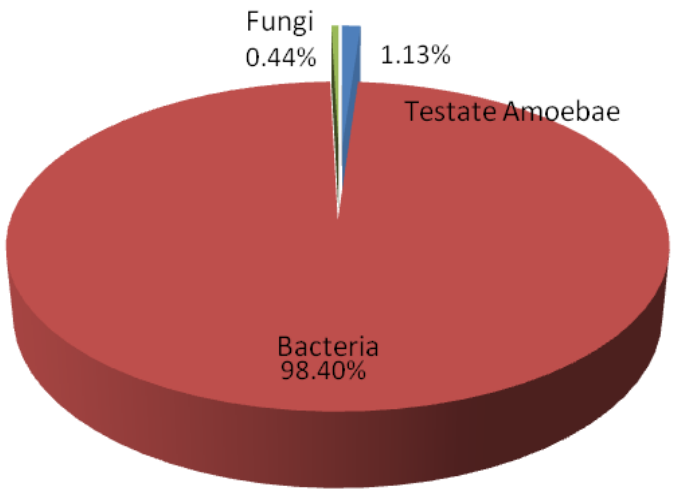


Figure 23: Relative Proportions of Testate Amoebae, Bacteria, and Fungi in Peatland Site Aurora 1.2

Control wetlands contained diverse and established vegetation communities with a higher number of taxa and a greater number of individuals when compared to sites amended with OSPM (Table 2, or the list in Appendix A). Percent organic content varied from 9.1 to 41%, (higher than percent cover seen in OSPM sites) common taxa included: *Typha latifolia, Potamogeton, Chara, Scirpus, Carex,* and *Lemna*, among other species that occurred in slighter proportions (Table 2, Appendix A). In open-water wetlands, samples were categorized as "vegetated" or "non-vegetated" based on the vegetation communities in the vicinity of the sampling zone. Samples taken from "vegetated" areas typically had higher percent organic content, compared to "non-vegetated" samples taken from within the same site, or "non-vegetated" samples taken from other sites. In some cases "vegetated" samples had low percent organic content values compared to other "vegetated" samples (from different wetlands). Samples were taken from every "zone" of vegetation that existed within a wetland. Discrepancies that exist between "vegetated" samples from different wetlands can be attributed to a particular sample taken from a "zone" of vegetation within a wetland, being deemed "vegetated" because it is the most dominant vegetation community in that particular wetland. This does not necessarily entail a thickly "vegetated" area but an area that is most vegetated within a particular site. An example of this is Loon Lake, a control site with percent organic content values of 9.1% in the "vegetated" sample and 2.05% in the "non-vegetated" sample. Though these results correspond with higher organic content in the "vegetated" sample, 9% is considered low organic content compared to other "vegetated" control type sites (which typically had organic content percentages greater than 29%).

Organic content ranged from 29 to 41% in open-water control wetlands with Loon Lake being the only "vegetated" sample from a control type site with less than 29% organic content. Although control sites typically had higher percent vegetation cover and consequently higher percent organic content (Table 2) due to the nature of these sites (naturally occurring) there is high variability in coverage and species composition of the vegetation communities (Table 3).

Testate amoebae assemblages in control sites were diverse, but less so than peatland communities (Figures 9 and 24). The data points extended across both axes of the DCA analysis, suggesting that a range of testate amoebae are able to colonize these sites (Figure 10). Open-water control wetlands were characterized by: *Centropyxis*, *Cyclopyxis* and *Difflugia* (Figures 9 and 24). Within these wetlands sites "vegetated" zones were rich in *Amphitrema*, *Arcella*, *Cyclopyxis*, and *Centropyxis*, whereas "non-vegetated" zones contained mostly *Difflugia*. *Centropyxids* have been found to be typically more abundant in the absence of pollution (Kauppila *et al* 2006) consistent with a control type site. *Centropyxis aculeata* and *C. platystoma* were common and have previously been associated with aquatic (Schonborn 1982) and wet conditions (Warner 1987). These taxa are adapted for growth in damp or wet environments and associated with marl lake sediments in Ontario, as well as with minerotrophic waters (Warner 1987). *Difflugia* have been found in bog pools and aquatic habitats (Charman *et al* 2000). This taxon exists in all site types, including OSPM sites where no other groups were able to colonize in sizeable proportions indicating robustness or durability of this group.

Figure 24 is showing a representative testate amoebae community in open-water control wetlands, and exemplifies 5 genus. Figure 25 shows the corresponding biomass proportions of this community, fungi dominate this system (64%) which is not prevalent in control sites, or any site type in this study. In most cases bacteria were dominant making up the greatest proportion of the microbial biomass studied.

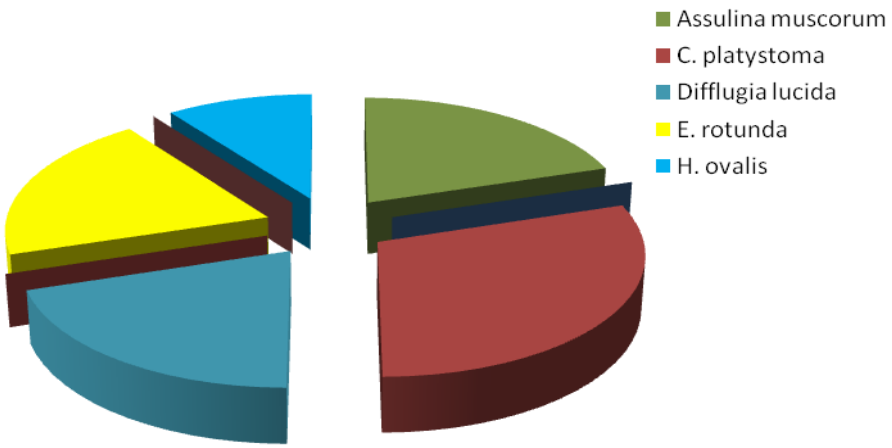


Figure 24: Testate amoebae community in a typical Open-water Control Site Loon Lake 2

