University of Alberta

Plant Productivity, Soil Microorganisms, and Soil Nitrogen Cycling in Peat Amendments used for Oil Sands Reclamation

by

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ABSTRACT

The objectives of this research were to measure seasonal soil nitrogen availability, and to characterize plant productivity and soil microbial community structure, in different peat amendments used in oil sands reclamation. Using resin-core incubations, net nitrification, nitrogen mineralization rates, and microbial biomass nitrogen (MBN) were measured to evaluate soil nitrogen availability. Net mineralization rates were highest in the fall, and low or negative in winter. A reduced proportion of MBN was associated with lower mineralization rates. Plant growth, assessed in greenhouse trials and by measuring *in-situ* understory cover, was fostered by the majority of peat amendments. Plant productivity was higher in the reclaimed peat materials than in the peat material sampled from a natural fen. Lastly, soil microbial community composition was characterized using phospholipid fatty acid fingerprinting. Results were variable and amendment specific. In summary, amendments showing greater mineralization rates had greater percent plant cover and total species *in situ.*

ACKNOWLEDGEMENTS

Starting my masters was a spontaneous decision; classes commenced a mere two weeks once I moved south from the North West Territories in late December 2003. Had I anticipated the work that would follow I might have run for the hills if it had not been the constant support from my family. Especially from my Dad who I called nearly daily for the last 30 months to ask questions about anything from tech support to looking at results with me. I would like to thank Justin for driving me to Fort Mac and building hundreds of PVC cores with me and for letting me be a nut sometimes. I would also like to thank Patrick who encouraged me to keep going and finish this despite what life was throwing my way.

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1.1 OIL SANDS HISTORY

Major, ongoing development of oil sands is occurring in Northern Alberta. Oil sands are located in the Athabasca, Cold Lake and Peace River regions of Alberta, with a collective landmass of nearly 141,000 km^2 (Alberta Energy, 2002). The discovery of the oil sands dates back 300 years, when local aboriginals used this resource to waterproof their canoes (Canadian Center for Energy, 2003). Extraction of the resource proved to be problematic until new technologies were developed. The Great Canadian Oil Sands Company, now Suncor Energy Inc., began production in 1967 (Canadian Center for Energy, 2003). Syncrude Canada Ltd. followed and constructed a much larger mine that began operation in 1978.

The Alberta oil sands contain 10 to 12 % bitumen, 80 to 85 % mineral matter (including sand and clay), and 4 to 6 % water (Alberta Energy, 2002). The bitumen in the oil sands is a heavy, black, viscous mixture of petroleum hydrocarbons that is upgraded into crude oil before it can be processed to produce gasoline and diesel fuels. Open-pit mining techniques are employed to recover the bitumen deposits near the surface. Syncrude Canada Ltd., Suncor Energy Inc. and Albian Sands Energy Inc. currently operate near Fort McMurray, Alberta. These three companies are committed to reclamation, with the goal to achieve self-sustaining ecosystems with capabilities equivalent to those under pre-disturbance conditions (Oil Sands Vegetation Reclamation Committee, 1998).

1.2 PEAT AND OILS SANDS RECLAMATION

Peat is defined as an accumulation of organic residues ensuing from the incomplete decomposition of plant debris under saturated conditions (Belanger et al., 1988). The Canadian System of Soil Classification recognizes three Great Groups for Organic soils based on the decomposition stage of the peat materials they contain: Fibrisols, largely composed of unaltered fibric peat; Mesisols, which contain mesic peat;

and Humisols, mainly comprised of humic peat (Soil Classification Working Group, 1998). Fibric peat contains organic materials that are still readily identifiable as to botanical origin, is usually light yellowish brown to pale brown in color, and loose and spongy in consistency. Mesic peat has been partially altered and is in a decomposition stage intermediate between fibric and humic peats. Lastly, humic peat is at the most advanced stage of decomposition and contains few recognizable plant fibers. The Fibrisol and Mesisol great groups dominate the peat bogs present in the oil sands area (Turchenek and Lindsay, 1982).

The challenge of land reclamation is to create a soil-like profile suitable for plant growth. In oil sands reclamation, the soil-like profile is constructed using tailings sand, mature fine tails, overburden (lean oil sands, glacial till, glacio-lacustrine materials, muskeg etc.), composite tails and reclamation material, with an organic cap used as a surface treatment (Fung and Macyk, 2000). A variety of reclamation techniques and amendments have been utilized to date and these continue to be modified based on ongoing research results.

Peat is used as an organic cap in oil sands reclamation mainly due to its availability in the pre-mining area. As the peat material is stripped from drained bogs prior to mining, some of the underlying mineral layer is taken with it (25 to 50 % by volume). This process is called over-stripping and creates the peat mixes used in reclamation amendments in the oil sands. Comprehensive studies on the uses of peat amendments were conducted during the 1960s, 1980s and 1990s (Lucas et al., 1965; Belanger et al., 1988; Riley, 1994). These studies demonstrated that peat caps increase soil water holding capacity, improve plant root penetration and retain nutrients (Lucas et al., 1965). Logan (1978) studied the use of peat mixes as soil amendments for oil sands reclamation. He concluded that peat mixes when used in combination with nitrogen fertilization could improve plant growth, and his findings have significantly contributed to current reclamation practices.

1.3 BOREAL FOREST ECOLOGY

The boreal forest occupies more than 60 % of the total forested area in Canada and Alaska and can have limiting growth conditions due to low soil nitrogen availability, moisture deficits, and low temperatures (Binkley and Hogberg, 1997). Key factors in soil development in the area may include poor drainage and thick surface layers of organic material. A thick layer of peat can insulate the mineral soil, causing lower soil temperatures and discontinuous permafrost (Johnson et al., 1995). The oil sands region lies in the natural subregion of the Central Mixedwood area, and the ecological area is the boreal mixedwood (Beckingham and Archibald, 1996). Table 1.1 gives detailed climatic data for the oil sands region, which has long, cold winters and warm summers, with marked differences between day and night temperatures.

Typical trees found in the boreal mixedwood include aspen (*Populus tremuloides* Michx.), white birch (*Betula papyrifera* L.), and black spruce (*Picea mariana* (Mill.) BSP.), and white spruce (*Picea glauca* (Moench) Voss) (Beckingham and Archibald, 1996). Common understory shrubs include prickly rose (*Rosa acicularis* Lindl.), low-bush cranberry (*Viburnum edule* Michx.), saskatoon (*Amelanchier alnifolia* Nutt.), and buffalo-berry (*Shepherdia canadensis* (L.) Nutt.). Common forbs are bunchberry (*Cornus canadensis* L.), wild sarsaparilla (*Aralia nudicaulis* L.), and dewberry (*Rubus pubescens* Raf.) (Moss, 1993).

The particular mix of plants and soils that currently comprises the boreal forest has developed over the last 12,000 years (Johnson et al., 1995). Plants that first colonized this area were the ones able to quickly take advantage of the vast areas of exposed land. These species were adapted to dispersal over great distances, and they could thrive in relatively simple communities (Johnson et al., 1995). Today, many species retain these pioneer characteristics.

Where an entire ecosystem needs to be reclaimed, return to a functioning sustainable ecosystem faces many challenges. Invasive species tend to be adaptable and may thrive in harsh environments (Ebbert and Byrd, 2002). Once established, they often out-compete native species for nutrients, and may thwart revegetation efforts. Hence, the

re-establishment of native species is a key element in the return to a functioning, sustainable ecosystem.

1.4 FACTORS INFLUENCING NUTRIENT DYNAMICS

Soil moisture is recognized as one of the controlling factors in pedogenesis, as it directly affects the rate of weathering and biological processes, including mineralization rates. Soil organic matter content typically increases with increasing moisture (Jenny, 1994). Moisture content fluctuates with season, with the highest soil moisture content in the boreal forest occurring in mid-May, due to snowmelt inputs, low evapotranspiration, and high precipitation relative to the rest of the year (Stottlemyer and Toczydlowski, 1995). Soil moisture is also affected by aspect. Increased solar energy on southern aspects will increase daytime air temperatures and evaporation, resulting in lower available soil moisture (Hutchins et al., 1976).

Temperature influences microbial activity and total microbial biomass in the soil (Campbell et al., 1973), with the optimum temperature for the microbial community responsible for N mineralization ranging from 0 to 35°C (Stanford et al., 1973). Offord (1999) found that in an organic horizon in a mixedwood boreal forest, maximum N mineralization rates occurred at 12°C, but that the optimum temperature for mineralization in the Ae horizon was 22°C.

1.5 NITROGEN CYCLE

Nitrogen is often the most limiting nutrient for plants in boreal forest soils (Kaye and Hart, 1997). It is critically important for organisms, as it is one of the most abundant elements in their tissues (Gale Group, 2001). The availability of biologically useful forms of nitrogen is a common limiting factor in plant productivity. Thus, nitrogen is crucial in maintaining a sustainable ecosystem (Raison and Stottlemyer, 1991). As such, the production of bioavailable N influences reclamation success. For this reason, nitrogen availability was used in this thesis as one of the primary indicators to assess reclamation success.

Nitrate and ammonium dissolved in soil water are the main forms of nitrogen that plants assimilate from the environment (Gale Group, 2001). Diazotrophs, in symbiosis with some leguminous and nonleguminous plants, however, can fix atmospheric nitrogen into ammonia. In addition, soluble organic nitrogen is becoming increasingly recognized as an important nitrogen source for plant nutrition in boreal forests (Persson and Nasholm, 2001).

Mineralization corresponds to the release of inorganic N from organic forms of N, and is the combination of ammonification and nitrification processes.

Ammonification and *nitrification*:

Organic N $\rightarrow$ NH_4^+ $\rightarrow$ NO_2^- $\rightarrow$$NO_3^-$

where ammonification corresponds to the release of ammonium from organic forms of N, and nitrification is the oxidation of ammonium into nitrate via nitrite (Figure 1.1). Net N mineralization rates can further be defined as gross N mineralization rates (or production) minus consumption. Low estimates of net N mineralization from field incubation studies do not necessarily correspond to low gross mineralization rates. Mineralized N can be simultaneously "consumed" by microbial immobilization, nitrate leaching, or denitrification (Jansson and Persson, 1982).

Schimel et al. (2004) suggested that microbial populations continue to mediate nitrogen transformations in soils throughout the winter months, even though process prevalence may differ according to the time of year. During the growing season, for example, net N mineralization rates are often low or negative, indicating that microbial N immobilization is the dominant process during this season (Chapin et al., 1988; Jonasson et al., 1999; Schmidt et al., 1999). These studies further indicate that soil N dynamics essentially differ between the cold season (September through May) and the warm season (June to August). Immobilization during the growing season changes to mineralization during the cold season (Schimel et al., 2004), creating a supply of bioavailable N utilized in spring at the start of the growing season.

1.6 RESEARCH OBJECTIVE AND EXPERIMENTAL APPROACH

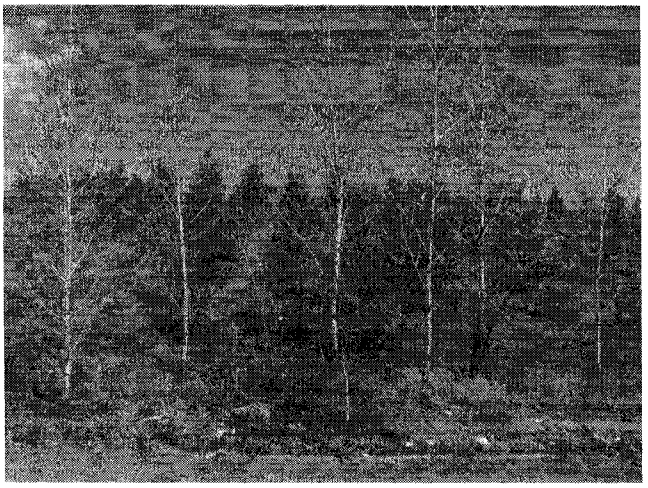
Five sites (i.e., reclamation treatments) were selected to represent a range of the decomposition degrees of peat (fibric, mesic, humic) that are used during reclamation in the Athabasca oil sands. Photographs of all sites are included at the end of this chapter as reference (Pictures 1.1 to 1.9). The main objectives of the study were:

1) To determine and compare the seasonal variability in labile soil N, and net nitrification and mineralization rates in these different peat amendments; and

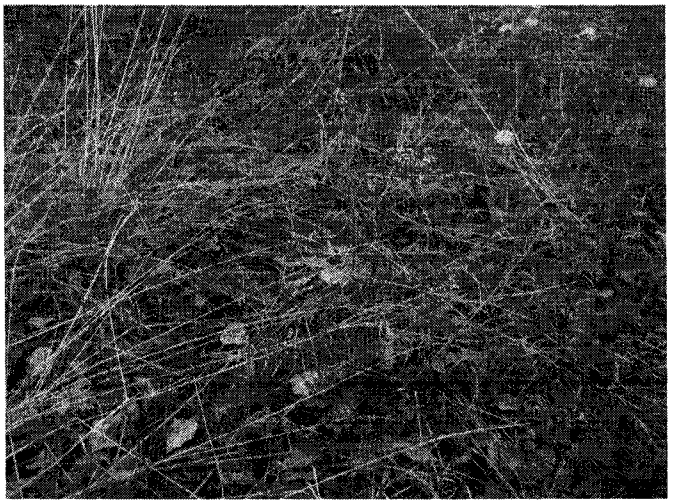
2) To characterize potential plant productivity, plant communities, and soil microbial community structure in various peat-mineral amendments used in oil sands reclamation

To address Objective 1, a transplant incubation experiment using the resin-core incubation method was set up in June 2004. Field incubation of samples allowed assessment of seasonal variability in nitrogen availability throughout the year. Laboratory analyses were conducted on all samples to determine soil moisture, pH, dissolved organic C and N, microbial biomass C and N, and net N mineralization rates. To address Objective 2, a greenhouse experiment was designed to study the direct effect of peat composition on plant growth. The chosen plant species was *Calamagrostis canadensis* Michx. (Blue-joint), a native species that grows in the northern boreal forests, and which has the potential to develop on all the study sites. In addition, surveys were conducted at each site to provide descriptive statistics for plant communities. The goal of reclamation is to return the post-mined areas into self-sustaining ecosystems with plant communities similar to naturally occurring communities. Finally, phospholipid fatty acid (PLFA) analysis was used to fingerprint the structural composition of soil microbial communities.

This thesis is organized into four chapters. The first chapter provides a general background and introduction to the major issues studied in this thesis. Chapter 2 presents the results of a field based study comparing nitrogen availability in different peat amendments. Chapter 3 examines the variability among peat treatments in both the soil microbial community structure, and composition of the plant communities. Chapter 4 summarizes the overall study.



Picture 1.1: Humic/mesic site, June 2004, planted with hybrid poplar and jack pine.



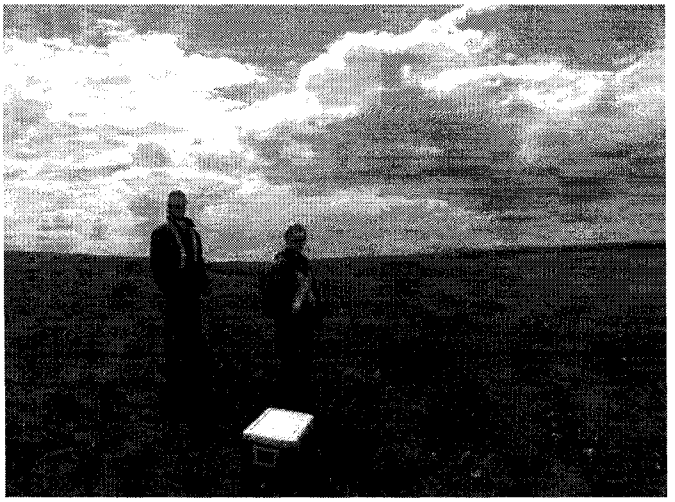
Picture 1.2: Humic/mesic site, close up of the under-story, June 2004.

Picture 1.3: Mesic 1 site, June 2005.

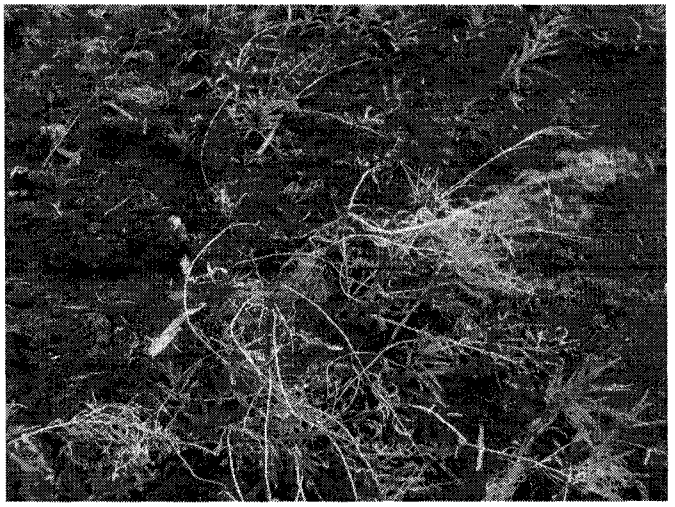
Picture 1.4: Mesic 2 site, June 2004.

Picture 1.5: Mesic 2 site, June 2004.

Picture 1.6: Mesic 3 site, taken in June 2004.



Picture 1.7: Fibric site, taken in June 2004.



Picture 1.8: Fibric site, taken in June 2004 soil close-up.

Picture 1.9: The Natural site, a sedge fen, June 2004.

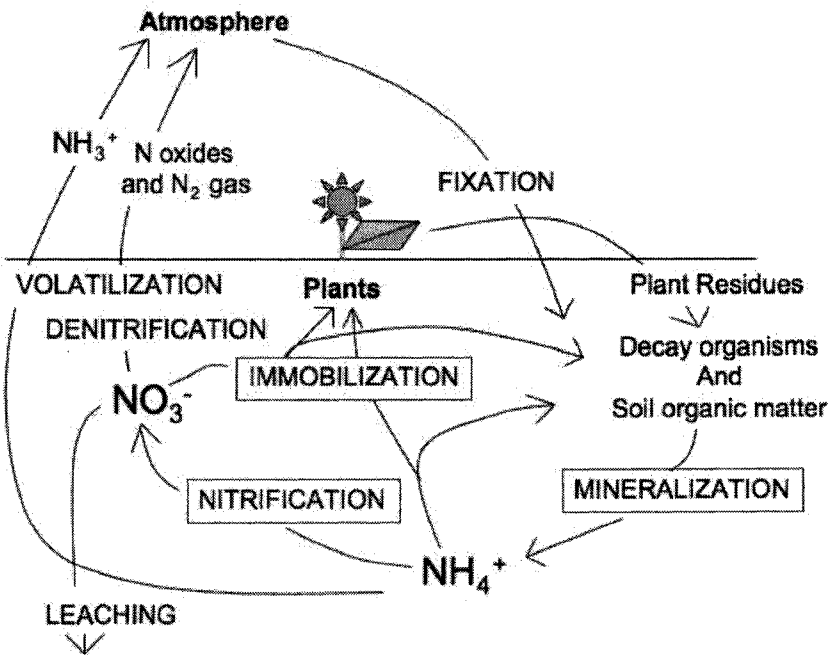


Figure 1.1: Schematic representation of N cycling (modified from Hausenbuiller, 1985).

Table 1.1: Summary of climate data for 1991 for the Boreal Mixedwood ecological area of Northern Alberta (modified from Beckingham and Archibald, 1996).

Summer[a]	
Mean temperatures (°C)	13.7
Minimum temperatures (°C)	7.2
Maximum temperatures (°C)	20.2
Total precipitation (mm)	238
Growing degree days	1,147
Numbers of days <0 °C	9
Winter[b]	
Mean temperatures (°C)	-11.9
Minimum temperatures (°C)	-17.2
Maximum temperatures (°C)	-6.5
Total precipitation (mm)	63
Annual	
Total precipitation (mm)	389
Mean Temperature (°C)	1.5

[a]Summer is defined as May, June, July and August
[b]Winter is defined as November, December, January, and February
Note: The data for the Boreal Mixedwood ecological area are based on average values derived from the Low- and Mid-Boreal Mixedwood ecoregions.

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Chapter 2: NITROGEN AVAILABILITY FROM PEAT AMENDMENTS USED IN BOREAL OIL SANDS RECLAMATION

2.1 INTRODUCTION

The Athabasca oil sands deposits are one of the largest reserves of hydrocarbons in the world, containing almost one trillion barrels of bitumen (Kimball et al., 2000). Bitumen deposits located near the surface are being recovered by open-pit mining techniques, which to date are impacting about 150 km^2 of land around Fort McMurray in northeastern Alberta. It is anticipated that by 2023 the disturbance may be as much as 10 times the currently affected area (Alberta Energy, 2002). Following mining, the challenge of land reclamation entails reconstruction of landforms and re-establishment of functioning ecosystems through the creation of soil-like profiles using salvaged mineral and organic materials. The reconstructed landscapes must support a mosaic of boreal forest communities similar to those that existed prior to disturbances (Syncrude Canada Ltd., 1981). In this regard, a key component of successful reclamation is the quality of the organic material in the reconstructed soils and, in particular, its ability to supply available nutrients for plants.