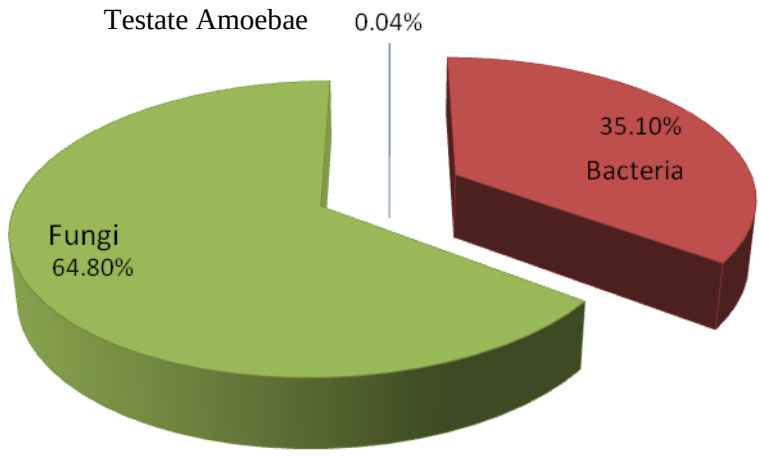


Figure 25: Relative Proportions of Testate Amoebae, Bacteria, and Fungi in Control Site Loon Lake 2

CCA analysis suggests that *Difflugia, Bullinularia,* and *Nebela* occur in environments with high vegetation cover percentages. *Difflugia* are considered more robust and have been reported in the literature as being abundant in "impacted sites" (Kauppila *et al* 2006) which typically contain less established vegetation communities. This inconsistency may be due to the fact that *Difflugia*, which is a common genus found in all wetland types, is one of only a few taxa that are capable of colonizing, but are not exclusive to OSPM sites. Also *Difflugia*, when present in unaffected (control) sites appear to be most abundant in the "non-vegetated" sample.

Hyalosphenia elegans and *Nebela carinata* were identified as species found in sites with deeper water. In a study conducted by Nguyen-Viet *et al* (2007(1)) *Nebela carinata* was identified as a species sensitive to contamination and typically found in more pristine sites, however in this study this species was present in only one site (reference: High Sulphate).

Using the output from this analysis (CCA), a Spearman's Rho correlation coefficient analysis was used to determine the importance of specific environmental parameters measured throughout the field season on the testate amoebae ordination. Characteristically, hydrology, and more specifically water table depth has been the most important factor influencing testate amoebae assemblages in research conducted in peatland sites. In this study thirty of thirty-five samples were taken from open-water sites where all communities are completely submerged eliminating water table depth as a foremost driving force affecting testate amoebae communities in open-water sites. Multi-meter data taken throughout the field season (Table 3) revealed pH values of 6.92 to 8.18, and dissolved oxygen levels varying between 78 to 161% in all open-water control and reference wetlands, with no clear

relationship to site type. However, conductivity values were elevated in OSPM sites when compared to all other sites.

Spearman's Rho revealed conductivity as a highly significant environmental variable in shaping the ordination of testate amoebae assemblages in open-water sites. Turbidity was also a significant contributor and has been observed in previous studies as a factor shaping testate amoebae assemblages (Gilbert *et al.* 1998(2), Muqi and Wood 1999, Escobar *et al* 2008). Dissolved oxygen (DO) can be related to vegetation cover, principally submerged vegetation, where DO levels would be higher in heavily vegetated areas due to photosynthesis, low DO may or may not be a stressor for testate amoebae communities and colonization.

Landscapes in open-water control and peatland sites were different at each sample location consequently, dissimilarity in landscape lends itself to accommodating different assemblages of flora and fauna due to the different niches that can be filled. Divergence in terms of vegetation community, sediment type (sandy, clay, and peat), location and proximity to roads, as well as topography could justify differences in testate amoebae community composition in these sites.

Reference sites had organic matter content values ranging from 5.3 to 31%. Vegetation communities in these sites were not largely different from those of control sites (9 to 41%) in terms of species composition and percent cover. The most common testate amoebae taxa in these sites were *Centropyxis*, *Cyclopyxis* and *Englypha*. This assemblage is comparable to control type wetlands (control sites: *Centropyxis*, *Cyclopyxis* and *Difflugia*). On average more taxa were present in "vegetated" samples when compared to "non-

vegetated" samples, also consistent with the control sites. In reference sites no particular species were linked to "vegetated" or "non-vegetated" samples. *Centropyxis aculeate, C. platystoma,* and *Cyclopyxis arcelloides* were common in both "vegetated" and "non-vegetated" sites. On average "vegetated" reference areas had lower organic content values than control wetlands, less than 20% in Control Reservoir, Shallow Wetland-1, South Ditch-1, and Bill's Lake-1. Reference type wetlands with low organic content values had one commonality in their design; the severe pitch of the basin in each of these wetlands could potentially reduce the capacity for plants to colonize these sites.

Figure 26 shows a representive community of testate amoebae present in a open-water reference site, in which 4 taxa are represented. This community is comparable to the control community of Loon Lake 2 (Figure 24). Figure 27 is the corresponding biomass values for reference site, Duck Pond, this graph indicates that bacteria is prevalent in most open-water sites, contrary to Loon Lake 2.

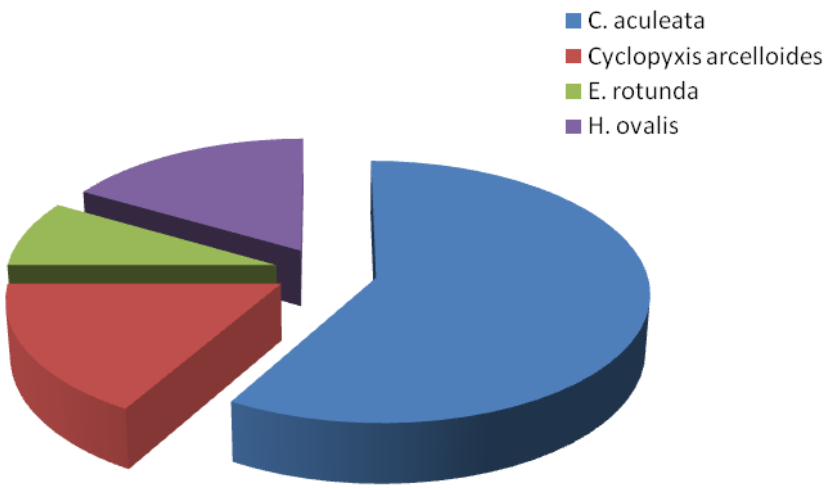


Figure 26: Testate amoebae community in a typical Reference site Duck Pond

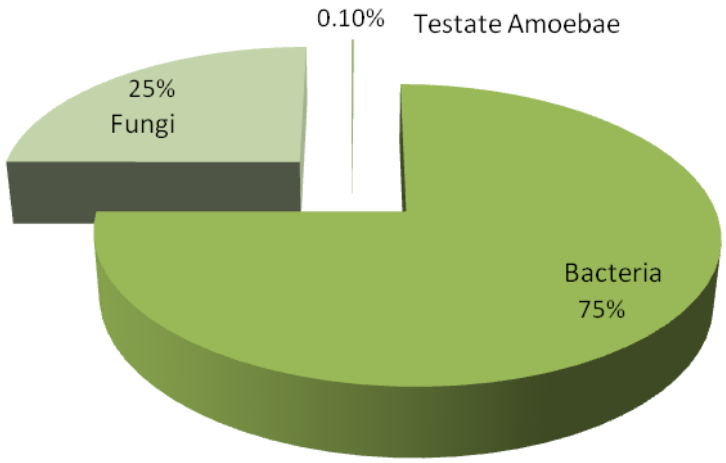


Figure 27: Relative Proportions of Testate Amoebae, Bacteria, and Fungi in Reference Site Duck Pond

Reference sites (Figure 10) showed a more narrow range of data points in terms of testate amoebae assemblage (bordered by an ellipse to show the scope) than peatland and to a lesser extent control wetlands, indicating a smaller assemblage of species colonizing these sites (Figures 9, 10 and 26). Since reference sites were either created by oil sands operators

or occur opportunistically after land has been reclaimed, these sites varied less in landscape compared to control sites (in terms of location, proximity to roads/other wetlands, sediment type). Differences between control and reference sites were minor albeit their surroundings and structural dissimilarity, testate amoebae assemblages including dominant taxa and vegetation communities were virtually indistinguishable.

OSPM sites showed the least diversity in testate amoebae communities (Figures 9, 10 and 28) with all data points being centered in the graph indicating a smaller range of testate amoebae capable of living in this site type. In these sites dissolved oxygen values were recorded as less than 100% in all cases (with the exception of 4MCT). The amount of dissolved oxygen in the water is important to aquatic life, suspended particles in the water column (common in OSPM wetlands, with turbidity values of 2 to 3) may absorb heat from sunlight, thus raising water temperature which in turn lowers dissolved oxygen levels (Hochman 1988). Also suspended particles can prevent sunlight from reaching plants below the surface as a result decreasing the rate of photosynthesis, so less oxygen can be produced by plants (Hochman 1988). Low dissolved oxygen values may severely reduce the diversity and population of aquatic communities, including plant and microbial communities that may be establishing in new wetlands (Hochman 1988).

Figure 28 and 29 are showing a testate amoebae community composition and biomass ratio present in a typical OSPM site. Demonstration Pond 2 is the model, and shows only one taxa of testate amoebae, but in some cases (Jan's Pond) no taxa were detected in a sample, revealing testate amoebae as sensitive indicators of change in environmental conditions.

Figure 28: Testate amoebae community in a typical OSPM site Demonstration Pond 2

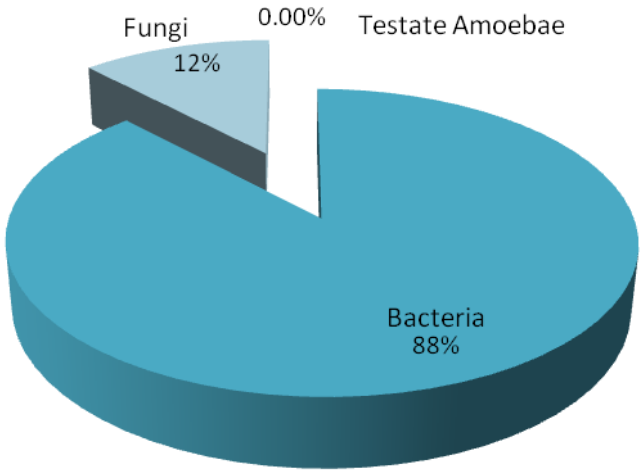


Figure 29: Relative Proportions of Testate Amoebae, Bacteria, and Fungi in OSPM site Demonstration Pond 2

4MCT recorded higher dissolved oxygen values compared to other OSPM sites, as well as an elevated organic content percentage. These characteristics may be attributed to sampling limitations. In 4MCT the only "safe" sampling areas is within arms' reach of a board walk, in a rich *Typha latifolia* zone which would increase the dissolved oxygen, and percent organic content in this sample due to rooted aquatic plants.