Nitrogen (N) is the nutrient most often limiting for plants in natural boreal soils (Kaye and Hart, 1997). Field measurements of N transformations in boreal forest and peatland soils typically show low rates of net N mineralization and nitrification (Table 2.1). Deciduous and mixed boreal forest floors are often reported to have higher soil N availability than coniferous forest floors; these differences have been related to lower nitrogen concentrations as well as higher lignin content in coniferous litter (Côté et al., 2000; Lindo and Visser, 2003; Jerabkova et al., 2006). Low mineralization and decomposition rates in peatlands have commonly been assumed to be due to anoxic conditions in these soils. However, a recent study testing whether placement of litter in upland or peatland sites affected decomposition rates indicated a minor effect of site on N dynamics in decomposing litter (Moore et al., 2005). Differences in intrinsic litter quality, including low nutrient concentrations and the presence of antibiotic metabolites, may override site factors and be the main contributors to low decay rates in peatland litters (Johnson and Damman, 1991; Aerts et al., 2001; Moore et al., 2005). Peat is

naturally abundant around Fort McMurray and is being used as the main organic amendment during reclamation. While peat may serve as a source of slow-releasing N fertilizer for several years as it decomposes (Lucas et al., 1965), there is a concern that it may not supply enough N to sustain plant growth during oil sands reclamation.

Schimel et al. (2004) showed that in tundra ecosystems, soil microbial populations continue to mineralize N throughout the winter months. While net N mineralization rates were often negative during the growing season, indicating microbial N immobilization, there was a shift to net mineralization during the cold season, creating a supply of bioavailable N that was used in the spring at the beginning of the growing season (Chapin et al., 1988; Jonasson et al., 1999; Schmidt et al., 1999; Schimel et al., 2004). McMillan (2005) was the first to compare N mineralization rates in reclaimed soils in the oil sands region around Fort McMurray to that of an undisturbed forest site. Her study, however, only measured rates from May to July. The overall objective of this study was to measure soil N availability throughout the growing and non-growing seasons in a range of peat amendments used for reclamation in the oil sands region. Specifically, the objective was to examine seasonal variability in labile soil N pools, and net nitrification and mineralization rates in these amendments.

2.2 MATERIALS AND METHODS

2.2.1 Experimental Area

The experimental area (57° latitude and 111° longitude) is located within the northern boreal forest region. The region has long, cold winters and warm summers, with marked differences in air temperatures among seasons (Beckingham and Archibald, 1996). The mean annual air temperature is 1.5 °C, with an average winter temperature of –11.9 °C, and an average summer temperature of 16.8 °C (Environment Canada, 2002). The annual precipitation is 455.5 mm, the majority of which is rainfall (342.2 mm).

Some of the typical trees in the northern boreal forest around Fort McMurray are trembling aspen (*Populus tremuloides* Michx.), white birch (*Betula papyrifera* L.), black spruce (*Picea mariana* (Mill.) BSP.), and white spruce (*Picea glauca* (Moench) Voss);

Beckingham and Archibald, 1996). Common understory shrubs include prickly rose (*Rosa acicularis* Lindl.), low-bush cranberry (*Viburnum edule* Michx.), saskatoon (*Amelanchier alnifolia* Nutt.), and buffalo-berry (*Shepherdia canadensis* (L.) Nutt.). Common forbs are bunchberry (*Cornus canadensis* L.), wild sarsaparilla (*Aralia nudicaulis* L.), and dewberry (*Rubus pubescens* Raf.). Soils in the experimental area are primarily organic soils overlying glacial deposits, with the Fibrisol and Mesisol great groups occurring more commonly than the Humisol great group (Turchenek and Lindsay, 1982). Other soils in the area are Gray Luvisols and Dystric Brunisols. Gray Luvisols are typically associated with lacustrine deposits and till, whereas Dystric Brunisols tend to develop on coarser parent material such as glaciofluvial outwash and eolian sands (Turchenek and Lindsay, 1982; Lanoue, 2003).

2.2.2 Study Sites

In consultation with the industrial partners, five reclaimed sites were chosen to span a range of representative reclamation practices at each of the mining operations, including typical peat amendments on a variety of slope orientations (Table 2.2). Peat used for reclamation in the oil sands region is salvaged from the area prior to the start of mining by over stripping drained peatlands such that 25 to 50 % (by volume) of the peat amendment is comprised of mineral material. The peat amendment is then placed 15 to 50 cm thick on various mineral substrates, including tailings sand (a by-product of the mining process obtained following caustic hot water extraction of the oil-impregnated sand), as well as overburden (i.e., mineral materials removed during surface mining operations to gain access to the oil-impregnated sands). Underlying materials also include lean oil sands (<8 % bitumen), and secondary material, which is mineral material with high pH and clay content from Pleistocene deposits. Overburden may encompass lean oil sands that contain less than 10 % oil, Cretaceous silts, shales, and sandstones in addition to various Pleistocene glacial deposits (Lanoue, 2003). More specifically, reclamation at the Mesic 1 site, located within the Albian Sands Energy Inc. Muskeg River Mine about 75 km north of Fort McMurray, consists of a peat mix (20 cm) with a sandy loam texture overlying 50 to 60 cm of tailings sand over lean oil sands. The Suncor Energy mine site, 20 km north of Fort McMurray, hosts sites Humic/mesic and

Mesic 2. Reclamation at the Humic/mesic site consists of a 20 cm peat mix of a sandy loam texture over 80 cm of tailings sand. At the Mesic 2 site, the peat mix overlies 80 cm of lean oil sands mixed with secondary material and overburden with a clay loam texture. Reclamation is similar at the Mesic 3 and Fibric sites, located on the Mildred Lake Mine site at Syncrude Canada Ltd., about 35 km north of Fort McMurray. Both sites include 20 cm of peat mix capping Cretaceous overburden, however, the peat amendments are of different origins at the two sites.

The experimental design precludes statistical analysis of the effect of peat type alone, as it was not possible to identify replicated reclaimed sites that only varied with respect to peat type. Instead, the present study aims to serve as reference in the monitoring of nitrogen availability from a range of peat mixes typically used in oil sands reclamation. Mesic peat is well represented and present at the Mesic 1, Mesic 2 and Mesic 3 sites, while more humified (humic/mesic) material is found at Humic/mesic, and fibric peat at the Fibric site (Table 2.2). The sites further span a range of time since reclamation (1988 to 2004), and aspects (south to north facing). Revegetation practices have been implemented at the Humic/mesic, Mesic 2 and Mesic 3 sites, while the Mesic 1 and Fibric sites have been left to regenerate naturally. In addition to the five reclaimed sites, an undisturbed (natural) sedge fen was selected within 2 km of the Mesic 1 site as representative of the type of peat used for reclamation at Albian Sands.

2.2.3 Field Methods

The following N availability indicators were measured or calculated: extractable ammonium (NH_4-N) and nitrate (NO_3-N); dissolved organic nitrogen (DON); net ammonification, nitrification, and mineralization rates; and microbial biomass nitrogen (MBN). Total labile N was further defined as the sum of NH_4-N + NO_3-N + DON + MBN. Net ammonification, nitrification and mineralization rates were assessed through field incubations using the resin-core technique. A transplant experiment was designed to isolate the influence of the peat amendment composition from that of climatic differences among sites during field incubation, and all soil materials were incubated at the Mesic 1 site. This site was chosen as the incubation location for two reasons: (1) it

was bare of plants thus no reclamation efforts would be damaged by our study, and (2) it had been instrumented with a weather monitoring station.

Five plots (10 m by 10 m) were established at each study site in May 2004. Samples were taken at six randomly selected locations per plot. Two sampling events were chosen for initial (baseline) analyses, one in May 2004 to measure the early growing season, and one in August 2004 to represent the end of the growing season. In addition, within each plot, a pair of intact soil core replicates was collected using PVC tubes (7.6 cm diameter) as close together as possible (approximately 3 cm apart) to a depth of 7 cm. One set of cores (5 cores per site) was returned immediately to the laboratory for baseline analysis in May and August 2004, and the remaining soil cores were left intact in the PVC tubes and incubated at the Mesic 1 site. A nylon stocking bag containing 20 g of mixed-bed ion-exchange resin (J.T. Baker no. M-614) was fixed to the top of the core to trap atmospheric deposition and one was attached to the bottom of the core as a leachate trap (Binkley and Matson, 1983; Binkley, 1984). In the August 2004 baseline sampling event, soil samples were taken for bulk density determination using plastic vials of a know volume, for a total of 30 samples per site.

A uniform 6 m long by 4 m wide plot was selected for incubation at the Mesic 1 site (Diagram 1, appendix A). Because of the logistics of recovering soil cores buried under snow, it was necessary to cluster the sets of cores for each incubation period so that only one snow pit was dug during the winter harvest, reducing the impact of disturbance (Schimel et al., 2004). Cores were buried 5 to 15 cm apart and no soil was placed on top of the cores to allow for the top resin bag to trap atmospheric deposition. Samples were incubated in periods consistent with climatic seasonal variation in the region (Table 2.3): the summer incubation corresponded to the growing season; the fall to plant senescence that occurs after the growing season; winter to frozen soil conditions; and the spring to soil thawing and the beginning of plant growth.

Net ammonification, nitrification and mineralization rates were calculated by subtracting the initial (pre-incubation) concentrations of inorganic N in the soil cores from the post-incubation concentrations, and adding the concentration of inorganic N leached into the resin bags attached to the bottom of the soil cores (Schimel et al., 2004).

Pre-incubation concentrations for the summer and fall seasons were obtained from the initial (baseline) set of cores sampled at the sites in May and August 2004, respectively. For the winter and spring seasons, where a baseline set of cores could not be easily sampled, concentrations in the resin-soil cores at the end of the previous incubation were used instead as the initial concentrations.

2.2.4 Laboratory Analysis

All field moist samples were sieved to 4 mm prior to analysis, except for the bulk density and moisture content determination, where unsieved samples were weighed, dried at 105 °C for 48 hours, and reweighed (Kalra and Maynard, 1991). Soil pH values were determined with a glass electrode using the paste method (Thomas, 1996); a 0.01 M $CaCl_2$ solution was added to the field-moist samples because results from this procedure are more reproducible than with water (Kalra and Maynard, 1991).

For soil samples, NH_4-N and NO_3-N were extracted using a 0.5M K_2SO_4 solution (1:10 soil: K_2SO_4) as described by McMillan (2005). The resin samples (20 g) were rinsed with deionized water and then extracted with 100 ml of 1M KCl. Filtrates were kept frozen until analysis for NH_4-N and NO_3-N concentrations using a Technicon Auto Analyzer II (Technicon Industrial systems, Tarrytown, New York). The chloroform fumigation extraction method (CFE) was used to quantify microbial biomass carbon (MBC) and nitrogen (MBN) as described by Horwath and Paul (1994). The CFE technique is applicable to soils with a low pH (Voroney et al., 1993), such as the Fibric site in this study. All filtrates were kept frozen until analysis for dissolved organic carbon (DOC) and total soluble nitrogen (TN) concentrations using a Shimadzu TOC-VTN instrument (Mandel Scientific Company Inc. Ontario, Canada). Dissolved organic nitrogen (DON) was calculated by subtracting NH_4-N and NO_3-N concentrations from TN. The MBN values were further calculated as DON after fumigation minus DON before fumigation, and MBC as DOC after fumigation minus DOC before fumigation (Jerabkova et al., 2006). Finally, a homogenized subsample (approximately 1 to 2 g) of air-dried soil was ground into a fine powder (150 μm) using a ball grinder, and then analyzed for total C and N content with a Carlo-Erba elemental analyzer (model NA-1500, Carlo-Erba Inc., Milan, Italy).

2.2.5 Statistical Methods

Significant interactions existed between reclamation treatments and seasons, therefore, data were analyzed separately using one-way analysis of variance (ANOVA) to determine if significant differences existed: (1) among peat materials within a given season, and (2) among seasons for each peat material. Data were analyzed using SAS version 8.01 (SAS Institute Inc. 1999-2000, Cary, NC). Data were tested for homogeneity of variance using the HOVTEST option in SAS, and a conservative multiple comparison test, Tukey LSD, was used as a post-hoc test (α=0.05).

2.2.6 Meteorological Data

Meteorological data were collected from stations set up at the research sites (note: the Fibric site had no meteorological station). Average monthly air temperatures and total monthly precipitation from November 2003 until June 2005 are presented in Tables 2.6 and 2.7, whereas individual site data can be found in Table B.4 in the appendices. Meteorological data for the Fort McMurray airport for the study period were compared to the Canadian Climate Normals (Environment Canada 2006; 1971-2000) for that station to determine the representativeness of monthly air temperature and precipitation within the study period. The same monthly meteorological parameters from the study sites were then compared to those for the same (study) period at the Fort McMurray airport.

For the Fort McMurray station the mean temperature was an average of the maximum temperature recorded in a 24-hour period and the minimum values for a period of the same length (Environment Canada, 2006). The average monthly temperature for the Experimental Sites was calculated using the average daily temperatures for those sites. All precipitation was measured using a standard Canadian rain gauge, a cylindrical container 40 cm high and 11.3 cm in diameter. The precipitation was funneled into a plastic graduated cylinder that served as the measuring device. In the winter, the snowfall was the measured depth of newly fallen snow, measured using a snow ruler. Measurements were made at several points that appear representative of the immediate area, and then averaged. Precipitation was the water equivalent of all types of precipitation.

2.3 RESULTS

2.3.1 Characteristics of the Peat Materials

Soil samples (0-7 cm) collected from the Fibric amendment and the Natural site were organic (i.e., contained > 17 % (wt) total C), and had a significantly higher C content but a lower bulk density than the peat amendments at the other sites (Table 2.4). Material from the Natural site further contained significantly greater total N than the peat amendments present at the reclaimed sites, while the Fibric amendment exhibited a significantly higher total C:N ratio but lower pH (4.1). There were no significant differences among the other reclaimed sites, with the exception of the C:N ratios that were higher for the Mesic 2 and 3 materials than the Mesic 1 and Humic/mesic amendments.

Dissolved organic carbon (DOC) concentrations and microbial biomass carbon (MBC) values were significantly higher for the Natural site when compared to the reclaimed peat amendments (Table 2.5). Pre-incubation labile N concentrations (i.e., the sum of DON, MBN, NO3-N and NH4-N concentrations) were also higher for the material from the Natural site and Mesic 3, and significantly so when compared to the Mesic 1 and 2 amendments. With the exception of the Mesic 3 amendment following the winter incubation period (as measured in April 2005), post-incubation labile N concentrations remained significantly higher in the material from the Natural site than in the reclaimed materials following the fall, winter and spring incubations (Figure 2.1).

Among the peat amendments, the Mesic 1 and 2 treatments showed the lowest pre-incubation labile N concentrations, and in particular were significantly lower than the Mesic 3 amendment in May 2004 (Table 2.5), and following the fall incubation in November 2004 (Figure 2.1). The Mesic 1 and 2 amendments had significantly lower MBN concentrations than the other peat amendments as well as the material from the Natural site (Table 2.5). When expressed as a percentage of total labile N concentrations, MBN values were significantly lower for the Mesic 1 (28 %) and Mesic 2 (21%) amendments than the other peat materials (61-69 %). Finally, the Mesic 1 and 2 amendments showed significantly lower DON concentrations when compared to the Natural site, but there were no significant differences among reclaimed peat treatments

(Table 2.5). When expressed as a percentage of total labile N concentrations, DON values were significantly higher for the Mesic 1 (59 %) and Mesic 2 (46 %) amendments than the other peat materials (21-30 %).

A commonality among sites was the small proportion (<20 %) of pre-incubation labile N present in inorganic N (NO3-N + NH4-N) form (Table 2.5). Among peat amendments, NH_4-N concentrations were significantly lower for the Mesic 1 and 2 than the Mesic 3 and Fibric amendments. NO_3-N and NH_4-N concentrations were combined and converted into a percent of total labile N concentrations (Figure 2.2). Materials from the Natural site and the Mesic 3 amendment typically had a higher percentage of NO_3-N + NH_4-N concentrations as compared to the other reclaimed treatments, although an opposite trend was seen following the spring incubation (as measured in June 2005) when material from the Natural site showed a lower value than all reclaimed treatments. On the other hand, higher values for the Mesic 3 amendment hold true for all incubation periods (fall $p = <0.0001$, winter $p = 0.002$, spring $p = <0.0001$), and pre-incubation NO3-N concentrations were also significantly higher for this amendment as compared to all other peat materials (Table 2.5).

To further compare N availability among peat materials, net ammonification, nitrification and mineralization rates were reported on a total-N basis (i.e., $\mu g\ g^{-1}$ total $Nday^{-1}$) as recommended by Lapointe et al. (2005). Differences in net N mineralization rates among materials were in large part determined by differences in net nitrification rates (Figure 2.3). Net ammonification rates showed smaller fluctuations and fewer differences among peat materials than net nitrification rates. Material from the Mesic 3 had significantly higher net nitrification and mineralization rates than all other sites in the fall. Material from the Humic/mesic amendment had a significantly higher net mineralization rate than all other reclaimed materials during summer ($p = 0.001$ Humic/mesic vs. Mesic 1; $p = 0.004$ Humic/mesic vs. Fibric), the second highest rate in the fall ($p = 0.017$ Humic/mesic vs. Mesic 1; $p = 0.010$ Humic/mesic vs. Mesic 2; $p = 0.005$ Humic/mesic vs. Fibric), but a significantly lower rate during winter ($p = 0.020$ Humic/mesic vs. Mesic 1; $p = 0.011$ Humic/mesic vs. Fibric). Similarly, net nitrification rates for the Humic/mesic amendment were significantly higher in the summer ($p = <0.001$ Humic/mesic vs. Mesic 1; $p = <0.001$ Humic/mesic vs. Fibric; $p = 0.017$

Humic/mesic vs. Natural), and the second highest in the fall ($p = 0.009$ Humic/mesic vs. Mesic 1; $p = 0.007$ Humic/mesic vs. Mesic 2; $p = 0.003$ Humic/mesic vs. Fibric), but significantly lower in the winter ($p = 0.013$ Humic/mesic vs. Mesic 1; $p = 0.049$ Humic/mesic vs. Mesic 2; $p = 0.022$ Humic/mesic vs. Fibric).

Pre-incubation and post incubation moisture contents indicated higher values for the Natural site than for the reclaimed peat materials at all times (Table 2.5 and Figure 2.4). Material from the Fibric site also had a tendency to be wetter than the other sites with significantly higher values following the winter and spring incubations.

2.3.2 Seasonal Variability

Labile N concentrations showed consistent seasonal N variations for all reclaimed peat materials, with the highest values typically seen following the fall incubations in November 2004 (Figure 2.1). These results for fall were significantly higher than summer values at the Humic/mesic ($p = 0.002$), Mesic 1 ($p = 0.0045$), Mesic 3 ($p = 0.026$), and Fibric sites ($p = 0.006$). Fall labile N concentrations also were higher than the winter and spring concentrations, and significantly so for the Mesic 1 and Mesic 2 peat materials (Figure 2.1). In contrast, there were no significant differences among seasons for the material from the Natural site.

Similarly to what was observed for labile N concentrations, net mineralization and nitrification rates were higher in the fall than the other seasons in the materials from the Humic/mesic, Mesic 1, Mesic 3, and Natural sites (Figure 2.3 a, b). In contrast, the Fibric site had significantly lower net mineralization and nitrification rates during the fall incubations when compared to the summer and winter periods. Again, with the exception of the Fibric peat material, no distinct seasonal patterns existed in net ammonification rates as these values tended to hover around zero (Figure 2.3 c). Finally, the pattern that emerged for NO_3-N + NH_4-N concentrations (as a percent of total labile N concentration) was higher values during the non-growing season, i.e. following either the fall or winter incubations as compared to summer and spring values (Figure 2.2). The Humic/mesic and Mesic 3 materials showed higher values in the fall, while materials from the Mesic 1, Mesic 2, Fibric and Natural sites had higher values in the winter. Moisture contents in the Mesic 1, Mesic 2, Fibric and Natural sites also were significantly higher following the

winter incubation than in spring, although no other consistent seasonal patterns were observed (Figure 2.4).

2.3.3 Climate Data

Comparing monthly air temperature averages for the Fort McMurray Airport during our experimental period (November 2003 – June 2005) to the Canadian climate normals for the airport (1971-2000) revealed that monthly air temperatures were typically within 2°C from the monthly normal temperatures, with temperatures being both higher and lower than normal (Table 2.6). However, two months were considerably warmer (December 2003 was 4.4°C warmer and February 2004 was 3.1°C warmer). The largest difference in air temperatures occurred in May 2004 where the Fort McMurray temperature was 4.7°C below normal; this month was the beginning of the incubation period for our experiment. The study period was broken down into overwinter and growth season averages (Tables 2.6 and 2.7). Average temperature for the four periods were within 1°C of the normals. In 3 of the 4 periods, the temperatures during the experimental period at the Fort McMurray airport were higher than normal. The average site values for monthly air temperatures were similar to those for the airport data at the same period: all monthly temperatures were within 2°C of the Fort McMurray values. Air temperatures were both higher and lower. For all four periods, the experimental sites were approximately 1°C warmer than the Fort McMurray airport.

Monthly precipitation was below normal at the airport for the study period compared to the normals (Table 2.7). The overwinter period for November 2003 to April 2004 received 10.5 mm (9 %) more precipitation than normal. The 2004 growing season at Fort McMurray received 192.1 mm precipitation, which is 150 mm (44 %) lower than the normal for that period. The precipitation for Fort McMurray for the overwinter period from November 2004 to April 2005 was 99.0 mm, or 14.6 mm (13%) below normal. Lastly, May and June 2005 received 83.5 mm, 28.2 mm (25 %) below normal. Precipitation at the experimental sites was also lower in 2 periods than that at the airport. The November 2003 to April 2004 overwinter period was considerably lower at the experimental sites, however 21.6 mm may not be an accurate estimate of precipitation as the meteorological station had just been installed at the Mesic 1 site and Albian Sands

staff who recorded the data stated that early values collected in this period may not have been accurate. The 2004 growing season for the study sites received 138.6 mm of precipitation, 53.5 mm (28 %) below that at the Fort McMurray station for the same period. The November 2004 to April 2005 overwinter period had precipitation within 1 mm of that at the Fort McMurray airport station. Lastly, the study sites received 20.4 mm (24 %) more precipitation during May and June 2005 than the Fort McMurray airport. Precipitation varied among experimental sites (Table B.4). In particular, the Mesic 1 site received at least 140 mm less precipitation than the other sites did during the 2004 growing season.