Characteristically fewer plant species were found colonizing OSPM sites than any other site type (Table 2, full list in Appendix A). The species found in these sites were consistently more robust species associated with succession and bioremediation such as; *Typha latifolia* and *Potamogeton* (Fassett 1940, Smith 1992, Fraga and Kvet 1993). In a study by Combroux *et al* (2002), *Potamogeton* and *Chara* were found to be dominant in early successional communities near the River Ain in France, consistent with communities present in OSPM sites, indicating a natural robustness of these species. These typical early successional communities were later replaced with more mature communities, with greater diversity and abundance which were consistent with control and reference sites in this study. This discovery indicates that though OSPM sites were of comparable age to control and reference sites, the vegetation communities in these sites are more analogous to early successional sites in other studies, suggestive of hindered development of vegetation communities in wetlands impacted by OSPM.

In OSPM sites, organic content ranged from 3.02 to 55.38% with most values falling below 20%. Reduced organic content and vegetation community diversity appears to have a negative correlation with testate amoebae assemblages. More taxa of testate amoebae typically occurred in “vegetated” samples, reduced vegetation and percent cover was associated with a reduction in testate amoebae community in this study (Table 3 and Figure 9).

Two OSPM sites with percent organic content greater than 50 % were Sustainability Pond North and Sustainability Pond South. These sites are located within twenty feet of each other and were difficult to sample as the edges of the basin (similar to the reference sites)

were tilted, but in contrast to the reference sites, these sites had greater organic matter content (greater than 50%). Due to the extreme steepness, samples were limited to an area in close proximity to the perimeter where the emergent vegetation may have skewed the percent organic content in these samples.

Elevated conductivities were another commonality in OSPM sites, as compared to lower levels in control and reference sites (Table 3). Studies by Holowenko *et al*. (2002) revealed elevated levels of naphthenic acids in tailings (associated with OSPM wetlands and as high as 88mg L^{-1}), which may contribute to the prominent conductivity levels measured in OSPM sites in the 2007 and 2008 field seasons. Indirect effects of excess dissolved solids (higher conductivity) are primarily the elimination of desirable plants and habitat-forming plant species (Brooks and Corey 1964). Mike's Pond (an OSPM site) registered the highest conductivity value: 4689µS/cm, this site is also associated with depressed organic content percentage (3.02%, one of the lowest recorded in this study). Additional sites with high conductivity levels were Jan's Pond, Demo Pond and 4MCT with values of 2229, 2233 and 2290µS/cm respectively, but do not appear to be as severely affected in terms of organic content (values ranging from 6 to 20% organic content). This suggests a threshold value of conductivity at which point vegetation and testate amoebae communities become adversely affected by elevated conductivities, which is expected to be between 3000 and 4000 µS/cm. Other factors must be considered with conductivity, such as naphthenic acid toxicity, Mike's Pond has a toxicity of 55±11mg/L (Farwell *et al* 2009) compared to Demo Pond registering a naphthenic acid level of only 8.9±2.7mg/L (Farwell *et al* 2009) which may be impacting organisms differently.

In this study Mike's Pond ordinated separately in all multivariate analysis than all other OSPM sites. In Figure 10 Mike's Pond shows a different community of testate amoebae with greater biomass (1.74μg of C/g, Table 6) compared to other open-water OSPM wetlands. This site ordinates more closely with peatland site Aurora 2.3 and control site Small Beaver Pond in the DCA indicating a different range of testate amoebae colonizing this site when compared to other OSPM sites. CCA analysis reveals Mike's Pond as more closely related to emergent vegetation and to Aurora site 1.2. Mike's Pond is a highly toxic site among all OSPM sites with elevated conductivity and naphthenic acid toxicity (Farwell *et al* 2009) but with greater abundance and diversity of testate amoebae. This site is isolated from other sites, and is in a reclaimed landscape, no differences were seen in terms of bacterial and fungal biomass indicating a possible effect at higher trophic levels, potentially the absence of a predator for testate amoebae permitting them to be present in higher abundance.

OSPM sites were of comparable age to control and reference wetlands which indicated that plant communities and testate amoebae became established in OSPM sites more slowly than the reference and control site types. This could be a symptom of a number of different causes.

OSPM sites are on active mine sites with surrounding landscapes being changed or altered relatively frequently resulting in an unstable terrain that may hinder the establishment of vegetation and testate amoebae communities. Also, there are few surrounding plant communities from which propagules could originate from, thus reducing the number of plants able to colonize these sites. Due to the fact that OSPM sites have all been created and amended by oil sand operators and typically are grouped for easier access by researchers,

they are relatively close to roads and exist in landscapes that have been recently or are currently disturbed, it was expected that OSPM sites contained fewer taxa of testate amoebae. The prominent taxa included *Arcella, Difflugia*, and *Hyalosphenia*, (Figures 9 and 10). The expectation was that more robust species (in terms of vegetation and testate amoebae) were likely to be present in OSPM wetlands due to compromised ecological conditions. In a study by Kauppila *et al* (2006), *Diffligia protaeiformis* was associated with sites impacted by Copper mine water on the eastern shore of Lake Retunen in eastern Finland. This finding was also supported by Asiolo *et al* (1996). Gehrels *et al* (2006) and Nguyen-Viet *et al* (2007(2)) found that testate amoebae communities in salt marshes with elevated conductivities and lead were characterized by the following succession of dominant taxa: *Arcella catinus, A. discoides, Nebela militaris, Trinema lineare, Centropyxis acueleate, C. cassis, C. platystoma*, and *Difflugia pristis*. While *Difflugia* were present in all site types, they were the dominant taxon in OSPM sites. *Difflugia* and *Arcella* have been noted for their prominence in many sites, but also for their robustness and likelihood to colonize sites impacted by metals or high conductivities (Nguyen-Viet *et al* 2007, Gehrels *et al* 2006). This study is consistent with literature reporting *Difflugia* and *Arcella* as common taxa in affected sites.