2.4 DISCUSSION

2.4.1 The Effects of Reclamation on Soil Properties

A variety of studies have examined N mineralization in boreal forest ecosystems even though few of these studies have actually used *in situ* incubation experiments (Aerts et al., 1999; Cote et al., 2000; Lindo and Visser, 2003). Boreal forest systems are generally regarded as being N limited, and low net N mineralization rates have been linked to high immobilization of N by microorganisms rather than to low release (i.e., gross mineralization) rates (Carmosini et al., 2003; McMillan, 2005; Jerabkova et al., 2006). Net N mineralization rates as reported in the literature range from -0.43 to 3.58 $\mu g\ N\ g^{-1}\ day^{-1}$ (Table 2.1). Results of this study are at the low range of the spectrum. The approximate average net N mineralization rate at our reclaimed sites is 0.035 $\mu g\ N\ g^{-1}\ day^{-1}$, and the highest rate as measured for the Mesic 3 amendment in the fall corresponded to 0.74 $\mu g\ N\ g^{-1}\ day^{-1}$.

Salvaging organic material for reclamation purposes causes serious soil disturbance. Disturbance of the soil causes dramatic changes in the taxonomic and functional diversity of the microbial community (Buckley and Schmidt, 2001; DeGrood et al., 2004). Tan et al. (2005) found that soil disturbance (soil compaction from forestry) inhibited the activities of aerobic bacteria. The reduction or lack of forest canopy has also been found to reduce microbial biomass in both partial and clear-cut sites (Lindo and Visser, 2003). Microbial communities mediate nutrient cycling (DeGrood et al., 2004),

thus, a disturbed community may not function the same as a natural community. The effects of disturbance may vary depending on the type of disturbance and the material. However, lower net mineralization rates in our reclaimed sites in comparison to those in the undisturbed sites found in the literature may be attributed to disturbance (Table 2.1). Table 2.5 indicates a variety of chemical parameters that differ among the reclaimed and natural sites in this study.

A strong similarity among peat materials was that the ammonification rates did not contribute much to net N mineralization rates. The patterns found in net N mineralization were in large determined by the net nitrification rates. This pattern was consistent among all sites studied which emphasizes that in these peat amendments ammonium is a very transient form of N and moves through the cycle quickly to become nitrate. This result is not typical to what is reported in the literature for undisturbed boreal soils (Carmosini et al., 2003; Jerabkova et al., 2006; McMillan, 2005; Westbrook and Devito, 2004). Van Cleve and Alexander (1981) stated that nitrate is rarely measured in significant concentrations in the soil or in soil water of the boreal forests. A comparison of net nitrification and net mineralization rates in table 2.1 shows that nitrification accounts for less than 55 % of net mineralization rates in all the studies listed above. Therefore, ammonification is typically driving mineralization in undisturbed boreal forests. However, there is evidence that disturbance to the forest canopy in boreal ecosystems can increase the importance of net nitrification relative to mineralization. Lindo and Visser (2003) observed an increase in nitrification in disturbed sites following clear cut harvesting. Pedersen et al. (1999) found the importance of net nitrification relative to mineralization greatly increases following clear-cutting in upland forest soils. They also reported a progressive reduction in NH_4-N from uncut to partial-cut to clear-cut sites in both coniferous and deciduous forest stand types. Research on peat amendments being used to re-create boreal forest ecology is limited, but these results appear to be consistent with other types of boreal forest disturbance.

2.4.2 Characteristics of Peat Materials

Results for net mineralization and nitrification rates depict a separation among the peat materials, with materials from the Humic/mesic and the Mesic 3 sites showing

stronger N fluctuations than the other materials and higher available N. Soil characteristics were analyzed using the May 2004 baseline results. Many significant differences occurred among sites, however, this experiment was not designed to make quantitative comparisons regarding these differences. An exception was the Fibric and Mesic 3 sites, where the main difference in amendments at their construction was peat type. By comparing these two sites, we may get a general idea of the differences between fibric and mesic peat types. The Fibric site differed significantly in all aspects of soil characteristics from the Mesic 3 material except for soil moisture (Tables 2.4 and 2.5). In general, the Fibric site showed soil characteristics that were distinct from those at all other sites. The literature provides evidence that the acidic nature of the Fibric material could contribute to its low net mineralization rates. Some researchers have concluded that NO_3^- production is prevented in peatland soils with low pH (Chapin, 1996; Nieminen 1998). Ste-Marie and Pare (1999) reported that forest floor pH appeared to be an important control over net nitrification. In their study, Jack pine forest floor had the lowest pH and lower net nitrification than aspen forest floor. They also found that, in general, forest floors that had low pH also had low net mineralization rates. Myrold (2005) also showed that nitrification was typically inhibited at low pH. Thus, there is evidence that in both upland and peatland ecosystems, acidic conditions may reduce net nitrification rates.

Typically, in pre-incubation materials, MBN corresponded to the highest portion of the labile N pool, whereas nitrate and ammonium comprised the smallest portion of the pool. Microbial biomass mediates nutrient cycling (Vestal and White, 1989) and regulates the transformation and storage of nutrients (Horwath and Paul, 1999). Therefore, lower proportions of MBN could potentially contribute to the lower nitrification and mineralization rates observed for peat materials from the Mesic 1 and Mesic 2 sites. It has been suggested that higher microbial N concentrations are an indicator of higher N availability (Myrold, 1987; Myrold et al., 1989; Wardle, 1992).

2.4.3 Differences among Seasons

Research on peat prescriptions being used in the oil sands area is limited. McMillan (2005) conducted research regarding N mineralization in our study area but did

not analyze seasonal N patterns. There is a variety of literature on mineralization in tundra soils that indicates that net N mineralization reaches minimum values after the growing season, and not during (Chapin et al., 1988; Kielland, 1990; Jonasson et al., 1999; Schmidt et al., 1999; Schimel et al., 2004). In contrast, our study found that net nitrification and mineralization rates tended to be significantly higher during the fall season, indicating that mineralization was not the strongest during the growing season. Lapointe et al. (2005) found that N mineralization was partly driven by the timing of senescence and the chemical quality of the litter type. Their study was located in natural boreal forest stands and their results showed that aspen stands had greater potential net mineralization of N in the spring than in the fall. Although the specific timing of senescence is not known for our study sites, it is interesting to note that a seasonal change from summer to fall may influence mineralization. In terms of the balance between N mineralization and immobilization, mineralization typically dominated the N cycle as evidenced by the majority of positive results for measured net mineralization rates.

There was a shift to stronger immobilization in the winter season when net mineralization values were closer to zero or even negative. While there is growing evidence that soil microorganisms can contribute to measurable N mineralization and nitrification during the winter season (Carmosini et al., 2003), there is also evidence that low temperatures reduce nitrification and mineralization in the soil. Van Cleve et al. (1993) found minimal mineralization rates during the winter seasons, and Myrold (2005) found that low temperatures limited nitrification. Temperature influences total microbial biomass and microbial activity within the soil (Campbell et al. 1973). Increasing soil temperature increases the decomposition rate of soil organic matter, which influences the mineralization rate (Bonan and Van Cleve, 1991). The overall microbial community responsible for nitrogen mineralization is most active between 0 and 35°C (Stanford et al., 1973). Our study also revealed an increase in the proportions of inorganic N in the non-growth seasons (fall and winter) to well above the 15 % seen in the pre-incubation analysis. This seasonal shift in the proportion of inorganic N was observed in all materials. The shift to decreased mineralization could be related to below freezing temperatures, and may provide a possible explanation for decreased mineralization in the winter season.

Net N mineralization rates have been found to be positively correlated to moisture content (Kowalenko and Cameron, 1976; Stottlemyer and Toczydlowski, 1999; McMillan 2005). McMillan (2005) studied reclaimed sites in the oil sands within close proximity to some of our study sites. Therefore, precipitation values that vary from normals for the region have the potential to impact the N mineralization rates at our study sites. One might expect higher mineralization rates in a year with more normal precipitation. An accurate estimation of precipitation at the experimental sites may not have been obtained for the overwinter period from November 2003 to April 2004. However, had they behaved similar to the Fort McMurray station, the sites would have had increased soil moisture at the beginning of the 2004 growing season (May). Less precipitation than normal for the region occurred during May 2004 to June 2005, both at the Fort McMurray airport and at the study sites, especially for the 2004 growth season. This likely reduced the net N mineralization rates that were measured during this period.

2.5 SUMMARY

A strong pattern that occurred for all materials was that net ammonification rates were exceeded by net nitrification rates. This leads to the conclusion that ammonium may have been a transient form of nitrogen, quickly shifting to nitrate, which consequentially drove N mineralization. The pattern of nitrification influencing mineralization rates is more consistent with literature from disturbed sites. Thus, reclamation may increase the importance of nitrification relative to mineralization. Furthermore, it appears that reclamation may also reduce net N mineralization rates in comparison to undisturbed sites.

Materials from the Mesic 3 and Humic/mesic sites had the strongest seasonal fluctuations in N mineralization and the highest levels of N mineralization compared to the other reclaimed sites. Although there are a variety of other potential reasons for increased mineralization not explored by this study, there was evidence of a few possibilities specific to the peat material in question. The acidic nature of the fibric peat material may have inhibited nitrification rates, leading to reduced N mineralization. For

the Mesic 1 and Mesic 2 peat amendments, a reduced proportion of MBN appeared to be influencing lower net mineralization rates.

In terms of seasonal N variability, the N mineralization was the strongest during the fall season after the growing season but prior to the below freezing temperatures of the winter season. Low temperatures in the winter season may have resulted in the low to negative N mineralization rates.

Lastly, the incubation period for this study received lower than normal precipitation for the region as reported at the Fort McMurray airport. Our study sites experienced similar reduced precipitation. This has the potential to decrease net N mineralization rates and as such the rates reported in this study may be lower than those in years that receive normal precipitation.

Table 2.1: Examples of net nitrification and mineralization rates in boreal forest floors as reported in the literature.

Source	Location	Plants	Incubation Period	Net Nitrification $\mu g\ N\ g^{-1}\ day^{-1}$	Net Mineralization $\mu g\ N\ g^{-1}\ day^{-1}$
Westbrook and Devito (2004)	Northwestern Ontario	Jack pine and black spruce	August (24 days)	-0.01	0.19
McMillan (2005)	Northeastern Alberta	Aspen	May	-0.03	0.46
			June	0.03	1.18
			July (30 days)	0.03	0.31
Jerabkova et al. (2006)	Northwestern Alberta	Mixed (aspen and white spruce)	June to August (6 weeks)	1.04	3.58
		White spruce		0.12	0.92
		Aspen		0.98	1.81
Carmosini et al. (2003)	Northwestern Alberta	Aspen	May to October (20 weeks)	0.28	3.51
Brais et al. (2002)	Northwestern Quebec	White spruce, balsam fir, and white birch	June to July (6 weeks)	Not reported	-0.43
			August to June (40 weeks)	Not reported	0.09
			June to October (18 weeks)	Not reported	1.93
Ste-Marie and Houle (2006)	Quebec	Black spruce	September to October (40 days)	0.06	-0.03
Tan et al. (2005)	Northern British Columbia	Aspen	May	0.4 – 1.0	1.5
			July (30 days)	-0.03	-0.40

Table 2.2: Selected characteristics of the five reclaimed study sites

Site	Reclamation Date (Year)	Aspect	Revegetation Practices	GPS Location
Humic/ Mesic	1988	S	Initial barley nurse crop (1997) then hybrid poplar and jack pine	N 56°58. W 111°30.
Mesic 1	2004	NE	None	N 56°16. W 111°28.
Mesic 2	2003	W	Initial barley nurse crop (2003)	N 56°55. W 111°24.
Mesic 3	2000	N	Initial barley nurse crop (2000) then white spruce and aspen poplar	N 56°59. W 111°37.
Fibric	2002	N	None	N 56°59. W 111°38.

Table 2.3: Climactic conditions at the Mesic 1 site during the field incubation experiment.

	Summer	**Fall**	**Winter**	**Spring**
Incubation period	May 25-Aug. 31, 2004	Aug. 31-Nov. 14, 2004	Nov. 14 2004-April 17, 2005	April 17-June 19, 2005
Incubation length (days)	99	75	155	63
Monthly Average Air Temperature (°C)	13.5	1.9	-13.0	10.4
Total Seasonal Precipitation (mm)	18.7	63.2	31.8	145.0

Table 2.4: Selected physical and chemical characteristics[a] of the peat materials. Values are averages with standard deviations indicated in parentheses, and different letters indicate significant differences among peat materials at $\alpha = 0.05$.

Site	Bulk Density Mg m^{-3}	pH	Total N %	Total C %	C:N	Moisture content (%)
Humic/ mesic	0.91 (0.13) a	7.3 (0.06) a	0.16 (0.03) c	3.07 (0.37) b	19.80 (1.44) c	31.0 (17.6) bc
Mesic 1	0.78 (0.10) a	7.5 (0.04) a	0.37 (0.04) bc	7.07 (0.49) b	19.09 (1.54) c	24.8 (19.2) bc
Mesic 2	0.74 (0.16) a	7.2 (0.06) a	0.23 (0.02) c	5.93 (0.83) b	25.26 (1.52) b	15.4 (0.69) c
Mesic 3	0.71 (0.14) a	7.0 (0.16) a	0.23 (0.19) c	6.30 (5.68) b	26.31 (2.59) b	41.3 (11.6) bc
Fibric	0.30 (0.11) b	4.1 (0.32) b	0.56 (0.08) b	19.08 (1.97) a	34.38 (3.09) a	133.1 (9.57) b
Natural	0.16 (0.06) b	7.2 (0.07) a	1.21 (0.33) a	24.46 (5.31) a	20.53 (2.18) c	501.5 (135.2) a

a: as determined in May 2004, with the exception of bulk density that was measured in August 2004

Table 2.5: Concentrations of different forms of N and C in the peat materials as determined in May 2004. Values are averages with standard deviations indicated in parentheses, and different letters indicate significant differences among peat materials at $\alpha = 0.05$.

	DOC	MBC	Labile N[a]	DON	MBN	NO3-N	NH4-N
	µg-C g^{-1} soil	µg-C g^{-1} soil	µg-N g^{-1} soil	µg-N g^{-1} soil	µg-N g^{-1} soil	µg-N g^{-1} soil	µg -N g^{-1} soil
Humic/mesic	218 (26) **b**	111 (43) **b**	37.8 (14.3) **ab**	11.0 (2.8) **ab**	23.9 (11.8) **a**	1.6 (0.3) **b**	1.4 (0.3) **b**
Mesic 1	273 (11) **b**	36 (24) **b**	17.4 (3.5) **b**	9.9 (1.0) **b**	5.3 (3.5) **b**	1.3 (0.1) **b**	0.9 (0.1) **b**
Mesic 2	158 (9) **b**	73 (13) **b**	17.3 (2.9) **b**	7.7 (1.9) **b**	7.3 (3.4) **b**	1.4 (0.4) **b**	0.8 (0.1) **b**
Mesic 3	145 (22) **b**	146 (54) **ab**	51.2 (20.7) **a**	11.4 (4.9) **ab**	33.3 (14.3) **a**	4.7 (2.6) **a**	1.9 (0.8) **b**
Fibric	221 (27) **b**	189 (34) **ab**	39.9 (3.6) **ab**	9.1 (2.0) **b**	26.2 (4.1) **a**	1.5 (0.3) **b**	2.4 (0.7) **ab**
Natural	484 (194) **a**	338 (283) **a**	68.8 (18.2) **a**	16.3 (4.7) **a**	42.7 (18.7) **a**	2.0 (0.7) **b**	5.4 (3.6) **a**

[a]Labile N = N03-N + NH4-N + DON + MBN

Table 2.6: Comparison of air temperature for Fort McMurray from Environment Canada and the average Site Values for Mesic 1, 2, 3, and the Humic/mesic sites. Shaded cells are estimated values.

Date	**Fort McMurray Airport Normals** Average Air Temperature (˚C)	**Fort McMurray Airport** Average Air Temperature (˚C)	**Average Site Values** Average Air Temperature (˚C)	**Difference (Fort McMurray - Fort McMurray Normals**	**Difference (Site Values - Fort McMurray)**
Nov-03	-8.5	-9.4	-7.8	-0.9	1.6
Dec-03	-16.5	-12.1	-10.9	4.4	1.2
Jan-04	-18.8	-21.4	-21.0	-2.6	0.4
Feb-04	-13.7	-10.6	-10.5	3.1	0.1
Mar-04	-6.5	-6.5	-5.4	0	1.1
Apr-04	3.4	3	3.8	-0.4	0.8
May-04	10.4	5.7	6.1	-4.7	0.4
Jun-04	14.7	13.3	14.7	-1.4	1.4
Jul-04	16.8	18	20.1	1.2	2.1
Aug-04	15.3	12.7	14.3	-2.6	1.6
Sep-04	9.4	7.9	9.1	-1.5	1.2
Oct-04	2.8	1.1	2.0	-1.7	0.9
Nov-04	-8.5	-5.8	-4.2	2.7	1.6
Dec-04	-16.5	-18.5	-17.6	-2	0.9
Jan-05	-18.8	-19.1	-19.0	-0.3	0.1
Feb-05	-13.7	-11.6	-10.5	2.1	1.1
Mar-05	-6.5	-4.9	-4.0	1.6	0.9
Apr-05	3.4	5.3	6.3	1.9	1.0
May-05	10.4	10	10.8	-0.4	0.8
Jun-05	14.7	13.8	15.0	-0.9	1.2
Overwinter (Nov 03 - Apr 04)	-10.1	-9.5	-8.6		
Growing Season (May 04 - Oct 04)	11.6	9.8	11.1		
Overwinter (Nov 04 - Apr 05)	-10.1	-9.1	-8.1		
Growing Season (May 05 - June 05)	12.6	11.9	12.9		

Table 2.7: Comparison of precipitation for Fort McMurray from Environment Canada and the average Site Values for Mesic 1, 2, 3, and the Humic/mesic sites. Shaded cells are estimated values.

Date	**Fort McMurray Airport Normals** Monthly Total Precipitation (mm)	**Fort McMurray Airport** Monthly Total Precipitation (mm)	**Average Site Values** Monthly Total Precipitation (mm)	**Difference (Fort McMurray - Fort McMurray Normals**	**Difference (Site Values - Fort McMurray)**
Nov-03	22.2	12.9	7.1	-9.3	-5.8
Dec-03	19.3	18	0.1	-1.3	-17.9
Jan-04	19.3	38.3	1.6	19.0	-36.8
Feb-04	15.0	16.9	4.0	1.9	-13.0
Mar-04	16.1	15	6.6	-1.1	-8.4
Apr-04	21.7	23	2.3	1.3	-20.7
May-04	36.9	54.6	27.3	17.7	-27.3
Jun-04	74.8	16.0	4.5	-58.8	-11.6
Jul-04	81.3	36.5	33.4	-44.8	-3.1
Aug-04	72.7	17.0	14.0	-55.7	-3.0
Sep-04	46.8	56.0	46.3	9.2	-9.7
Oct-04	29.6	12.0	13.1	-17.6	1.1
Nov-04	22.2	15.0	17.0	-7.2	2.0
Dec-04	19.3	27.0	18.2	7.7	-8.8
Jan-05	19.3	14.5	14.2	-4.8	-0.3
Feb-05	15.0	11.0	11.1	-4.0	0.1
Mar-05	16.1	15.0	11.0	-1.1	-4.0
Apr-05	21.7	16.5	28.1	-5.2	11.6
May-05	36.9	22.5	39.0	-14.4	16.5
Jun-05	74.8	61.0	64.9	-13.8	3.9
Overwinter (Nov 03 - Apr 04)	113.6	124.1	21.6		
Growing Season (May 04 - Oct 04)	342.1	192.1	138.6		
Overwinter (Nov 04 - Apr 05)	113.6	99.0	99.6		
Growing Season (May 05 - June 05)	111.7	83.5	103.9		

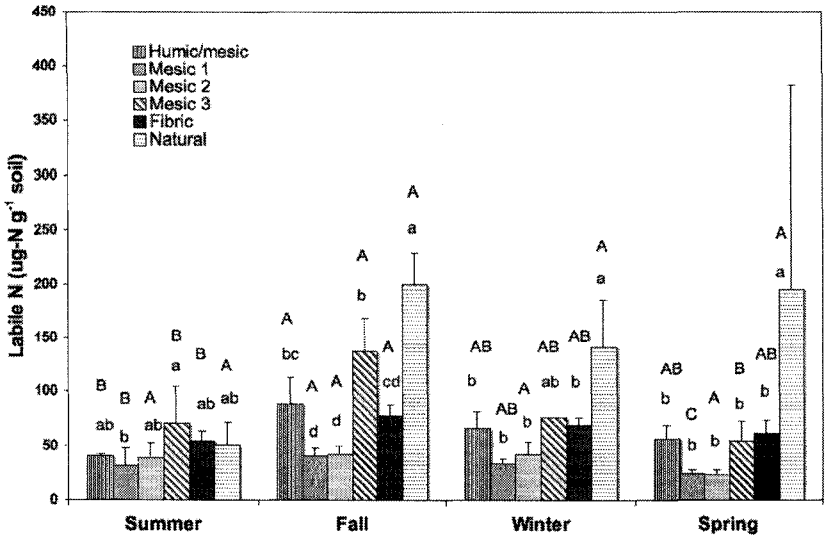

Figure 2.1: Labile N concentrations (μg-N g^{-1} soil). Sampling dates: Summer (August 2004 baseline), Fall incubation (Nov. 2004), Winter incubation (April 2005), Spring incubation (June 2005). Error bars represent 1 standard deviation from the mean (n=5). Lowercase letters indicate significant differences among peat materials within each season, while capital letters indicate significant differences among seasons within each material (α=0.05).

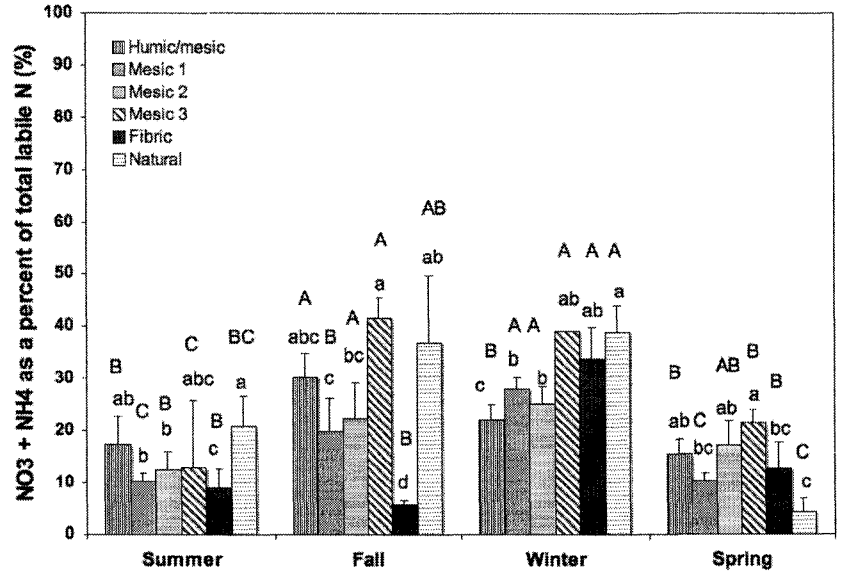

Figure 2.2: $N\text{-}NO_3$ + $N\text{-}NH_4$ concentrations (as % of total labile N). Sampling dates: Summer (August 2004 baseline), Fall incubation (Nov. 2004), Winter incubation (April 2005), Spring incubation (June 2005). Error bars represent 1 standard deviation from the mean (n=5). Lowercase letters indicate significant differences among peat materials within each season, while capital letters indicate significant differences among seasons within each material (α=0.05).

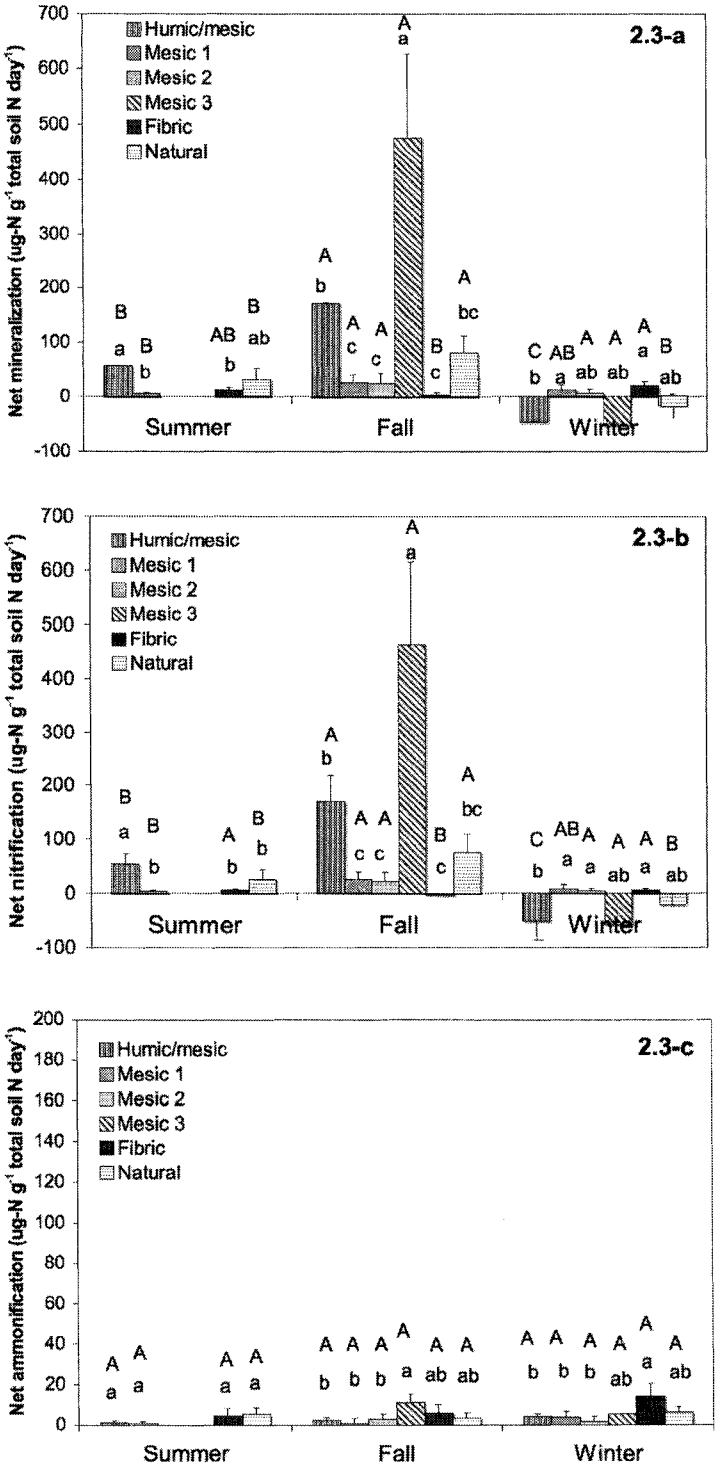

Figure 2.3: Net N a) mineralization, b) nitrification, and c) ammonification rates (μg-N g^{-1} total soil N day^{-1}). Error bars represent 1 standard deviation from the mean (n=5). Lowercase letters indicate significant differences among peat materials within each season, while capital letters indicate significant differences among seasons within each material (α=0.05).

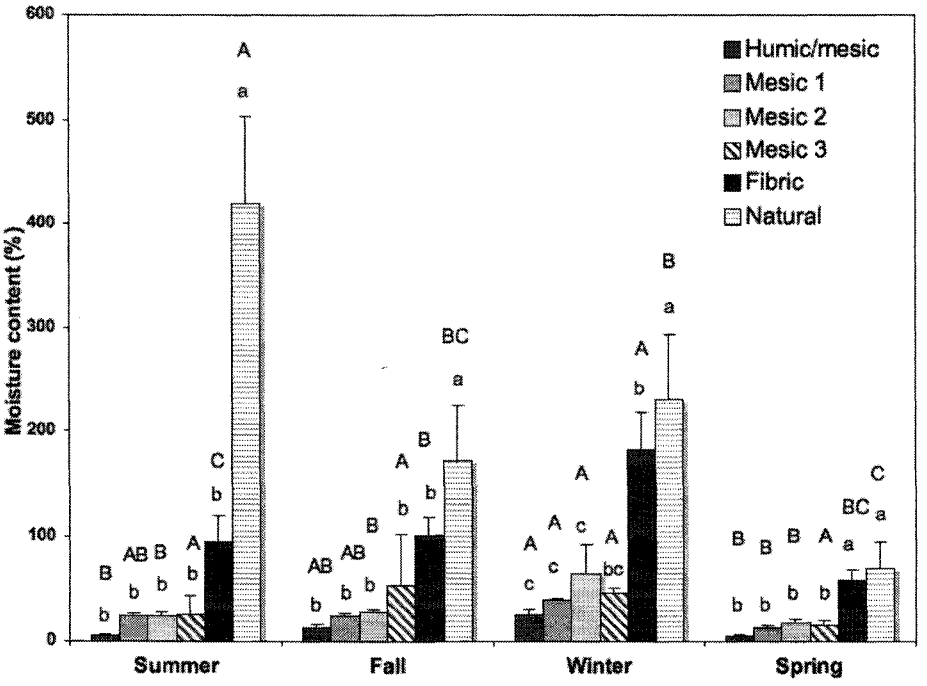

Figure 2.4: Soil gravimetric moisture content (g/g*100). Sampling dates: Summer (August 2004 baseline), Fall incubation (Nov. 2004), Winter incubation (April 2005), Spring incubation (June 2005). Error bars represent 1 standard deviation from the mean (n=5). Lowercase letters indicate significant differences among peat materials within each season, while capital letters indicate significant differences among seasons within each material (α=0.05).

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Chapter 3: PLANT PRODUCTIVITY OF AND SOIL MICROBIAL COMMUNITIES IN BOREAL OIL SANDS RECLAIMED SITES

3.1 INTRODUCTION

Abiotic factors, such as light and nutrient levels, determine plant species dominance to a great extent (Tilman, 1982). Soil microorganisms are also required by vegetation. Microbially mediated processes in soils affect ecosystem function in a variety of ways, for instance by altering soil organic matter turnover and nutrient cycling patterns (Vestal and White, 1989; Blair et al., 1990; Horwath and Paul, 1994; Couteaux et al., 1995). De Deyn et al. (2004) indicated that soil biota affect plant biomass production, plant species assemblages, and plant succession. Microbial communities aid in other ecosystem processes through their symbiotic associations with plants. In areas where soil conditions for plant growth are marginal, soil microorganisms are critical for supporting plant growth and revegetation success (DeGrood et al., 2004).

Soil disturbance dramatically changes the taxonomic and functional diversity of the microbial community (Buckley and Schmidt, 2001; Chow et al., 2003) and the impact of disturbance on microbial communities can be long lived (Mummey et al., 2002). The loss of topsoil results in a decrease in microbial biomass and alters microbial community composition, so much so that the microbial community will only recover following topsoil replacement (De Grood et al., 2004). Often the material used for organic amendments is transferred from other ecosystems and must function under different conditions, stressing newly reclaimed sites. For example, in oil sands reclamation, the peat material is transferred from lowland areas, often saturated with water, to drier upland slopes. Prescott (2002), comparing upland boreal sites, found that the amount and composition of leaf litter produced largely determines the composition of soil microbial and faunal communities, the amount of nutrients recycled, and the resulting availability of nutrients. As plant species in the parent peatlands are different than those that will develop on the reclaimed oil sands soils, it is hypothesized that these differences will have distinct effects on soil microbial communities. Because there is limited research on the microbial communities of oil sands reclaimed soils, there is a need for research in this area.

Succession refers to the more-or-less predictable changes in composition and abundance in an ecological community following disturbance of a site (McCook, 1994). Primary succession is defined as vegetation establishment on previously unvegetated terrain (Finegan, 1984). In contrast, secondary succession occurs in disturbed areas that have remnants of previous vegetation. Areas subject to primary succession must rely on colonizing plants, whereas areas undergoing secondary succession rely on existing viable seeds and vegetative plant parts (MacKenzie, 2006). Large disturbances created by mining operations are especially challenging to reclaim, and must utilize every resource that is available to facilitate successful re-colonization of plants, including seeds in the donor soil or organic amendments. The use of the seed and propagule bank found in soils enhances native vegetation establishment (Skousen et al. 1990; Standen and Owen 1999; Rokich et al. 2000; Zhang et al. 2001). MacKenzie (2006), conducting research in the oil sands, found that most species within the seed and propagule bank from peat donor soil were hydrophilic species, which will be less likely to establish on upland reclaimed sites. The results suggested that invading wind-dispersed species would tolerate the drier soil conditions; characteristic of reclaimed sites, and may dominate the site until more competitive species can establish.

Few studies have directly tracked the post-disturbance development of boreal forest stands in Western Canada (Strong, 2004). Studies tracking boreal forest succession are typically post clear-cut or post fire-disturbance (Oliver, 1980; Paré et al., 1993; Clark et al., 2003; Haeussier and Bergeron, 2004; Lee, 2004). This study assesses post-disturbance development following reclamation of oil sands sites in the boreal forest. The objectives of this study were to characterize potential plant productivity, plant communities, and soil microbial community structure, in various peat-mineral amendments used in oil sands reclamation.

3.2 MATERIALS AND METHODS

3.2.1 Study Sites

Five reclaimed sites were chosen to include representative reclamation practices at each of the mining operations. The time since reclamation ranges from 1988 to 2004, and slope aspects varied. An undisturbed (natural) sedge fen was selected as a reference. Both bogs and fens are common peatlands in the area used for peat salvage, the natural fen was chosen because of its accessibility. Reclamation at the Mesic 1 site, located within the Albian Sands Energy Inc. Muskeg River Mine consists of a peat mix (20 cm) with a sandy loam texture overlying 50 to 60 cm of tailings sand over lean oil sands. The Suncor Energy mine site, hosts sites Humic/mesic and Mesic 2. Reclamation at the Humic/mesic site consists of a 20 cm peat mix of a sandy loam texture over 80 cm of tailings sand. At the Mesic 2 site, the peat mix overlies 80 cm of lean oil sands mixed with secondary material and overburden with a clay loam texture. Reclamation is similar at the Mesic 3 and Fibric sites, located on the Mildred Lake Mine site at Syncrude Canada Ltd. Both sites include 20 cm of peat mix capping Cretaceous overburden, however, the peat amendments are of different origins. Revegetation practices have been implemented at the Humic/mesic, Mesic 2, and Mesic 3 sites, while the Mesic 1 and Fibric sites have been left to revegetate naturally. Chapter 2 describes site amendments and the major abiotic factors for each study site. These results, including pH, soil moisture, C:N, and total labile N, are summarized in Table 3.1.

3.2.2 Greenhouse Bioassay

A greenhouse experiment was designed to study the influence of different peat amendments on plant productivity. Plant height and biomass were measured to assess productivity. In late August 2004, approximately 19 L of soil was collected at each site (0-20 cm), from the top left corner of each of the five plots (10 m by 10 m) that had been randomly chosen for the incubation experiments (see chapter 2). Peat material collected from each site was placed in air - tight pails immediately after being collected, then it was transported back to the lab and stored at room temperature until the greenhouse

experiments commenced (a period of 9 months). Although the lengthy storage period may not have been ideal, salvaged peat is often stock piled and stored for lengthy periods before it is used for reclamation. Using a clean shovel, the soil was homogenized in large pails by stirring the peat material for approximately five minutes, and then placed in 4 L pots to approximately 3 cm below the top of the pot. Pots contained drainage holes at the base to allow excess water to drain.

Two greenhouse experiments were conducted: a bluejoint grass (*Calamagrostis canadensis* (Michx.) Beauv.) experiment and a natural seed-bank experiment. The temperature in the greenhouse was kept at 21°C consistently throughout the day and night. A variety of other experiments were being conducted in the same greenhouse, thus we did not have control of the greenhouse temperature. Glass ceilings in the greenhouse allowed for natural light conditions. Soils were watered with deionized water to prevent any additional ions from being added to the pots. Plates were placed under the pots to indicate when the soils were draining. The pots were watered every third day until water ran into the plates. The peat material for the *Calamagrostis canadensis* experiment was watered for two weeks prior to seeding; all species that emerged were removed. Twenty *Calamagrostis canadensis* seeds were planted per pot, using forceps to place the seeds just below the soil surface. After germination, plants were reduced to five per pot by pulling out additional plants, being careful to capture the root. Weekly height measurements were taken by straightening the plant against a ruler and recording the maximum height. Weekly photos were taken to visually monitor the physiological stages of growth. Day 50 data were chosen to represent maximum height data, i.e., the height that was recorded before the grass began to die back. The bioassay was terminated on the 92nd day of the experiment.

For the natural seed-bank experiment, plants were established from seeds and vegetative propagules in the soil. The experiment was conducted under the same greenhouse conditions as the *Calamagrostis canadensis* experiment. It began on May 24, 2005 and was terminated on August 24, 2005 at the same time as the *Calamagrostis canadensis* experiment. Biomass was determined by weighing the whole plant (above and below ground components) on the 92nd day of the bioassay (August 24, 2005). Using a high-pressure hose in a sink in the greenhouse, soil was rinsed from the roots of all

plants in each pot. It is possible that the pressure in the hose may have removed some of the small roots and roots hairs during this process. Samples were oven dried at 65°C for 24 hours, and then biomass was measured (g) by weighing the entire plant (roots and shoots). The dry plant material (roots and shoots combined) were ground to 150 μm using a ball grinder, subsamples of the ground material were analyzed for total C and N using a Carlo Erba NA 1500 Elemental Analyzer (McMillan, 2005). Total N (g) was calculated by multiplying the concentration of N by the biomass of the dried plant tissue.

Data were analyzed using a SAS statistical package, Version 8.01 (SAS Institute Inc. 1999-2000, Cary, NC). A one-way ANOVA PROC GLM was used to assess differences among peat amendments. Data were tested for homogeneity of variance using the hovtest in SAS. A conservative multiple comparison test was used (Tukey LSD) with α=0.05 since data for the natural seedbank bioassay were nonhomogenous, a variety of transformations did not create homogeneity of the variance. Therefore, the nonparametric Wilcoxon Rank Sum Test was performed on this data set using SAS.

3.2.3 Plant Measurements:

3.2.3.1 Field Sites and Methods

Plots from the 2004 soil incubation experiments (see Ch. 2) were used for the plant surveys. Quadrats were randomly placed at 3 locations within each of the 5 plots, creating 15 quadrats per site. Percent cover was estimated using a 0.25 m^2 quadrat (0.5 m x 0.5 m), with an internal grid (10 cm x 10 cm) to increase accuracy of cover estimations. The results were pooled resulting in one value per plot (n=5 per site). The Natural site was not surveyed, as wetland plant communities are distinct from upland plant communities.

Understory surveys were conducted in accordance with industrial methods employed by Suncor Energy (Amec, 2003). As opposed to using a rank system such as the Braun-Blanquet scale, the actual percent cover was recorded for higher accuracy, and then rounded to the nearest 5 (i.e., 5 %, 10 %, 15 %, etc.). Average percent plant living cover (by individual species), dead plant cover/litter, and non-living material (rock, bare

soil, wood) within each quadrat were estimated to equal a total of 100 % cover per quadrat. Litter is defined as all dead plant material that is on the ground, while dead plant matter is defined as all dead plant material that is still standing. Only plants rooted within the quadrat were used to estimate plant cover. However, if a plant was rooted in one grid and overhanging into another, it was counted in each area for the percent it covered in that sampling unit. The tree canopy cover was not assessed as all trees were planted, and industry personnel had already recorded planting intensities and methods.

The statistical procedures from the Braun-Blanquet scale were used. The mean percent cover of each species represented the abundance of that species, and percent frequency refers to the percentage of quadrats in which the species was observed. The prominence value of each species was calculated as the square root of percent cover multiplied by percent frequency (Archibald et al., 1996). The total number of species in the pooled quadrats was combined to determine species richness, and standard deviation was used to show variation of the number of species present in each quadrat.

According to Beckingham and Archibald (1996), ecological classification of boreal forest communities consists of an integrated hierarchical ecological classification (ecosite, ecosite phase, and plant community type). Ecosites are ecological units that develop under similar environmental influences (climate, moisture, and nutrient regime). An ecosite phase is a subdivision of the ecosite based on the dominant species in the canopy. Where a tree canopy may not be present, the tallest structural plant layer with greater than 5 % cover determines the ecosite phase. Ecosite phases may be subdivided into plant community types, which are the lowest taxonomic unit in the classification system. Trees must be greater than 6 m tall to be considered part of the tree canopy. There were not a sufficient number of trees that met these criteria for tree canopy at the study sites. The understory dominated the sites; thus, the lowest level of the classification system (plant community type) was used to characterize plant communities. Characteristic species are plant species that are present with a prominence value of 20 or greater (Beckingham and Archibald, 1996). Hence, the communities were described by the characteristic species in the forb layer. Detailed tables and descriptions of the data collected in the surveys can be found in the appendices. The characteristic species analysis highlights the most prominent species on each site.

Land capabilities were calculated using the Land Capability Classification System for Forest Ecosystems developed by the Cumulative Environmental Management Association (CEMA). There are five classes of land rated according to potential and limitation for productive forest use (CEMA 2006). Classes 1, 2, and 3 are capable of supporting commercial/productive forests, and Classes 4 and 5 being non-commercial/lower productivity forestlands. This system has been specifically calibrated for the use in the Athabasca oil sands region only. The physical and chemical soil parameters presented in chapter 2 were used to calculate the reclaimed amendments land capability class. Once the soil capability was calculated, the target ecosites for the reclaimed sites were determined using the Guidelines for Reclamation to Forest Vegetation (OSVRC 1998). This provided a method of comparison among sites because the different physical and chemical characteristics (such as aspect, capping depth, nutrient availability, pH etc.) were used for the soil capability calculations.

The indicator species method, developed by Dufrene and Legendre (1997), was also used; this method combines information on the concentration of species abundance in a particular group and the faithfulness of occurrence of a species in that particular group. Faithfulness indicates how often a species is present in the samples taken in a particular site. A completely faithful species would be present in all samples from a site. Unlike the characteristic species approach, a perfect indicator of a particular group should always be present and it should also be exclusive to that group (McCune and Grace, 2002). A benefit of this approach is that species that are less abundant on a site can be accounted for.

3.2.3.2 Statistical Methods

The vegetation data were analyzed using a variety of techniques to characterize the plant communities present at each site. Non-metric multidimensional scaling (NMS) was selected as the most appropriate ordination for illustrating coverage patterns and site type relationships in plant species space. In general terms, the closer the sites appear on these ordinations, the closer they are in coverage patterns and plant species composition (CEMA, 2006b). Therefore, sites that are further apart on the ordination can be considered to be more floristically different.