Percent moisture and bulk density (measured for all sites) depend strongly on the freshness of samples. Samples were transported to the laboratory from Alberta, and then preserved (at 4º Celsius) until they were processed, the likelihood of maintaining all moisture content is improbable. Therefore the usefulness of bulk density and percent moisture content

was reduced and percent organic matter content became the most important instruments in sediment analysis.

4.2 Biomass: Relationship of Testate Amoebae to the whole Microbial Community

In terms of biomass one group was dominant: bacteria made up a major proportion (>65% in open-water wetlands and >80% in peatlands) of the microbial community in all site types. The original expectation was that testate amoebae would make up a larger proportion of these communities. However, it should be noted that not all groups of microorganisms were considered in this study. Some of the groups that were not examined in this study include cyanobacteria, bacillariophyceae, micro algae, ciliates, and heterotrophic flagellates, which may have contributed to the microbial community in peatland and open-water sites altering the microbial proportions measured in this study. By changing the proportions each group contributes to the microbial community it may be possible to identify trends that are complicated and cannot be clarified with abundance values for bacteria, fungi and testate amoebae.

Testate amoebae biomass in peatlands was significantly higher ($P<0.05$) than all open-water site types (control, reference and OSPM) ranging from 5.63 to 747.44 µg of C/g of sample (mean of 242±316 µg of C/g of sample) but with no trends in relation to peatland site (wet versus dry in peatland site). These values were variable between site types, but comparable to values reported by Nguyen-Viet *et al* (2007(1)) for testate amoebae associated with *Sphagnum fallax* with biomass values between 14.9 and 524.9 µg of C/g of sample. Densities of testate amoebae in this study were comparatively lower than those reported by

Warner (1987), who estimated 10 000 cells per liter in an upland *Sphagnum magellanicum* site, with densities for most testate amoebae species less than 5000 individuals per g of sample (with a exceptions present in peatland sites Figure 9). Gilbert *et al* (1998(2)) reported testate amoebae percentages of 48% for a peatland microbial community in Puyde-Dome, France, which is substantially higher than peatland sites in this study where testate amoebae percentages averaged 2%. In the study conducted by Gilbert and colleagues (2008(2)) at least seven other groups were considered which increased the proportion of testate amoebae, albeit biomass values were comparable (0.56 to 1.63 µgC/ml). Although testate amoebae percentages are low in this study (compared to other groups studied, and what has been reported in the literature) it may be partially attributed to the few groups studied.

Testate amoebae biomass proportions in open-water wetlands fell below 1 µg of C/g of sample, with no trends in relation to "vegetated" versus "non-vegetated" samples. Little is known about the ecology of testate amoebae and their response to limnological variables, but Tolonen (1986) speculated that the principle environmental control on species distribution was oxygen concentration and surrounding vegetation. In a study of sub-tropical lakes in Florida, organic matter content in sediment emerged as the only variable that influenced the presence/absence of testate amoebae in Florida lakes (Escobar *et al* 2008). Organic-rich sites were reported to contain large numbers of testate amoebae, whereas sites characterized by sandy substrates yielded few or no testate amoebae (Escobar *et al* 2008). In this study fewer taxa were present in "non-vegetated" samples in reference and control sites when compared to "vegetated" samples.

Biomass values for testate amoebae were significantly different in sites amended with OSPM compared to those without OSPM (Table 6 and 7). These differences are supported by all analyses (DCA multivariate analysis, and raw species counts, Figure 9, 10 and Table 6), indicating that testate amoebae assemblages, including biomass are different in sites that have OSPM incorporated compared to those that do not. It is also important to note that the opposite trend is true for bacterial biomass with elevated biomass measured in sites amended with OSPM suggesting a different response to the same environmental status. It has been well documented that bacteria are able to thrive in environments that are typically unfavorable to other organisms (Gottschalk 1985). Bacteria also have been documented as successfully remobilizing nutrients from toxic substances such as naphthenic acids (Hadwin *et al* 2006) explaining their prevalence in these sites where no other organisms are able to succeed.

Testate amoebae assemblages and biomass are indicative of differences in wetlands that they colonize. Recent research indicates that testate amoebae have a complex response to environmental variables in open-water sites: they may be sensitive to pollution, pH, and temperature change (Patterson *et al* 1996, Reinhardt *et al* 1998). No differences between control and reference wetlands were detected in terms of testate amoebae biomass even though species assemblage varied somewhat by site type, indicating that testate amoebae species assemblage act as a better indicator of wetland establishment than general biomass measurements.

Bacterial biomass was significantly different between peatland sites and all other site types (Tables 6 and 7). Significantly more bacteria were measured in peatland sites with

values from 1455 to 9649 µg of C/g of sample compared to 226 to 2419 µg of C/g of sample reported by Nguyen-Viet *et al* (2007 (2)). The average of bacterial cells per slide in peatland was 1.1×10^4 compared to 7.29×10^6 cells per milliliter of sample in Gilbert *et al* (1998(2)). Despite the fact that absolute bacterial density and biomass were low in this study compared to literature values, this group comprised an average of 80% of the microbial community in peatlands and >65% in open-water sites.