In the first NMS ordinations, plant species percent coverage was grouped into native species and non-native species. Percent coverage of litter and bare ground were included in these ordinations to assess the basic patterns in cover types at each site. Percent cover ordinations were conducted for June, July, and August. Another ordination was conducted using species prominence data for each site to assess site type relationships in plant species space. For this ordination, replicates for each site were the three sampling events (survey data for June, July, and August); therefore, n = 3 for each site.

NMS ordinations were run using PCORD software (version 4 MjM Software Design, Gleneden Deach, OR) (Kruskal, 1964; Mather, 1976). All data were run with a random starting configuration and 40 runs were made with the data. Comparing final stress values among best solutions assessed the dimensionality of the data set. All final solutions contained two dimensions. NMS searches for the best representation of the data and then orders the objects along axes according to their similarities. The Sorensen (Bray-Curtis) distance measure, a normalization method, was used in the analyses (Hannam, 2006). The objective of using this technique is to reduce the data expressing a multi-dimensional relationship into a smaller number of dimensions by extracting the strongest correlation structures in the data (McCune and Mefford, 1999).

Multiple response permutations procedure (MRPP) statistics were used to statistically test distances in the ordination space between points corresponding to the differences in plant communities among sites. The chance-corrected, within-group agreement (A) describes within-group homogeneity. The test statistic (T) describes the separation between groups, and P is the probability (McCune and Grace, 2002).

Indicator species analysis (Dufrene and Legendre 1997) was performed to determine the prominence of individual species within each site using canopy cover as a measure for abundance; analysis was conducted with PC-ORD (McCune and Mefford, 1999). Indicator values corresponding to the combined frequency and relative abundance of each species were obtained for each treatment (MacKenzie, 2006). This index is maximum when all individuals of a species are found in a single group of sites and when the species occurs in all sites of that group; it is a symmetric indicator (Dufrene and

Legendre, 1997). Indicator values (IV) can range from zero (no indication) to 100 (perfect indication). Perfect indication means that presence of a species points to a particular group without error. A Monte Carlo permutation test with 1,000 interactions was used to test significance of the maximum indicator value. Indicator species were analyzed only on the results from the species prominence ordination to describe community types.

3.2.4 Phospholipid fatty acid (PLFA) analysis

Phospholipid fatty acid (PLFA) analysis examines essential membrane components to determine lipid profiles, which can provide insight into the soil microbial community structure (Leckie et al., 2004). The PLFA technique is used to fingerprint the structural composition of soil microorganisms (Fritze et al., 2000; DeGrood et al., 2004; Leckie et al., 2004), and the total number of PLFAs in a sample is used as a proxy indicator of microbial richness (Bradshaw, 1984).

3.2.4.1 Field Methods

Five soil samples were taken from each site at random midslope locations, to a depth of 7 cm, with metal soil cores (7.6 cm diameter) sterilized with ethanol, and stored in whirlpack bags in the freezer until they could be transferred back to the laboratory. They were then stored in a super freezer at -86°C until further analysis. There were two sampling events, the first occurred late June 2005 and early July 2005 and is referred to as the June/July sampling event; the second one occurred in August 2005.

3.2.4.2 Laboratory Methods

The above samples were freeze-dried and polar lipids were extracted using a modified Bligh and Dyer extraction (Frostegård et al., 1991; White and Ringelberg, 1998). The samples were taken from the super freezer, thawed enough to place them in clean glassware, refrozen in the regular freezer, and placed into the freeze drier. This was done in small batches to minimize the time each sample was out of the super freezer and not yet in the freeze drier. Freeze drying works by freezing the material and then

reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to gas (Kennedy and Cabral, 1993). The extracts were prepared by extraction with a single-phase chloroform mixture, lipid fractionation on a SPE Si column (Agilent Technologies, Wilmington, DE), and subjected to mild alkaline methanolysis (Hannam, 2006). The resulting fatty acid methyl esters (FAMEs) were then analyzed using an Agilent 6890 Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with a 25 m Ultra 2 (5 %-phenyl)-methylpolysiloxane column. The carrier gas used was hydrogen. Peaks were identified using bacterial fatty acid standards and MIDI peak identification software (MIDI, Inc., Newark, DE; Hannam, 2006).

Fatty acids were represented by X:YωZ, where X indicates the number of carbon atoms, Y is the number of double bonds, and Z represents the position of the first double bond from the aliphatic (ω) end of the molecule. Fatty acids with less than twenty carbons in chain length were included in calculating total microbial PLFAs (Hannam, 2006). The total number of PLFA at each site was used as an index of microbial richness. PLFA diversity was calculated using the Shannon index, $H' = -\Sigma(p_i \times \log(p_i))$ where H' is species diversity, and p_i is the mole fraction of individual PLFAs (Chipman and Johnson, 2001; Schutter and Dick, 2001; Hannam, 2006). PLFA evenness, a measure of the variability in the abundance of different PLFAs within a sample, was calculated as E = H'/ln(PLFA richness).

3.2.4.3 Statistical Methods

A one-way ANOVA PROC GLM was used to examine patterns in total PLFA numbers (nmol g^{-1}). A conservative multiple comparison test, Tukey, was used as a post-hoc test (α=0.05). Differences were considered statistically significant at $p<0.05$. Data were analyzed using SAS with statistical package version 8.01 (SAS Institute Inc. 1999-2000, Cary, NC).

The different PLFA peaks were treated as different species to compare samples. NMS ordinations were performed (Hannam et al., 2006) using PCORD software (version 4 MjM Software Design, Gleneden Deach, OR) and the MRPP statistical test was used to

test the distances in the ordination space between points corresponding to the differences in microbial communities among sites.

3.3 RESULTS

3.3.1 Greenhouse Bioassay

Results were similar for the plants from the *Calamagrostis canadensis* bioassay and natural seed-bank bioassay (Figures 3.1a and b). Plant material from the Mesic 1 pots had the lowest height and biomass throughout the 3-month duration of the bioassay experiment (Figure 3.1; Figure 3.2, $p = <0.0001$). Plants in pots from the Mesic 3 site had the significantly greatest height and biomass (Figures 3.1 and 3.2). The Natural site performed similar to the Mesic 1 site in both height and biomass (Figures 3.1 and 3.2). Pictures 3.1 to 3.12 provide a visual assessment of the greenhouse bioassays for each peat amendment on the 92nd day of the experiment. These images clearly show the lack of plant growth in the Mesic 1 material and a reduced plant growth in the Natural site material.

The total N (g) from *Calamagrostis canadensis* plant tissues followed the same statistical trends as biomass (Figure 3.3 a). The N concentration in plant tissues from the *Calamagrostis canadensis* experiment for the Mesic 1 site was significantly higher than those for the Mesic 3, Fibric, and Natural sites (Figure 3.3 b).

3.3.2 Plant Communities

The land capability class for the Mesic 1, Mesic 2, Mesic 3, and Humic/mesic amendments were a class 4 (conditionally productive). The Fibric amendment had a capability of class 5 (non-productive). Class 4 material with a 20 cm peat-mineral mix has target ecosites of g (Labrador tea-subhygric) and h (Labrador tea-horsetail). Class 5 materials have target ecosites l (marsh), j (poor fen), and k (rich fen) (OSVRC 1998). The majority of materials have similar capabilities for plant productivity, and all reclaimed materials can be described as having a low productivity potential.

All descriptive statistics from the plant surveys are given in tables in the appendices grouped by month (June, July, and August) and include species name, average percent coverage, percent frequency, and prominence. Table 3.2 lists the prominence of the characteristic species found at each site in the summer of 2005 (June to August) and indicates the prominence of bare ground, moss, and litter (when present). The Mesic 3 site had the highest number of characteristic species of all sites.

Sites that were further apart on the percent coverage ordinations can be considered to be more different in cover types (native species, non-native species, bare ground, and litter) (Figures 3.4, 3.5, and 3.6). The Mesic 3 and Humic/mesic sites were statistically similar in percent coverage in June and July as indicated by the MRPP statistics (June: $A = 0.05$, $T = -1.01$, $p = 0.1466$; July: $A = 0.05$, $T = -0.87$, $p = 0.1669$). In all sampling events (June, July and August) the sites consistently separated in the following order: Mesic 3, Humic/mesic, Mesic 2, Fibric, and then Mesic 1 (the Mesic 3 site was always the most different from the Mesic 1 site).

The species prominence ordination and the corresponding MRPP statistics indicated that all sites were significantly different from one another in plant species space, with relatively the same p values for all site comparisons (Figure 3.7, Table B.6 of Appendices). Within each site the data points grouped relatively close together. Similar to the percent cover ordinations, the Mesic 1 and Fibric site were the most different from the Mesic 3 site. Noteworthy indicator species from this data set are included in Table 3.2. Site Humic/mesic had twice as many indicator species as the other sites. The majority of indicator species found at all sites were plants that are native to the boreal forest.

3.3.3 PLFA

For the June/July sampling event, the Mesic 1 site had significantly lower microbial richness than all other sites (Figure 3.8), which were statistically similar. The PLFA NMS ordination (Figure 3.9) showed a general trend of each site grouping separately from one another, with the most overlap occurring between the Humic/mesic and Mesic 3 sites in June/July 2005. According to the MRPP analyses for the ordination,

the PLFAs differed significantly amongst all sites in the NMS ordination, with the exception of the Mesic 3 and Humic/mesic sites in June/July (June/July A = 0.065, T = -1.446, p = 0.083). PLFA diversity and evenness results are presented in table B.24 of the appendices

3.4 DISCUSSION

3.4.1 Plant Communities

A number of factors affect the distribution and abundance of plant species, including site conditions (Host and Pregtizer, 1992; Chipman and Johnson, 2001), and time since the last disturbance (De Grandpre et al., 1993; Pare et al; 1993; Halpern and Spies, 1995). Major disturbances to forests are quite common (Oliver, 1980), and a large body of research has been dedicated to the study of their reclamation. A major component of site reclamation is revegetation. *In situ* surveys were used to evaluate revegetation efforts and laboratory bioassays assessed revegetation potential in this study. It should be noted that some creative license was necessary in the interpretation of plant communities data due to the fact that there were a lot of genera in the species lists as not all plants could be identified to the species level.

Significant differences in plant community structure, height, and biomass occurred among sites. However, a comparison of the species lists in the appendices to that of the OSVRC (1998) species lists revealed that on average approximately 75% of vascular understory species cover has been observed on reclaimed oil sands. OSVRC (1998) found that species establishing after oil sand reclamation on sites that were not seeded or only seeded to annual barley were dominated by perennial sow thistle, fireweed, sweet clover and hawksbeard. Therefore, many of the species establishing on the reclaimed sites could be described as typical of early vegetation establishment in oil sands reclamation.

Although the ordinations and indicator species analysis depict each site as having variable plant communities, land capability classifications indicate that the majority of sites have the same potential for forest productivity. As succession continues to progress

on each site, target ecosites of g (Labrador tea-subhygric) and h (Labrador tea-horsetail) can be expected for the majority of sites. Class 4 capability is defined as land having severe limitations, some of which may be surmountable through management, and class 5 land has limitations that appear so severe as to preclude any possibility of successful forest production (CEMA 2006).

Based on the literature, one would expect the productivity of boreal forests to be largely limited by N availability (Mahendrappa and Salonius, 1982; Kaye and Hart, 1997). Ferris (2006) found that increasing soil N significantly impacts plant productivity. Nitrogen is important for developing plant tissues (Gale Group, 2001). During the summer and fall season, the Mesic 3 and the Humic/mesic sites had the highest plant available N of the reclaimed sites (Chapter 2). This result combined with the literature leads to the prediction that the Mesic 3 and Humic/mesic sites would have the greatest plant productivity. Field surveys showed that the Mesic 3 and Humic/mesic sites had a greater number of plant species than other sites. These two sites also showed greater plant biomass in the bioassays, the bluejoint experiment confirms that the increased biomass was not just a function of species composition. Higher N availability likely contributed to the higher plant productivity at these sites.

The Fibric site had reduced species numbers and higher percentages of exposed soil in comparison to other sites (Appendix B). This site had significantly lower net N mineralization rates than the Mesic 3 and Humic/mesic sites (Chapter 2). The fibric peat (being the least decomposed) had significantly lower pH in comparison to the other reclaimed sites. The differences in the soil characteristics at this site gave it a land capability class of 5, or severely limited. The literature provides evidence that the acidic nature of the Fibric material could contribute to its low net mineralization rates (Chapin, 1996; Nieminen 1998; Ste-Marie and Pare, 1999). Furthermore, the low pH may deter certain plants from establishing, and could have caused the low number of species found on site (Lucas et al., 1965). However, the greenhouse experiments provide evidence that certain species can tolerate the conditions of the fibric peat. Given optimal light, moisture, and temperature conditions in the greenhouse, plant height and biomass at the Fibric site were not significantly different from other sites. Furthermore, plant tissues did not have significantly lower amounts of N. It is difficult to predict a target ecosite for

this site because class 5 materials have target ecosites l (marsh), j (poor fen), and k (rich fen) (OSVRC 1998). This site was designed for an upland ecosite and wetland ecosites will not establish because of the topography.

Fireweed is an extremely common pioneer species in the boreal forest (Johnson et al., 1995); thus its prominence at this site is not surprising. Opportunistic species are native colonizers, which tend to establish on and can even dominate disturbed sites. A 20-year study on the recovery of boreal forest by Strong (2004) found that opportunistic species established during the first 2 years after disturbance but disappeared from the developing vegetation by year 10. Lucas et al. (1965) suggested that blueberry (*Vaccinium myrtilloides* Michx.) and certain conifers are adapted to acidic peat. Therefore, if active revegetation efforts were pursued at the Fibric site in the future, acid tolerant species could be planted and may successfully establish. In summation, the acidity of the fibric peat is suspected to have reduced N mineralization in the soils and reduced species richness on site. However, certain species are adapted to these conditions and may still thrive despite the limitations of this peat material.

In the bioassays these experiments produced synchronous results between biomass patterns in both the *Calamagrostis canadensis* and natural seedbank bioassays. This indicated that regardless of the plant species growing, the peat material had a consistent potential for plant productivity. In both the height and biomass assessments, the reclaimed sites performed better than the Natural site, and the plant tissues in the Natural site had low total N.

Optimum soil moisture content is close to field capacity (Stanford and Epstein, 1974; Zaman and Chang, 2004). A moisture content that exceeds this optimal water content will slow decomposition rates by decreasing the aerobic microbial community (Filonov et al., 1999). Perhaps the extremely moist field conditions of the Natural material (described in Chapter 2) decreased the aerobic microbial community.

One would expect to find plant species typical of disturbed boreal sites establishing on the reclaimed amendments. Plant communities as indicated by both the characteristic and indicator species analyses were variable and site specific; however, many of the plant species that were re-establishing were typical early successional

species associated with reclaimed oil sands (OSVRC, 1998). Furthermore, the majority of sites have the same target ecosites based on land capability of the peat materials; therefore, the plant communities at each site were not so unique. In instances where the characteristic species were hydrophilic, like *Equisetum* L. (horsetail), it is unlikely that these species will have much permanence on the drier upland slope (Mackenzie, 2006). Strong (2004) suggests that not all the initial species found on site remain in the evolving mature forest communities. The literature indicates that as forest stands mature, the pattern will shift to one of decreasing plant species diversity (Pitkanen, 1998; Clark et al., 2003).

The Mesic 1 site did not support plant growth in the field or in the bioassays. Not surprisingly, this site was consistently different from all other reclaimed sites. As the Mesic 1 site appears to be unique; it will be discussed separately later in the chapter.

3.4.2 Microbial Communities

Although there is a lack of research on microbial communities in reclaimed oil sands, there have been previous studies on microbial communities in the boreal forest. The literature suggests that boreal vegetation exerts a strong influence on the microbial community structure of forest soils (Saetre and Baath, 2000; White et al., 2005). Carney and Matson (2005) established that plant species diversity influenced soil microbial community structure, and Priha et al. (2001) found that the microbial communities may actually reflect the composition of the understory vegetation. Hannam et al. (2006) suggested that more diverse understory vegetation could potentially be related to greater microbial biomass. Furthermore, Prescott (2002) found a connection between soil microbial communities and leaf litter quality and quantity. Considering the results of the NMS ordinations for plant communities, one might expect to find significant differences among sites in microbial communities. The PLFA NMS analysis revealed significantly different microbial communities, with stronger similarities between the Mesic 3 and Humic/mesic sites both in total numbers of PLFA and community structure. Plant species percent cover results also found statistical similarities between the Mesic 3 and Humic/mesic sites. One could deduce that the plant communities may have influenced

different microbial communities to develop among sites. However, the results of this study cannot explicitly show that vegetation was influencing soil microbial communities and not vice versa.

The composition of the soil microbial community may influence post-disturbance regeneration success because soils with distinct patterns in microbial community structure also frequently exhibit differences in nutrient dynamics (Hannam et al., 2006). Although the results were not conclusive, Hannam et al. (2006) found evidence that microbial communities in the boreal forest floor had returned to pre-harvest levels five years post-disturbance. However, microbial communities can take much longer to recover (Mummey et al., 2002). Hannam et al. (2006) speculated that quick recovery could be attributed to the efforts made to reduce the soil disturbance, by harvesting trees in the winter. Reduced microbial biomass and altered PLFA biomarker concentrations have been reported in soils from other boreal forests 5 to 10 years post-disturbance (Baath, 1980; Pennanen et al., 1999).

Microorganisms have the ability to quickly adapt to changes in the environment (Rajendran and Nagatomo, 1999). Rapid changes in microbial community structure can be detected by changes in PLFA patterns (Zelles, 1999). A pot study investigating the influence of soil properties on three species of tree seedlings revealed that after only 3 months the composition of microbial PLFA of organic soil was considerably different among tree species (Priha et al., 1999). PLFA analysis for the June/July 2005 sampling event was significantly different from the August 2005 sampling event in the proportion of gram + bacteria, gram – bacteria, fungi, and actinomycetes (Appendix B). There were also dramatic shifts in the microbial biomass from June/July to August. It is not clear what specifically caused the changes between the June/July and August sampling events. The literature suggests a variety of reasons for such rapid changes. A combination of factors such as initial litter chemistry, populations of soil fauna, soil moisture, and soil temperature regimes, or seasonal changes in the proportion of vegetation may all play a role in fluctuating microbial communities (Fox and Van Cleve, 1983; Lindo and Visser, 2003).

3.4.3 The Unique Site

The Mesic 1 site was dramatically different from the other sites in plant productivity, species richness, and soil microbial diversity. One might expect that the Mesic 1 site would group with the Mesic 2 site, as these sites were reclaimed within a year of one another and contain the same type of peat mix. However, results showed that the Mesic 1 site was not as able as the Mesic 2 site to support plant growth. Results from chapter 2 indicated that the Mesic 1 site had lower net nitrification and net mineralization rates than the Mesic 3 and Humic/mesic sites, and limited seasonal fluctuations in net N rates. However, Figure 3.3 indicates that N concentration in the plant tissues of grass grown in the Mesic 1 material tended to be higher than those obtained from the other peat materials, a contradictory result to the hypothesis that soil N availability is lower for Mesic 1. Turkington et al. (1998) stated that it is difficult to predict plant responses to environmental change especially in already harsh environments. It is difficult to pinpoint what is causing the differences in plant growth between the Mesic 1 site and the other sites. Discussion about possible soil contamination has been considered, and Albian Sands Inc. is conducting tests to determine possible contamination sources. Hydrocarbon contamination may have occurred in the over stripping of the peat layer if oil impregnated sand was close to the surface. Even though ecosystems can be severely impacted by disturbance, the site should still be able to support opportunistic species.

3.5 SUMMARY

This study provides new information on reclamation of oil sands using peat amendments. The objectives of this study were to characterize potential plant productivity, plant communities, and soil microbial community structure, in various peat-mineral amendments used in oil sands reclamation. There were significant differences in plant productivity, plant communities and soil microbial communities among the range of peat amendments studied. Physical and chemical soil properties assessed in chapter 2 were considered when assessing which factors may limit or promote revegetation of reclaimed sites. No single soil characteristic could explain revegetation success. However, N availability and soil pH appear to be possible limiting factors in oil sands

reclamation. The peat material's ability for plant growth under optimal moisture and temperature conditions was consistent regardless of plant species growing, and plant productivity in the reclaimed material was higher than that of the natural material. Aeration of the peat material that occurs in the upland slopes may increase its productivity.

Boreal vegetation appears to influence the microbial community in the forest soils; individuality in microbial community structure might be related to the differences in plant communities. The individuality of the plant communities was reflective of the unique conditions and history of each site and did not provide much insight into performance questions. Lastly, most of the plant species that were re-establishing were typical early successional species associated with disturbed ecosystems in oil sands reclamation.

Table 3.1: Description of the reclamation amendments and site characteristics at the study sites.

Name	Reclamation Date (Year)	Aspect	Revegetation Practices	pH	Moisture (g/gx100)	C:N	Total Labile N (µg-N/g soil) May 2004	Total Labile N (µg-N/g soil) August 2004
Humic/ mesic	1988	S	Initial barley nurse crop (1997) then hybrid poplar and jack pine	7.3 (0.06) a	31.0 (17.6) ab	19.8 (1.44) c	37.8 (14.3) ab	40.8 (15.72) ab
Mesic 1	2004	NE	None	7.5 (0.04) a	24.8 (19.2) ab	19.1 (1.54) c	17.4 (3.5) b	32.1 (1.60) b
Mesic 2	2003	W	Initial barley nurse crop (2003)	7.2 (0.06) a	15.4 (0.69) b	25.3 (1.52) b	17.3 (2.9) b	38.9 (13.92) ab
Mesic 3	2000	N	Initial barley nurse crop (2000) then white spruce and aspen poplar	7.0 (0.16) a	41.3 (11.6) ab	26.3 (2.59) b	51.2 (20.7) a	70.7 (34.08) a
Fibric	2002	N	None	4.1 (0.32) b	133.1 (9.57) a	34.4 (3.09) a	39.9 (3.6) ab	54.6 (8.30) ab

Note: Values are means [n=6] (± standard deviation) and different letters indicate significance ($p < 0.05$). Values for pH, moisture content, and C:N are from the May 2004 baseline.

Table 3.2: Prominence of characteristic species for the summer of 2005 (n=5).

Site	Scientific Name	Common Name/Constituent	June	July	August
Humic/mesic	*Fragaria virginiana* Duchesne	Wild strawberry	<20.0	<20.0	21.8
	Taraxacum officinale Weber	Common dandelion	20.8	21.3	20.9
		Litter	55.8	42.5	38.9
		Moss	39.7	44.9	37.1
		Soil	36.9	23.8	37.6
Mesic 2	*Equisetum* L.	Horsetail	25.6	38.0	33.9
	Hieracium umbellatum L.	Narrow-leaved hawkweed	39.7	30.2	29.2
		Litter	48.5	54.4	52.8
		Soil	62.2	58.2	61.8
Mesic 3	*Calamagrostis canadensis* (Michx.) Beauv.	Bluejoint	29.6	29.6	<20.0
	Epilobium angustifolium L.	Fireweed	28.4	28.4	35.9
	Hordeum jubatum L.	Foxtail barley	<20.0	<20.0	28.5
	Koeleria macrantha (Ledeb.) J.A. Schultes f.	June grass	22.6	22.6	<20.0
	Sonchus uliginosus Bieb.	Smooth perennial sow thistle	28.1	28.1	45.6
	Trifolium hybridium L.	Alsike clover	27.1	27.1	<20.0
		Litter	53.6	53.6	24.0
Fibric	*Epilobium angustifolium* L.	Fireweed	49.1	49.0	49.3
		Litter	23.1	28.0	20.1
		Soil	74.2	75.2	82.2

Table 3.3: Indicator species determined from prominence values from the June, July and August 2005 vegetation surveys.

Site	Indicator Species	Common Names	Observed Indicator Value (IV)	p*
Humic/mesic	*Anemone canadensis* L.	Canada anemone	100.0	0.010
	Carex siccata Dewey	Hay sedge	100.0	0.010
	Festuca ovina L.	Rocky mountain fescue	52.5	0.037
	Fragaria virginiana Duschesne	Wild strawberry	92.5	0.010
	Galium boreale L.	Northern bedstraw	90.1	0.010
	Mertensia paniculata (Ait.) G. Don.	Tall lungwort	100.0	0.010
	Rosa acicularis Lindl.	Prickly rose	100.0	0.010
	Taraxacum officinale Weber	Common dandelion	50.7	0.010
		Moss	53.7	0.010
Mesic 2	*Equisetum* L.	Horsetail	61.2	0.008
	Hieracium umbellatum L.	Narrow-leaved hawkweed	61.2	0.014
	Rubus idaeus L.	Wild raspberry	76.0	0.016
	Salix L.	Willow	69.0	0.028
	Urtica dioica L.	Stinging nettle	70.1	0.012
Mesic 3	*Achillea millifolium* L.	Common yarrow	65.9	0.014
	Calamagrostis canadensis (Michx.) Beauv.	Bluejoint	82.3	0.005
	Sonchus uliginosus Bieb.	Smooth perennial sow thistle	57.0	0.014
	Trifolium hybridum L.	Alsike clover	87.3	0.027
Fibric	*Carex* L.	Sedge	53.0	0.041
	Epilobium angustifolium L.	Fireweed	46.6	0.006
	Potentilla norvegica L.	Rough cinquefoil	73.3	0.013

Note: Mesic 1 has no plant species growing on site. Indicator values (IV) can range from zero (no indication) to 100 (perfect indication). Perfect indication means that presence of a species points to a particular group without error. To determine indicator species only those results with significant p values were selected and displayed in the above table.

* proportion of randomized trials with indicator value equal to or exceeding the observed indicator value.

$p = (1 + \text{number of runs} \geq \text{observed})/(1 + \text{number of randomized runs})$ Note: complete species lists can be found in appendices.

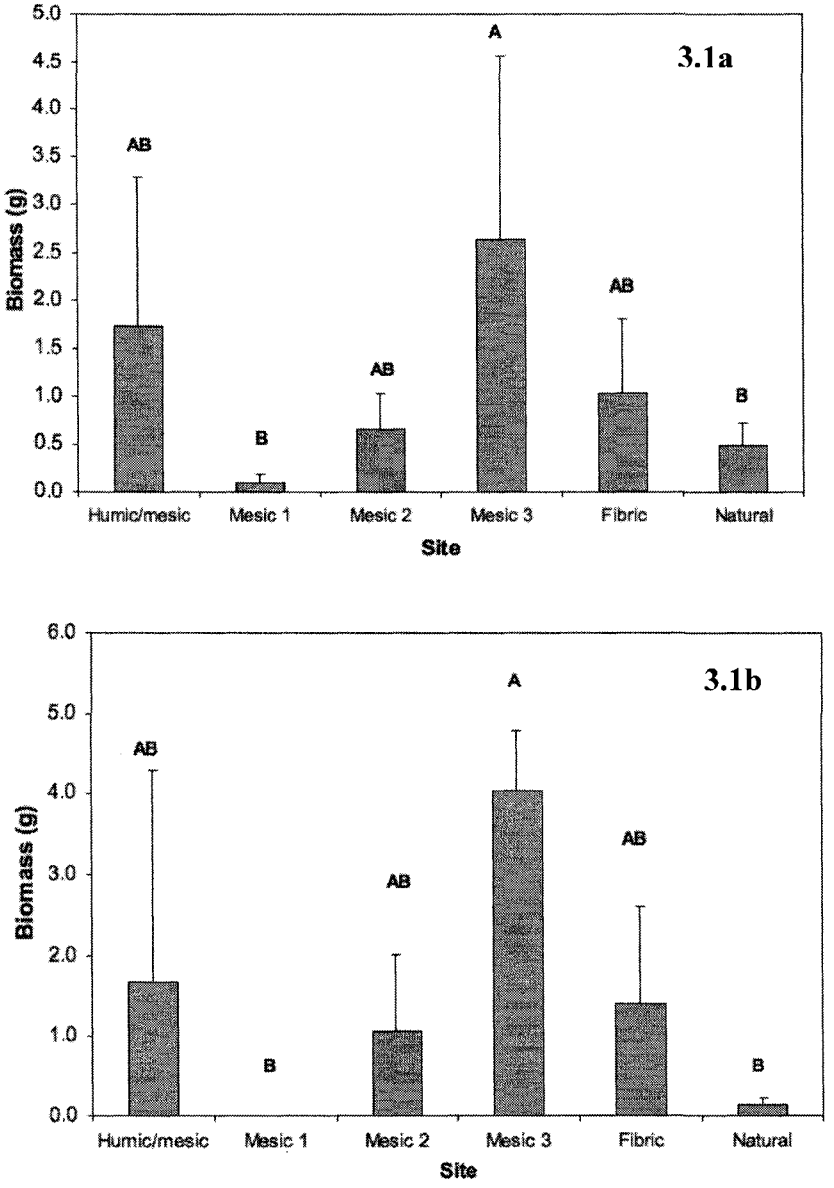

Figure 3.1: Average biomass (grams per pot) of a) *Calamagrostis canadensis* and b) the natural seedbank at day 92 of the greenhouse bioassay. Error bars represent one standard deviation from the mean; capital letters indicate significant differences at $p < 0.05$.

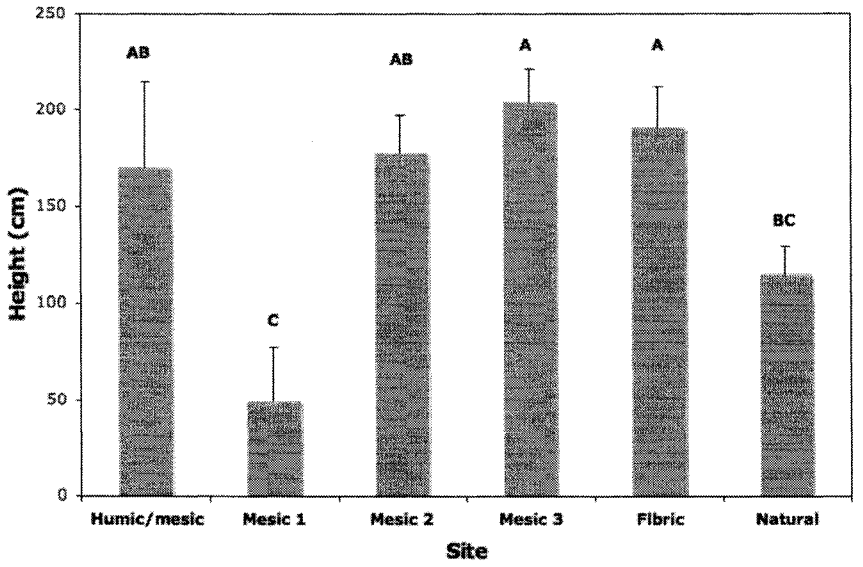

Figure 3.2: Average height of *Calamagrostis canadensis* at day 50 of the greenhouse bioassay. Error bars represent one standard deviation from the mean; capital letters indicate significant differences at $p < 0.05$.

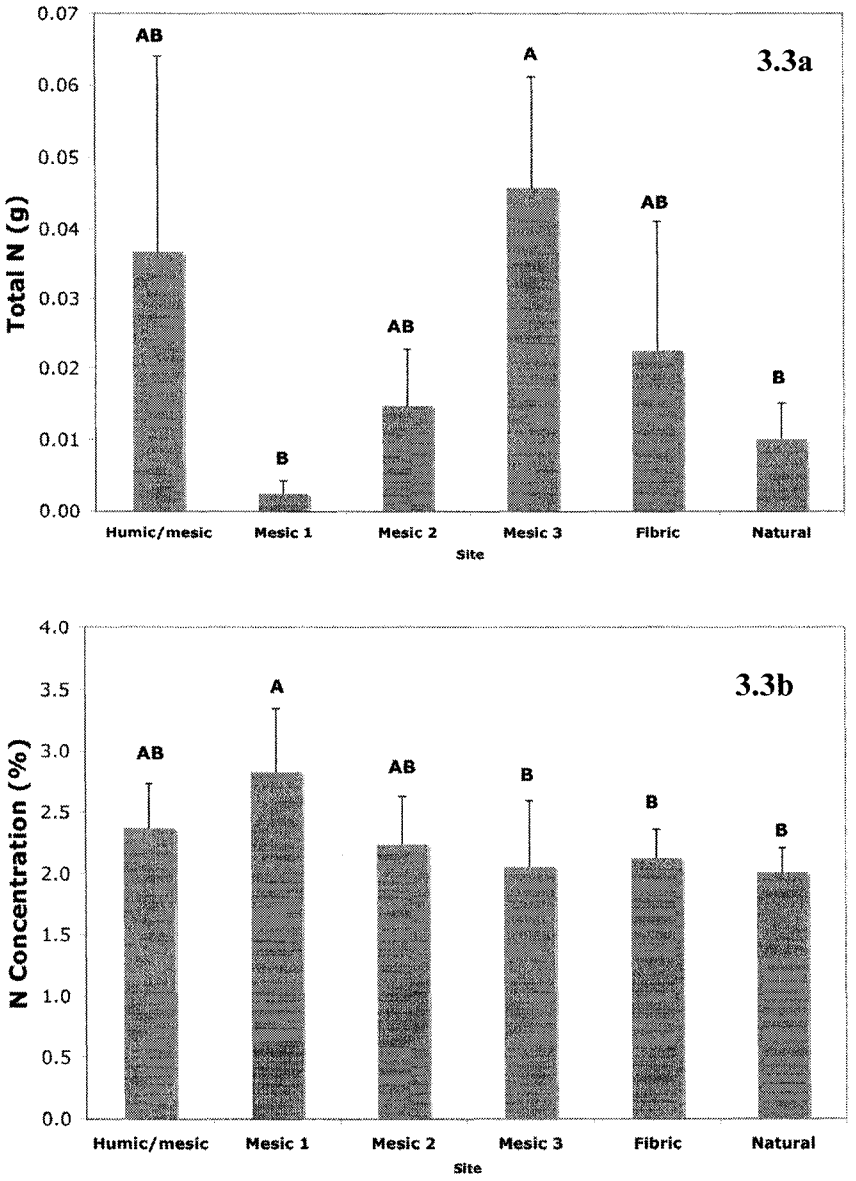

Figure 3.3: a) Total N (g) and b) N concentration (%) in *Calamagrostis canadensis* samples at day 92 of the greenhouse bioassay. Error bars represent one standard deviation from the mean; capital letters indicate significant differences at $p < 0.05$.

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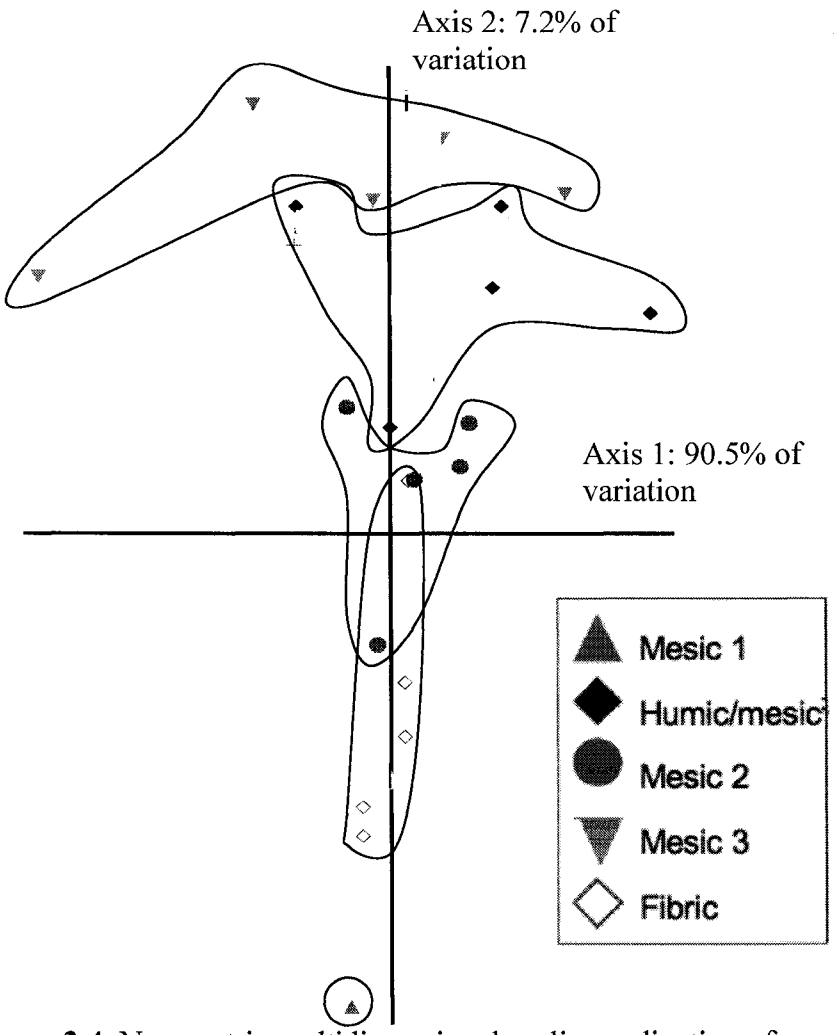


Figure 3.4: Non-metric multidimensional scaling ordination of percent coverage of native species, non-native species, litter and bare ground for each site (June 2005). Circles encompass all samples from each site.

Note: NMS ordination produced a solution with a stress of 5.96, which was achieved after 400 iterations.

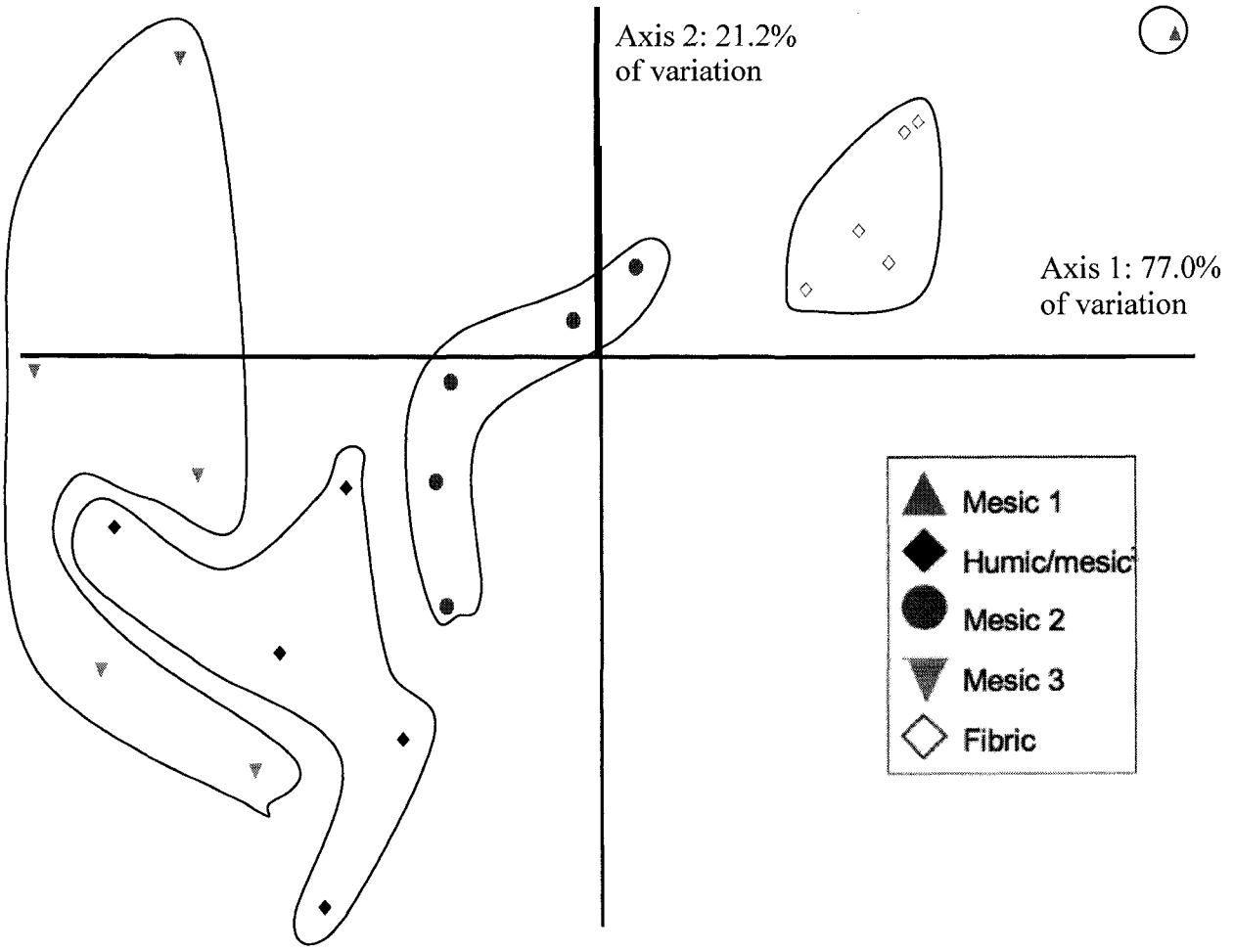


Figure 3.5: Non-metric multidimensional scaling ordination of percent coverage for native species, non-native species, litter and bare ground at each site (July 2005). Circles encompass all samples from each site.

Note: NMS ordination produced a solution with a stress of 7.70, which was achieved after 400 iterations.

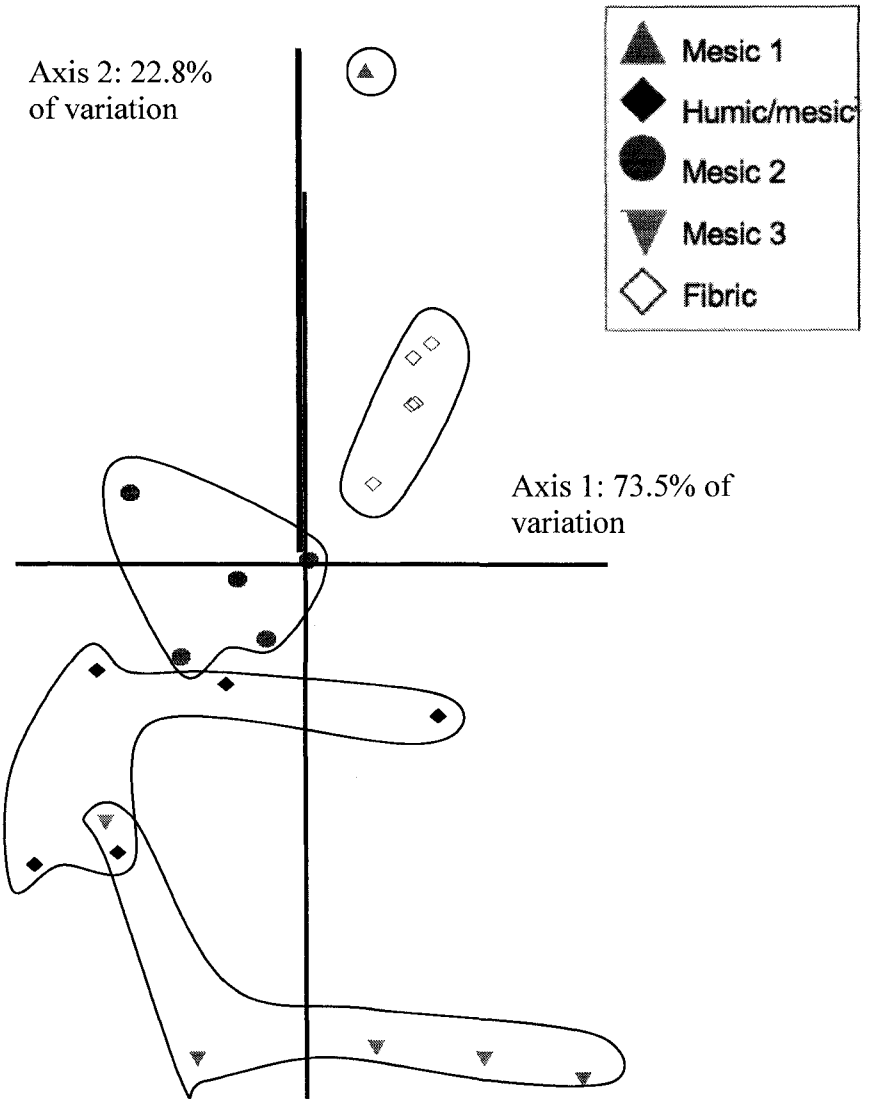


Figure 3.6: Non-metric multidimensional scaling ordination of percent coverage for native species, non-native species, litter and bare ground for each site (August 2005). Circles encompass all samples from each site.