Bacterial biomass in open-water site types fell below 1000 µg of C/g of sample with no significant difference between control, reference and OSPM. The standard deviation of biomass values (for all open-water site types) was considerably large suggestive of variable bacterial communities in open-water sites, as well as variation between samples.

Control sites that were considered "vegetated" had lower bacterial biomass values (from 81 to 324 µg of C/g of sample) compared to "non-vegetated" control samples (752 and 997 µg of C/g of sample). This trend is contrary to expectations as heavily vegetated areas would have additional organic substrates from which bacteria are able to produce chemical energy, also the sample locations were expected to have higher dissolved oxygen values (due to photosynthesizing plants) creating an environment more conducive to bacterial life. The exceptions to the trend were Loon Lake, and MacLean Creek. The "vegetated" sample from Loon Lake and MacLean Creek had higher bacterial biomass (consistent with expectations) in the "vegetated" sample. The commonality between these sites was low organic content in the "vegetated" sample, making these samples more comparable to "non-vegetated" samples from other wetlands.

During the isolation of the bacterial fraction from a sediment sample mechanical agitation was performed only after the sample was rinsed of large organics. Therefore there is a chance that low bacterial biomass in "vegetated" samples may be a symptom of cells being lost with larger organics. The possibility that bacterial cells were present on organics that were removed from the sample would reduce the accuracy of this study and unfortunately no standard (like lycopodium spores used for testate amoebae analysis) was used to indicate no loss of cells through processing. Running the same sample through mechanical agitation before filtration and comparing the resultant biomass to non-agitated samples from this study would provide insight into the adequacy of this methodology.

Despite bacterial biomass values not being significantly different between site types, the absolute biomass values for control sites were higher (3117.4 ±4867.8µg of Carbon/g of sample) than reference and OSPM sites (which appear to be comparable; 250.2 ±206.8, 360.1 ±262.0 µg of Carbon/g of sample respectively) (Table 8). In terms of proportion bacteria contributed most in sites amended with OSPM, which may have some ecological bearing as this group is able to thrive in conditions that are limiting for both fungi and testate amoebae.

Analysis of fungal biomass data revealed significant differences between peatlands and all open-water sites; fungal biomass in peatland sites was considerably higher than all other site types. No significant differences within open-water sites were detected, however fungal biomass in reference sites was intermediate (4.361 ±1.176*)* compared to control (5.093 ±1.770*)* and OSPM (3.407 ±1.350*)* sites indicating a possible transitional community stage (Table 6). No relationship existed with fungal biomass in connection to "vegetated" or "non-vegetated" samples.

Overall, biomass data (all biomass data considered together) were significantly different between peatland and all site types but no differences were found between any open-water sites. Peatland sites had elevated overall biomass ranging from 1505 to 13353 µg of C/g of sample. No distinction could be seen between peatland sites Aurora 1 and 2. In all cases (testate amoebae, bacteria and fungi), peatlands had higher biomass values symptomatic of the notable differences between peatlands and open-water wetlands. In all analyses differences were pronounced between peatland and open-water sites, emphasizing peatlands as a distinctive site type in this study. In control sites "non-vegetated" samples had moderately higher overall biomass, which was primarily contributed by bacteria, but in reference and OSPM sites no trends with relationship to "vegetated" or "non-vegetated" samples could be established.

Younger wetlands were expected to be negatively affected in terms of biomass. Variance in biomass measurements between young and older sites would indicate a successional change that could be used by oil sands scientists in order to keep an account of the progress of an affected wetland. Mann-Whitney tests (for non-parametric data) were used to determine differences between young and old wetlands, using young at ≤8yrs and Old at >8yrs (Tables 9 and 10) as well as young at <12yrs and Old at ≥12yrs (Table 11). This was done in order to determine if there were differences in intermediate and older aged communities. The standard of <8 years for a younger site (and ≥8 years for older sites) has been used in studies on invertebrate populations in the oil sands. In a study focusing on microbial communities it was expected that changes may be occurring more rapidly (1-2yrs) and substantially longer (>12yrs). Unfortunately few wetlands in this study are <8years,

while even fewer are younger limiting age as a measure of change in these communities in this study. Using younger sites in future research would be useful in clarifying successional changes in these microbial communities.

Reference and OSPM sites were expected to have the most noteworthy differences between young and old wetlands since they have been subject to more disturbances and changes than control type sites. In both cases (8 and 12 yrs) no significant differences were seen between young and old wetlands in any case.

4.3 Testate Amoebae as a Biological Indicator of Microbial Community Health

As open-pit mining continues, landscapes are destroyed every day and large quantities of tailings are produced when bitumen is separated from oil sands. The increasing scale of oil sand operations reveals the essential need for reclamation. Created and restored wetlands are intended to replace the functionality of former systems that have been lost through disturbance. Unfortunately, not all functions of a wetland are or can be considered during reclamation efforts and many wetland restoration projects have shown that structure does not necessarily imply function (Reinartz and Warne 1993) meaning that while these wetlands may appear to be similar to natural wetlands, there may be underlying deficiencies. Therefore, researchers are unable to assume the functionality of these created systems and require biological indicators to provide this information. By relating testate amoebae assemblages to general abundance values for bacteria and fungi, the health and progress of restored wetlands was expected to be clarified.

Testate amoebae act as biological indicators in many circumstances in both peatland (Warner 1987, Lüftenegger and Foissner 1989, Balik 1991, Charman *et al* 2000) and lacustrine ecologies (Escobar *et al* 2008) due to the fact that they were abundant and diverse, their isolation and identification was relatively easy using microscope techniques and test characteristics. For this study their trophic position at the top of the microbial food web was ideal for an early signifier of disturbance, or rehabilitation, as they consume the primary group which remobilizes nutrients making it bioavailable for higher trophic levels (Gilbert *et al* 1998(1), 1998(2), 2000). Testate amoebae have model characteristics for straightforward monitoring procedures and with easy sampling and identification techniques this group of organisms is well suited for the wetlands guideline for oil sand operators.

Testate amoebae assemblages and biomass daona were sensitive to changes in the ecological parameters, namely conductivity, dissolved oxygen and water turbidity as well as contaminant levels (OSPM). Sensitivity was manifested in variations in testate amoebae abundance, diversity and richness, and/or species assemblage, with the most robust species (*Difflugia*) being dominant in sites amended with OSPM (Figure 9). Unfortunately, changes in testate amoebae communities were not in harmony with changes in bacterial and fungal biomass suggesting, a disconnect or complexity in the relationship between testate amoebae and the rest of the microbial loop. While testate amoebae assemblages and biomass were lesser in OSPM sites, bacterial biomass was found to be elevated in OSPM sites. This trend suggests an intricacy to responses to environmental conditions and the dynamic between testate amoebae and bacteria that general abundance values cannot convey. Bacteria typically exist in specific niches, greater biomass in sites amended with OSPM does not

consequentially indicate improved physical condition of the microbial community. In this situation it is possible one or two groups of bacteria are able to tolerate OSPM conditions, and with no competition they have an advantage and are able to thrive. The bacterial community (both aerobic and anaerobic) establishes itself and plays a vital role in bioremediation of wastewater (Ali *et al* 2008). Bioremediation has emerged as one of the most important tools to eliminate or reduce the contamination caused by diverse compounds of anthropogenic origin that are spilled into the environment (Vasquez *et al* 2009), to which bacteria are the primary consumers.

It is clear that testate amoebae assemblages are changing in response to a changing environment. Unfortunately, this study was only able to focus on three groups; testate amoebae, bacteria, and fungi, limiting the links that can be drawn from the interaction of testate amoebae with the rest of the microbial community. Bacteria dominated all site types, their predominance sites impacted by OSPM suggest that bacteria are able succeed in conditions that are problematic for other organisms and remediate the environment. Further bacterial research may reveal specific species of bacteria most able to metabolize the toxic compounds (OSPM) which may ultimately be related to specific assemblages of testate amoebae. As tolerant species of bacteria dissipate, and other species of bacteria are able colonize it would be expected that fewer toxic elements are present in these sites, allowing testate amoebae, the middle trophic indicator to settle.

This study served as a starting point for microbial work in the Athabasca oil sands, highlighting the dominant players (bacteria) and flagging groups that may require more in-depth research to act as indicators of microbial community health (testate amoebae).

Investigation of other microbial groups that contribute to these changes may provide the information necessary to more readily understand and interpret the relationships between the changing testate amoebae assemblages and the microbial community as a whole. Investigation aimed at characterizing bacteria will identify central species, and their relationship to testate amoebae. Additional research will also reveal the mechanisms for why differences exist between site types in terms of testate amoebae as well as bacteria and processes that are required to restore created wetlands to a pre-industrial state. Based on this study, testate amoebae species assemblages cannot successfully operate as indicators for the wetlands guideline for oil sands operators, based on microbial community health in reestablishing wetlands impacted by oil sands processed materials but future research is expected uncover links between bacteria and testate amoebae.

4.4 Conclusions

Based on this study the following statements can be made about testate amoebae and their relation to bacteria and fungi in wetlands impacted by OSPM:

1) Testate amoebae, bacteria, and fungi were most abundant in peatland sites when compared to other site types.

2) Peatland sites were identified as dissimilar when compared to open-water wetlands in this study based on testate amoebae assemblages, and biomass for testate amoebae, bacteria, and fungi.

3) The primary monitored parameters affecting testate amoebae composition in peatland sites were soil moisturepH. Importantparameters affecting testate amoebae assemblagesin open-water sites were dissolved oxygen, turbidity and conductivity.

4) Open-water sites: control, reference, and OSPM maintained comparable values of bacterial, fungal, and overall biomass, with no significant differences between site types.

5) Bacteria contributed the greatest proportion of biomass in each site type suggesting their importance in wetlands impacts by oil sands processed materials.

6) Testate amoebae assemblages as well as biomass values showed sensitivity to a changing environment and contaminant levels suggesting promise as a bioindicator in wetlands impacted by OSPM.

7) In this study testate amoebae comprised a small part of microbial communities (<1%) compared to other groups considered and had opposing trends in terms of biomass in affected sites. This study suggests that testate amoebae community composition is distinctive though they comprise a small proportion of the whole microbial community.. Further work, some of which is suggested below, may help to reveal the true potential of testate amoebae as biomonitors in the rehabilitation of OSPM wetlands and oil sands mining landscapes. Testate amoebae and the rest of the microbial community probably hold the greatest promise as bioindicators in wetlands impacted by oil sands mining.

4.5 Recommendations

1) Further research is required to further characterize specific composition of bacterial communities and differences in relation to age of OSPM wetlands. Such work will provide insight into the microbial loop and the role of testate amoebae in the microbial loop.

a) This study included selected groups (i.e. bacteria, fungi); further work that includes additional microbial groups (i.e. cyanobacteria, bacillariophyceae, micro algae, ciliates, and heterotrophic flagellates) may provide further insights on role and position of testate amoebae in the whole microbial community. In OSPM-affected wetlands bacterial communities make up the majority of the community, unfortunately biomass is a crude measure and does not indicate the diversity and composition of the specific communities. With further research it can be determined if linkages exist between less diverse bacterial communities and testate amoebae, and more diverse bacterial communities and testate amoebae, thus allowing testate amoebae (a middle trophic indicator) to be indicative of activity at a bacterial level.

b) When considering sites for future studies including very young (<1yr), intermediate (1>12 yrs) and older sites (>12 yrs) of OSPM wetlands might lead to further insights on the position of testate amoebae in the microbial community as a whole .