Note: NMS ordination produced a solution with a stress of 10.12, which was achieved after 400 iterations.

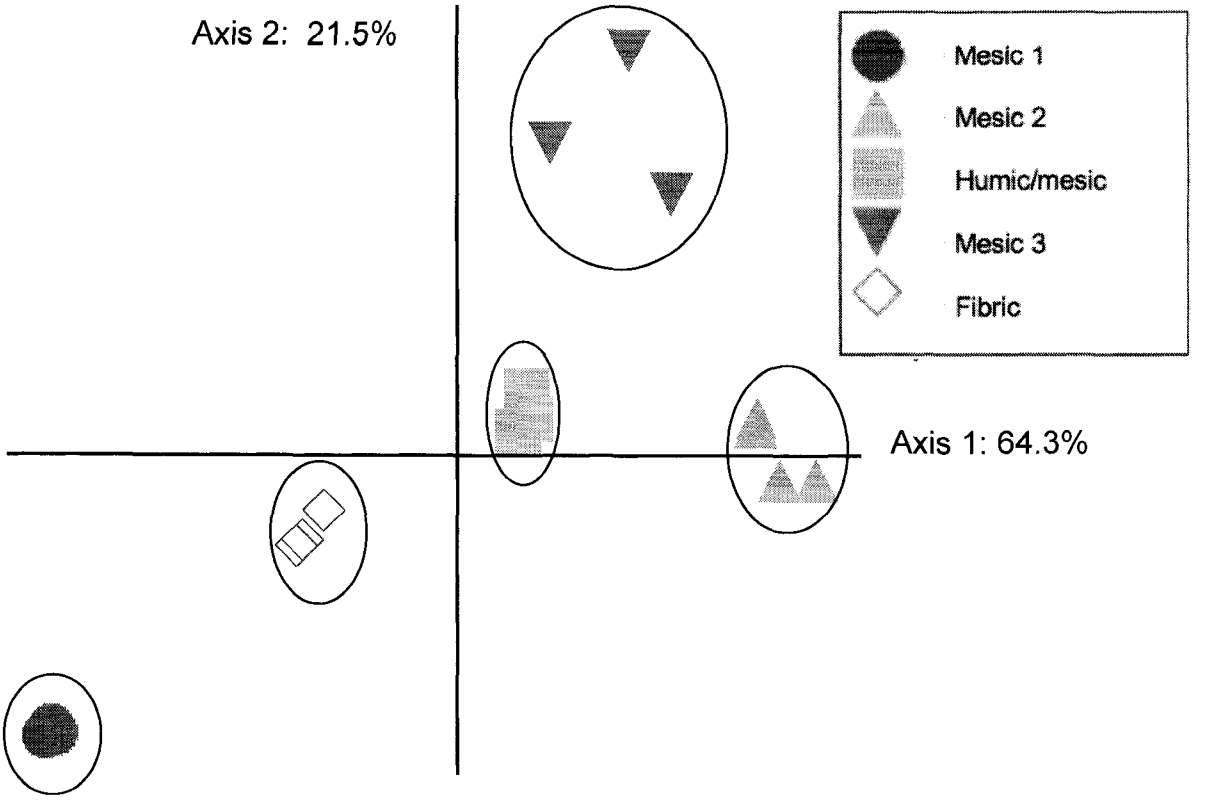


Figure 3.7: Non-metric multidimensional scaling ordination of sites in plant species space using the prominence data of all plant species. Circles encompass all samples from each site (June, July and August 2005 data are the sample replicates).

Note: NMS ordination produced a solution with a stress of 6.08, which was achieved after 400 iterations.

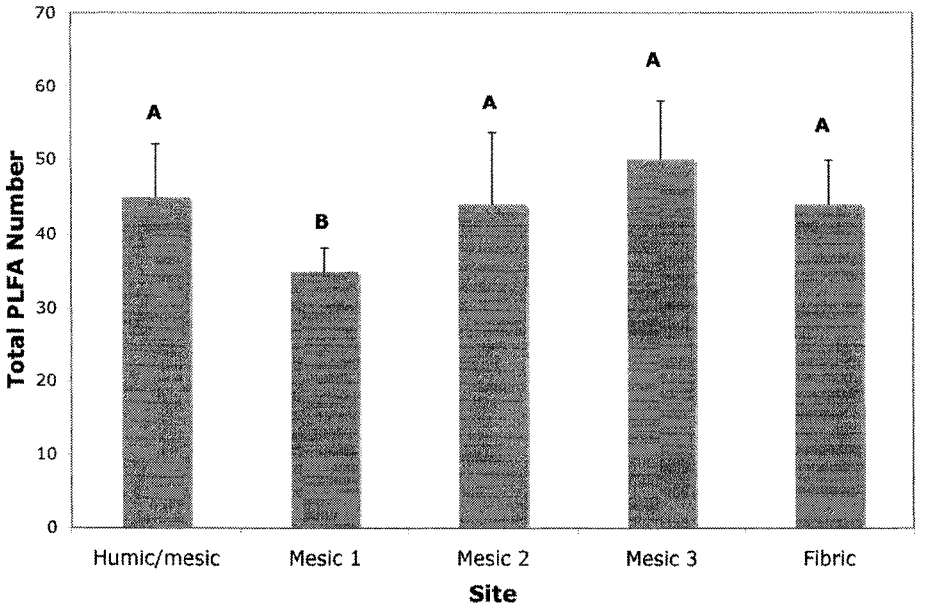


Figure 3.8: Microbial richness (total PLFA number) for June/July 2005. Error bars represent one standard deviation from the means; capital letters indicate significant differences at $p < 0.05$

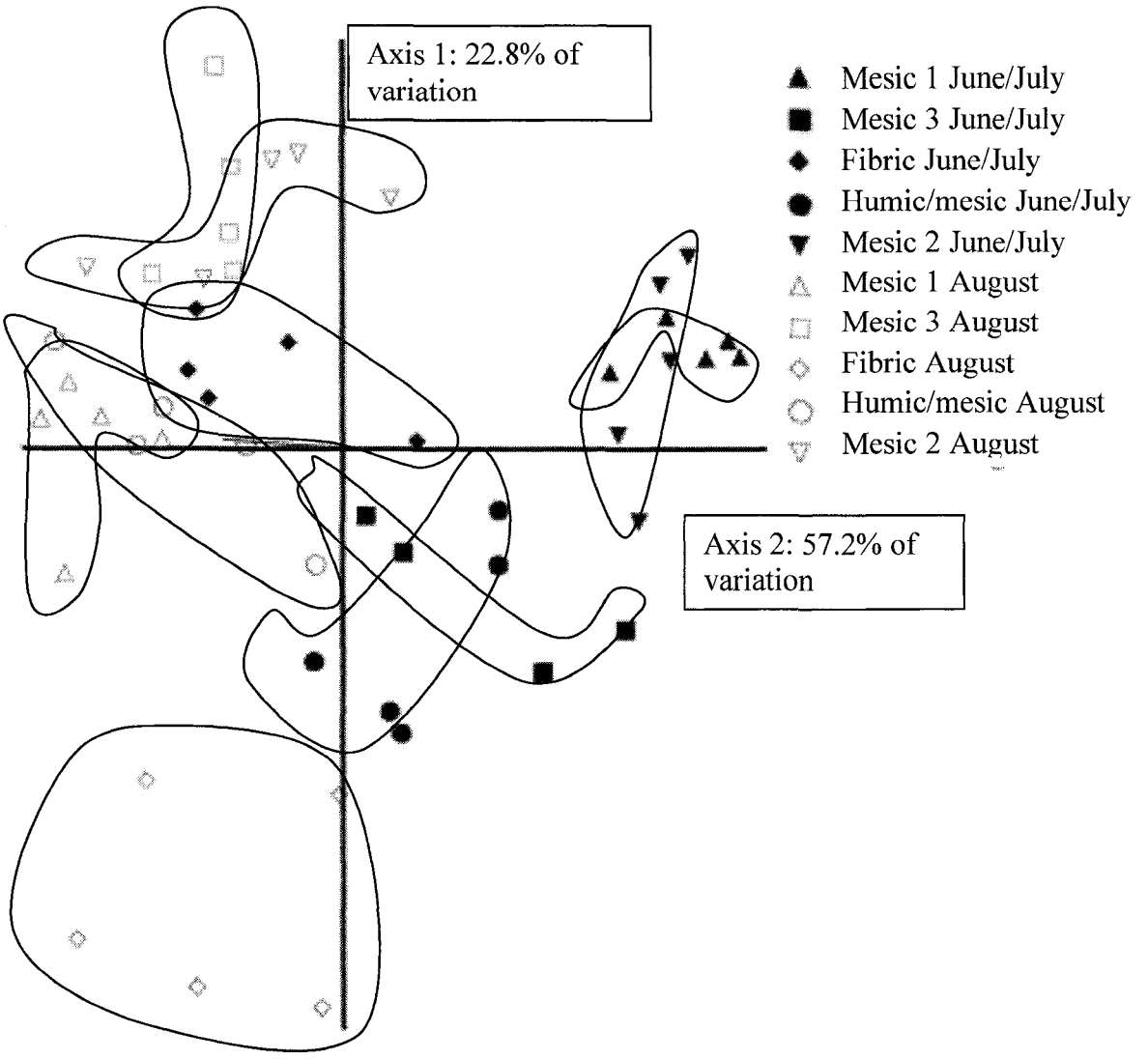
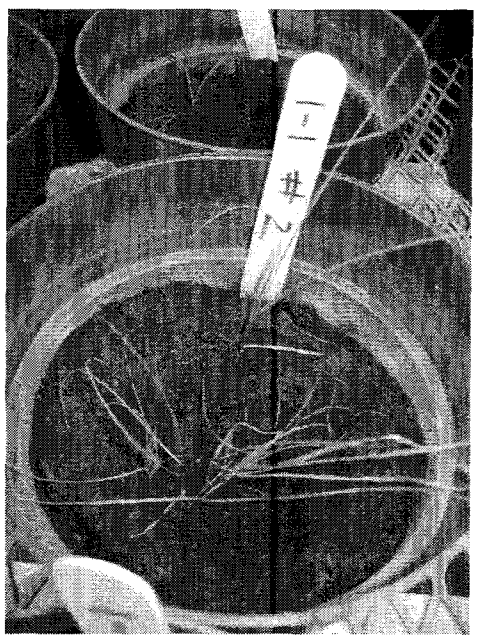


Figure 3.9: Microbial community NMS ordination for 2005.

Note: NMS ordination produced a solution with a stress of 12.78, which was achieved after 85 iterations.

Calamagrostis canadensis grass experiment, August 24th 2005 (92nd day of experiment):



Picture 3.1: Mesic 1 site.

Picture 3.2: Humic/mesic site.

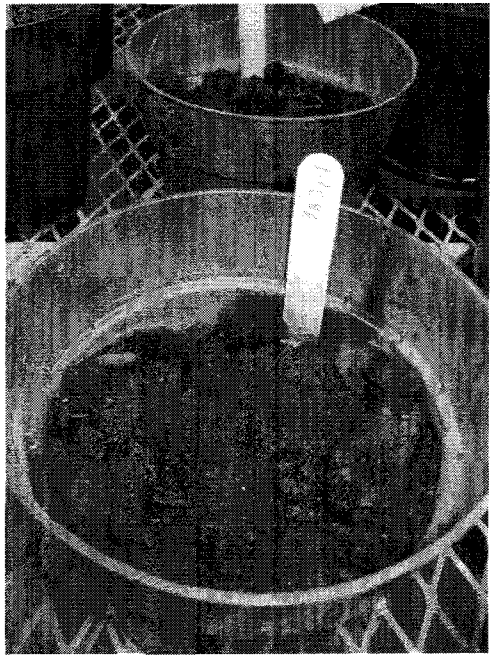
Picture 3.3: Mesic 2 site.

Picture 3.4: Mesic 3 site.

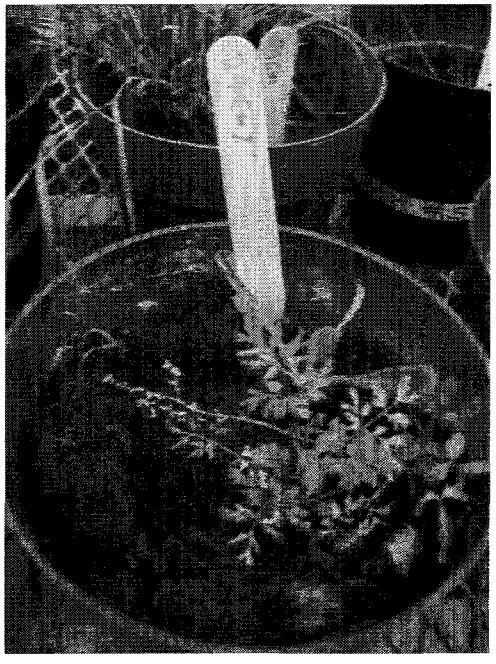
Picture 3.5: Fibric site.

Picture 3.6: Natural site.

Natural seedbank experiment, August 24th, 2005:

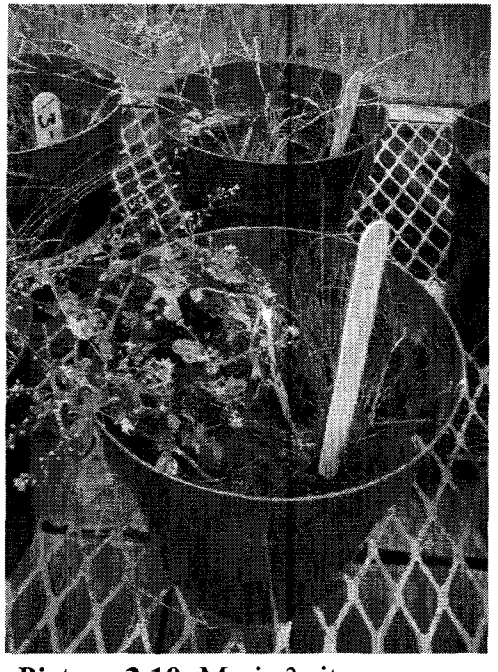


Picture 3.7: Mesic 1 site.

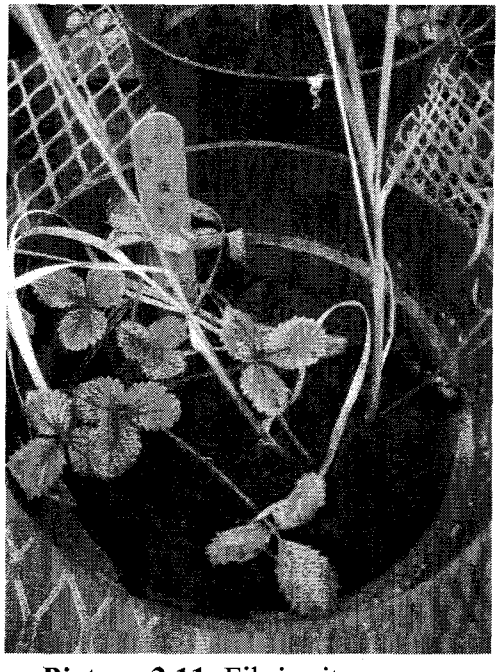


Picture 3.8: Humic/mesic site.

Picture 3.9: Mesic 2 site.



Picture 3.10: Mesic 3 site.



Picture 3.11: Fibric site.

Picture 3.12: Natural site.

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Chapter 4: SUMMARY

4.1 SUMMARY

Peat amendments are widely used in oil sands reclamation. The main objectives of this study were: (1) to measure seasonal soil N availability in a range of peat amendments used for reclamation in the oil sands region, and (2) to characterize plant productivity, plant communities, and soil microbial community structure in the same amendments. Net nitrification and mineralization rates and microbial biomass C and N were measured as well as parameters that influence them (soil moisture, pH, and total C and N) in an attempt to understand the factors controlling nitrogen availability in these soils. Following the characterization of nitrogen availability, on site plant community surveys and greenhouse bioassays were conducted to assess the ability of the amendments to support plant growth. Lastly, composition of the soil microbial communities was characterized using PLFA analysis.

4.1.1 Nitrogen Availability from Peat Amendments used in Boreal Oil Sands Reclamation

The overall objective of this chapter was to measure soil N availability throughout the growing and non-growing seasons in various peat amendments used for oil sands reclamation. Specifically, the objective was to examine seasonal variability in labile soil N and net nitrification and mineralization rates in these amendments.

In the peat amendments, mineralization was the strongest in the fall, following the growing season, and was low and negative in the winter. Negative values indicate that immobilization was the dominating process during the winter. The literature provides evidence that below freezing temperatures in the winter may reduce net N mineralization. However, further investigation is required to determine why the N mineralization levels peak in the fall. It is speculated that as plants senesce, a large sink for bioavailable N is reduced, resulting in a peak in soil N mineralization. Also, as plants senesce the fine root turnover provides a source of labile N for microbes.

Materials from the Mesic 3 and Humic/mesic sites showed the strongest seasonal fluctuations and the highest levels of N mineralization compared to the other materials. Although there are potentially a variety of other reasons for decreased mineralization not

explored by this study, there was evidence of a few possibilities specific to the peat material in question. The acidic nature of the fibric peat material may have inhibited nitrification, reducing N mineralization. For the Mesic 1 and Mesic 2 peat amendments, a reduced proportion of microbial biomass nitrogen (MBN) in comparison to other forms of N in the labile N pool (NH_4-N + NO_3-N + DON + MBN) appeared to be lowering net mineralization rates. The Mesic 1 site separated from the other sites, showing limited seasonal variability, low net nitrification and net mineralization rates, and low labile N. This study was not able to pinpoint specific causation for the low mineralization rates at the Mesic 1 site.

Ammonium was a very transient form of nitrogen at all sites, quickly shifting to nitrate, which consequentially drove N mineralization. The pattern of nitrification influencing mineralization rates is more consistent with that reported in the literature for disturbed sites in the boreal forest than for undisturbed sites.

4.1.2 Plant Productivity and Soil Microbial Diversity in Boreal Oil Sands Reclamation

The overall objective of this chapter was to assess plant productivity using bioassays, plant communities using *in situ* surveys, and microbial community structure via PLFA analysis, in various peat amendments. The majority of peat amendments fostered plant growth under optimal conditions, and productivity in the reclaimed peat material was higher than that of the natural material. Aeration of the peat that occurs on the upland slopes may increase the productivity of the peat by making the soil more hospitable for aerobic microorganisms.

The literature on boreal forests suggests that plants exert a strong influence on microorganisms. The differences in the species composition in the plant communities at each site were reflective of the unique conditions and history of each site. Further examination of the plant species at each site revealed that the plant communities' contained typical early successional species associated with reclaimed oil sands sites.

4.1.3 Synthesis

The different peat amendments exhibited different N mineralization rates, which are known to be related to plant growth. *In situ* plant surveys revealed differences in

plant growth that were consistent with results from the soil analyses. Sites with greater N mineralization had higher percent cover and total species. However, greenhouse bioassays indicated that when temperature and moisture restrictions were removed, the majority of reclaimed peat materials were capable of supporting plant growth, with no significant differences in height or biomass between the materials capable of supporting plants. This shows that although N mineralization rates may be important, they are not the only factor limiting plant growth. Despite differences in plant species establishing on each site, many species were typical disturbed and early successional species in the boreal forest. Given time, the primary successional species that were establishing will likely be replaced as the planted trees begin to mature.

The Humic/mesic site had high levels of N mineralization in the fall, high plant productivity, and high plant species richness. This might lead to the conclusion that the Humic/mesic amendment is a preferable reclamation treatment; however, it is important to note that this site had had the longest elapsed time since reclamation. Based on the design of this study, and a single year of study, one cannot be sure whether the results at this site are representative of that peat in general, other sites of the same age as this site, or both. These results may in fact be representative but we cannot make any firm conclusions with our data. Furthermore, the Mesic 3 site had significantly higher N than the Humic/mesic site in the fall, despite being considerably younger, and it also had high plant productivity and species richness. This indicates that nitrogen availability or age are not the only factors contributing to plant productivity. This study alludes to factors that contribute to the successful revegetation of a site but cannot exclusively distinguish which single factor is the most important. More likely a combination of factors will create optimal reclamation conditions.