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Appendix A. Detailed Vegetation Data from the 24 Wetland study Sites

Wetland	Wetland Type	Water Depth	Vegetation
Test Pond 9 (TP9)	OSPM	60cm, very turbid	40% *Typha latifolia*
Shallow Wetland (SW)	REF	12cm, clear 40cm, clear 50cm, less clear	*Typha latifolia* 50%, *Scirpus* 15%, *Hippuris* 5%, *Utricularia* <1, *Lemna* <1 *Utricularia* 10%, *Hippuris* 70%, *Scirpus* 10%, *Carex asturica* <5, *Lemna* <1, *Typha* <5 *Ceratophyllum* 80%, *Hippuris* 10%, *Utricularia* <1, *Potamogeton filiformis* 5%
South Ditch (SD)	REF	12cm, clear 56cm, clear	*Scirpus* 10%, *Typha* 30% *Charales* 85%, *Potamogeton filiformis 10%,*
Natural Wetland (NW)	OSPM	10cm, less clear 3cm, turbid	*Potamogeton filiformis* 70% *Carex rostrata* 55%
4 Meter Consolidated Tailings (4MCT)	OSPM	15cm, turbid	N/A
High Sulphate (HS)	REF	32cm, less clear 7cm, less clear	*Chara* – 75%, *Typha* 60%
Peat Pond (PP)	REF	33cm, less clear	*Chara* 85%
Bill's Lake (BL)	REF	35cm, turbid 65cm, turbid	*Lemna* 20%, *Chara* 5%, *Typha* 15%, *Ceratophyllum* 90%
Beaver Pond (BP)	CONTROL	32cm, less clear	*Chara* 35%
Mike's Pond (MP)	OSPM	22cm, less clear	*Potamogeton* 45%
Duck Pond (Duck), formerly South West Corner Waste Area 11	REF	39cm, clear	*Utricularia* 10% *Typha latifolia 90%*
Sandpit Wetland (SP)	REF	35cm,less clear	*Scirpus* 40%, *Typha latifolia* 50%
Sedimentation Wetland (SED)	CONTROL	54cm, less clear	*Potamogeton* 40%
MaClean Creek (MC)	CONTROL	9cm, clear	*Lemna* 20%
Loon Lake (LL)	CONTROL	11cm, clear 28cm, clear	*Scirpus* 15%, *Typha latifolia 25%* *N/A*
Sustainability Pond North (SUS N)	OSPM	32cm, turbid	*Typha latifolia* 60%
Sustainability Pond South (SUS S)	OSPM	36cm, turbid	*Typha latifolia* 45%
Demo Pond (DP)	OSPM	60cm, turbid 42cm, turbid	*Potamogeton* 90% *Scirpus* 80%
Small Beaver Pond (SB)	CONTROL	40cm, less clear	*Carex rostrata* 40%, *Lemna* 10%
1 Meter Consolidated Tailings (1MCT)	OSPM	11cm, turbid	*Typha* 25%
Control Reservoir (CON RES)	CONTROL	14cm, less clear	*Typha* 25%, *Scirpus* 30%
V Notch Weir (V NOT)	CONTROL	12cm, less clear	*Scirpus* 40%
Jans (JANS)	OSPM	17cm, turbid	*Typha* 20%, *Scirpus* 10%

Appendix B. Analysis Using ImageJ

For bacteria the following steps were followed in ImageJ;

Step 1. Open Image

Step 2. Convert color image into an 8-bit greyscale image (image→type→8-bit)

Step 3. Invert the greyscale image (edit→invert Or press ctrl+shift+I)

Step 4. Subtract the background (image→adjust→brightness & contrast)

Step 5. Threshold the image (In the Brightness & Contrast menu click on Thresh and carefully adjust the brightness slider so that everything that was in the original image appears in the thresholded image.)

Step 6. Watershed (Process→Binary→Watershed. This algorithm uses a density profile to determine if one object with a peninsula should actually be two objects.

Step 7. Analyze Particles for bacteria (Analyze→Analyze Particles)

Step 8. Subtract masks from the original image to check for missed particles (Edit→Invert (or ctrl+shift+I) then revert your original image to the saved version by going to File→Revert (or ctrl + r). Process→Image Calculator and subtract mask from your original (check "Create New Window")

For fungi the following steps were followed in ImageJ;

Step 1. Open Image

Step 2. Set Scale (Go to Analyze→Set scale in the ImageJ main window and enter the following values. Distance in Pixels: 1 – use scale bar (select the length of the scale bar with the line tool)

Known Distance: The value from the metadata (indicate the known length of the scale bar) Pixel Aspect Ratio: 1 – leave this value, Unit of length: micrometer, Global: Checked (this ensures the scale is constant for all pictures).

Step 3. Select Line tool – right click on the picture of the line tool to select the segmented line, this allows the tool to follow the length of the hyphea measuring through bends and curls.

Step 4. Measure (ctrl-m will measure and record the measurement in a results box).

The number of bacteria cells in a field of view ranged from $4.3 \text{ X } 10^{8}$ to $2.2 \text{ x } 10^{11}$ and the total length of fungal hyphae per field of view ranged from eighteen to one hundred and fifty-one micrometers.