4.2 PROJECT LIMITATIONS/FUTURE RESEARCH

One limitation of this project was the lack of replication of the fibric and humic peat types used in the peat amendments. The experimental sites were established before this study began, and could not be modified. Because of lack of replication, conclusions regarding differences among peat types provide the reader with only a basic picture of

differences among peat materials that need to be confirmed with further research. Ideally, a minimum of three sites reclaimed with each type of peat would be needed to test how these amendments function relative to one another. Furthermore, one would have to control the aspect, slope degree, vegetation, etc. However, in industrial research projects such as this that would require a very large number of study sites to include combinations of all these different variables, these requirements are often not easily met. Thus, speculation about the general functioning of such systems based on the preliminary evidence provided is necessary.

Although we were able to conduct the experiment over one full year, time constraints limited us from repeating this experiment. Another problem associated with only having the experiment conducted over one year is how climatic data compare to the climate normals for that region. In order to state that mineralization is indeed the strongest in the fall season, or that mineralization results were not influenced by abnormal climatic conditions, the experiment ideally should be repeated.

The plant community survey methods used in our study were consistent with industrial practices. However, if future research were conducted on the plant communities, using a method such as the Shannon Weaver's diversity index would allow the researcher to make more comparisons to the literature. Finally, it would be beneficial to analyze reclaimed sites in the oil sands that have had longer time since reclamation to formulate a hypothesis of the potential trajectory of plant re-establishment on these sites. It would be valuable to monitor these sites on a long-term basis to determine if in fact they were on a trajectory returning to a forest ecosystem.

To determine if the rapid fluctuation seen in the PLFA between sampling events is characteristic only of newly reclaimed sites, more studies comparing recently reclaimed sites to more mature reclaimed forest communities should be conducted. This would help to determine if microbial community structure will stabilize as these communities mature.

4.3 RECOMMENDATIONS

The mesic and humic peats appear to provide a more hospitable environment for early successional species than the fibric peat. The fibric peat site had a lower plant species richness that is likely due to the acidic nature of the material. Few species are adapted to such conditions, and the literature indicates that low pH reduces N mineralization rates.

Research analyzing older reclaimed slopes (e.g., greater than 25 years since reclamation) may provide information about the trajectory of our research sites. For example, older sites that have used fibric peat for reclamation material may provide some insight as to whether the acidity of this material will affect tree growth. If there is evidence that tree growth is hindered, then testing the pH of the peat material prior to its use for reclamation is recommended. A less acidic peat source (such as the mesic peat in this study with a pH near 7) may be more beneficial for reclamation than more acidic peat (such as the fibric peat with a pH near 4). Another technique to increase the peat pH is to mix it with alkaline mineral material available in the oil sands region. Another recommendation would be to tailor revegetation prescriptions to include acid tolerant species for sites reclaimed with fibric peat. This may enable the use of such material for reclamation and provide diversity in forest developing communities.

Results from the field plant surveys showed that non-native species with a prominence over 20 were found on some sites; they are non-native species whose introduction causes, or is likely to cause, economic or environmental harm and they can threaten reclamation success (Bigelow et al., 2002). When these species become established on a site, they can out-compete and displace native species, reduce wildlife habitat potential, alter natural ecosystem processes, and potentially limit overall biodiversity. Despite providing ground cover for newly reclaimed sites, these non-native species may pose a threat to the establishment of native species and could potentially degrade the quality of these landscapes. Generally, over time, one can expect poor survival of non-native species as a result of limited light from the growing trees (Lieffers and Stadt, 1994). In situations where non-native species are the characteristic species on a site, these species should be monitored to ensure their prominence does not increase. If

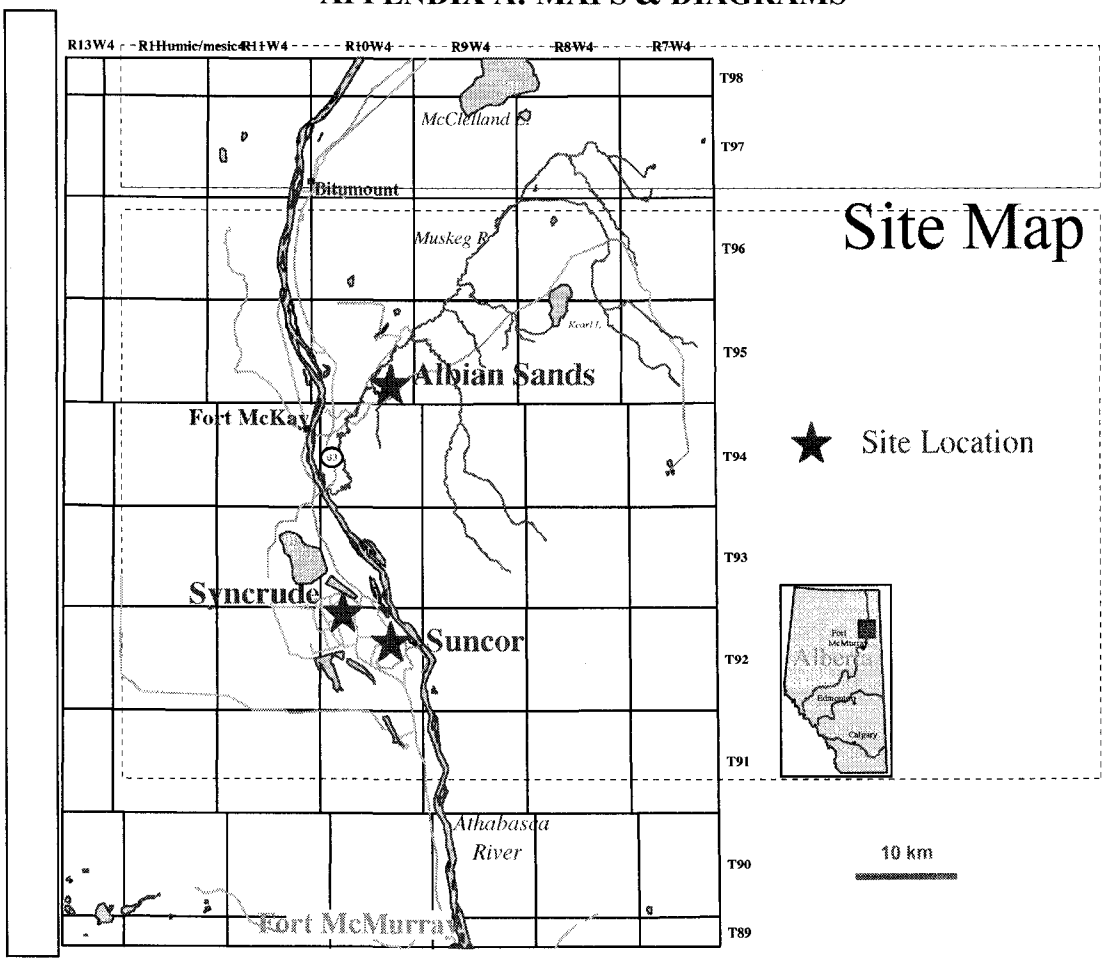
there is evidence that such species are competing with trees, then they should be controlled.

4.4 LITERATURE CITED

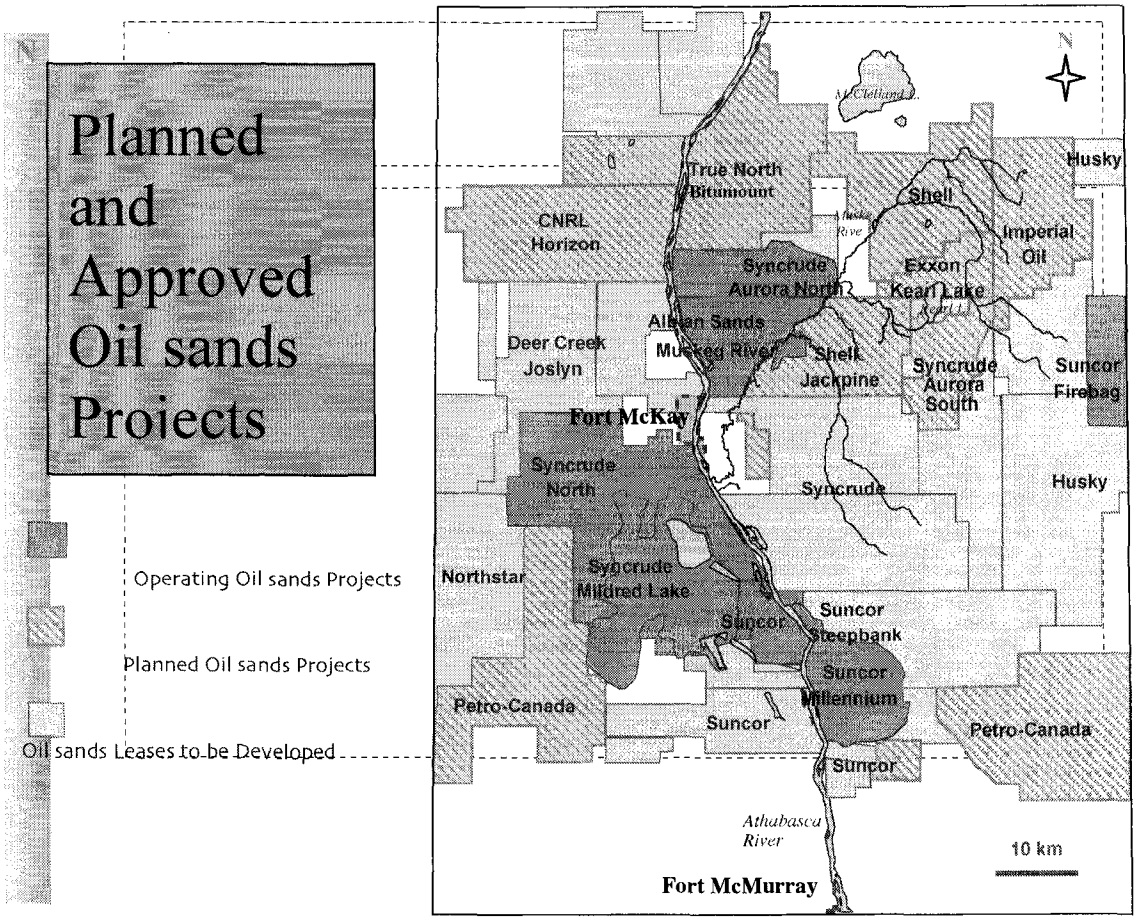
Bigelow, D., B. Vanden Born, and S. Bayley. 2002. Weed identification in Alberta. Agriculture industry, Alberta Environment Health and Safety, and Telus. 25 pp.

Lieffers, V.J., and K.J. Stadt. 1994. Growth of understory *Picea glauca, Calamagrostis canadensis*, and *Epilobium angustifolium* in relation to overstory light transmission. Can. J. For. Res. 24(6): 1193-1198.

APPENDIX A: MAPS & DIAGRAMS



Map 1: Location of companies' infrastructure (map courtesy of Birch Mountain Resources Ltd.).



Map 2: Planned and Approved Oil sands Projects (courtesy of Birch Mountain Minerals Ltd.)

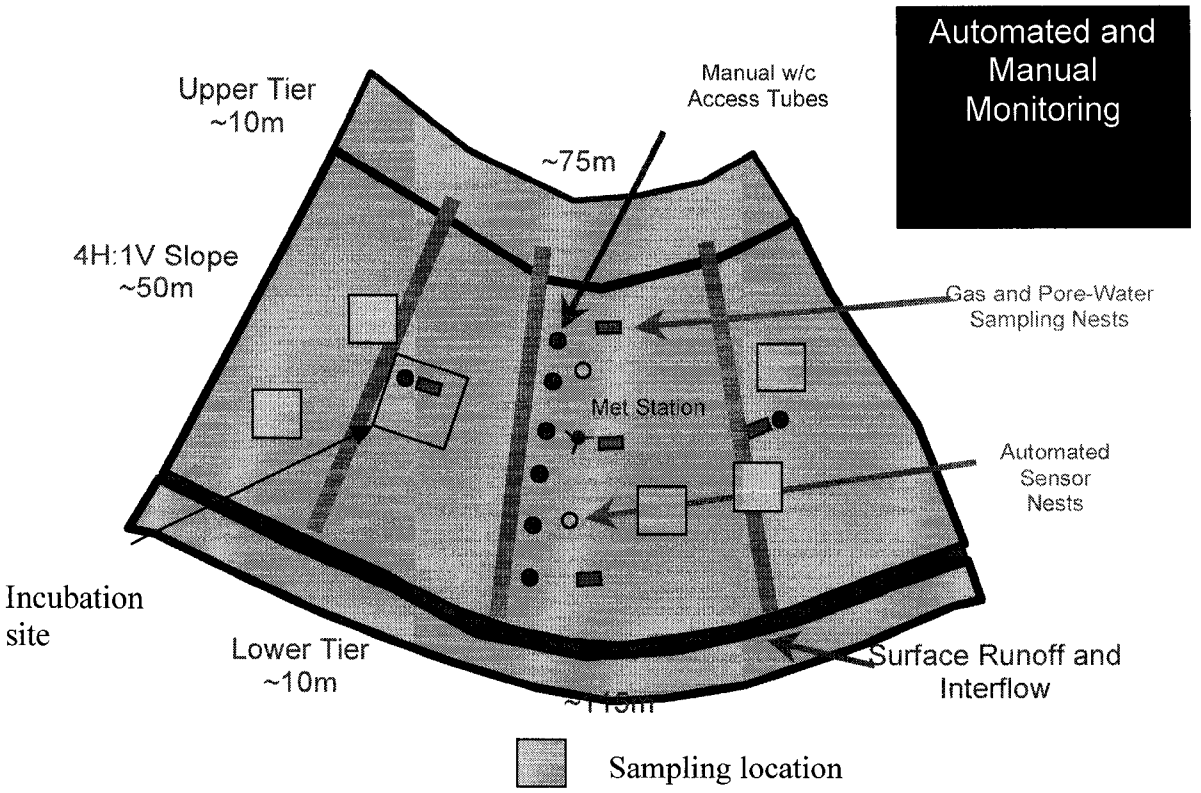
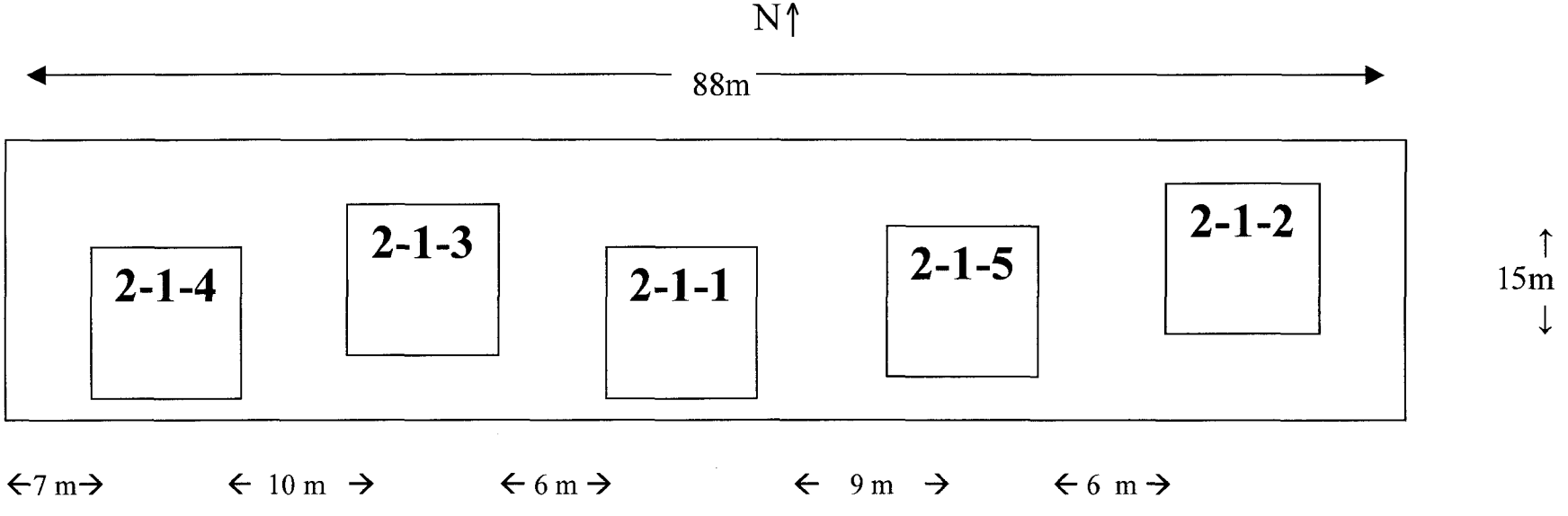


Diagram 1: Illustration of Mesic 1's instrumented slope (courtesy of Albian Sands Inc.)

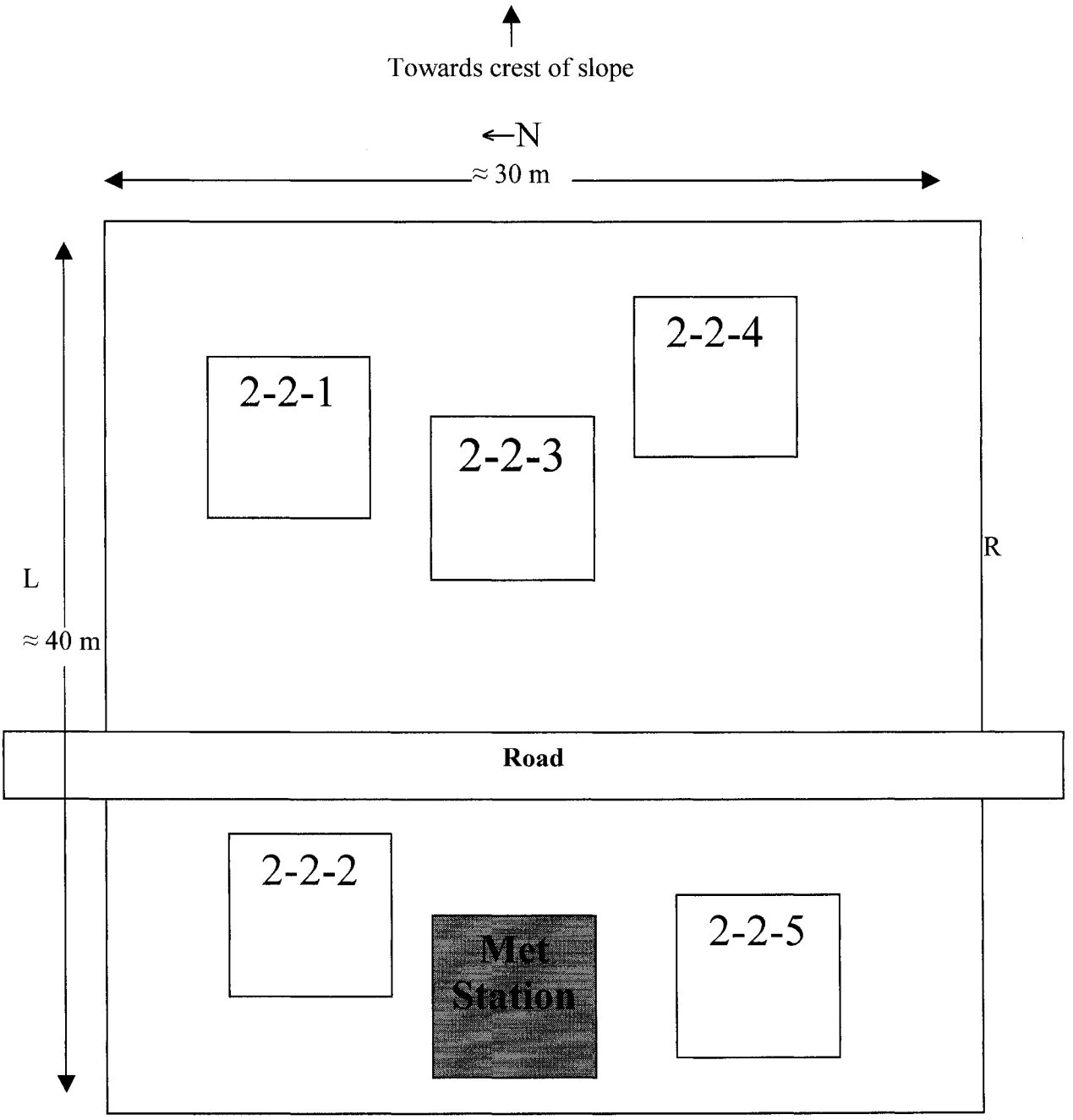
HUMIC/MESIC SITE

The diagram, below is of the Humic/mesic site (field code 2-1) located at Suncor's dyke 2W, the site was 88 m long and 15 m wide. The Humic/mesic site was a south-facing slope that was reclaimed in 1997. There was a 20 cm peat cap over tailings sand. The site had been seeded to lodgepole pine and hybrid poplar. This particular vegetation prescription is not native to the area and has been discontinued. The plot size for each of the replicates (i.e. 2-1-4) is 10 m x 10 m.



MESIC 2 SITE

The diagram below is of site Mesic 2 (field code 2-2), located at Suncor's dyke 11A. This slope was west-facing with a 20 cm cap of peat mix containing mesic peat. The cap was placed over secondary material over lean oil sands mixed with secondary material and overburden. This site was very large both in height and width. There are three benches on the slope; the top of the site was at the crest of the hill and was not included in the study. The road on the map separates the first and the second benches. All the replicates were located in mid-slope positions. Wayne Tedder of Suncor, and Mike O'Kane of O'Kane Consulting were consulted in the decision of where the replicates would be located. The plot size for each of the replicates (i.e. 2-1-4) is 10 m x 10 m.

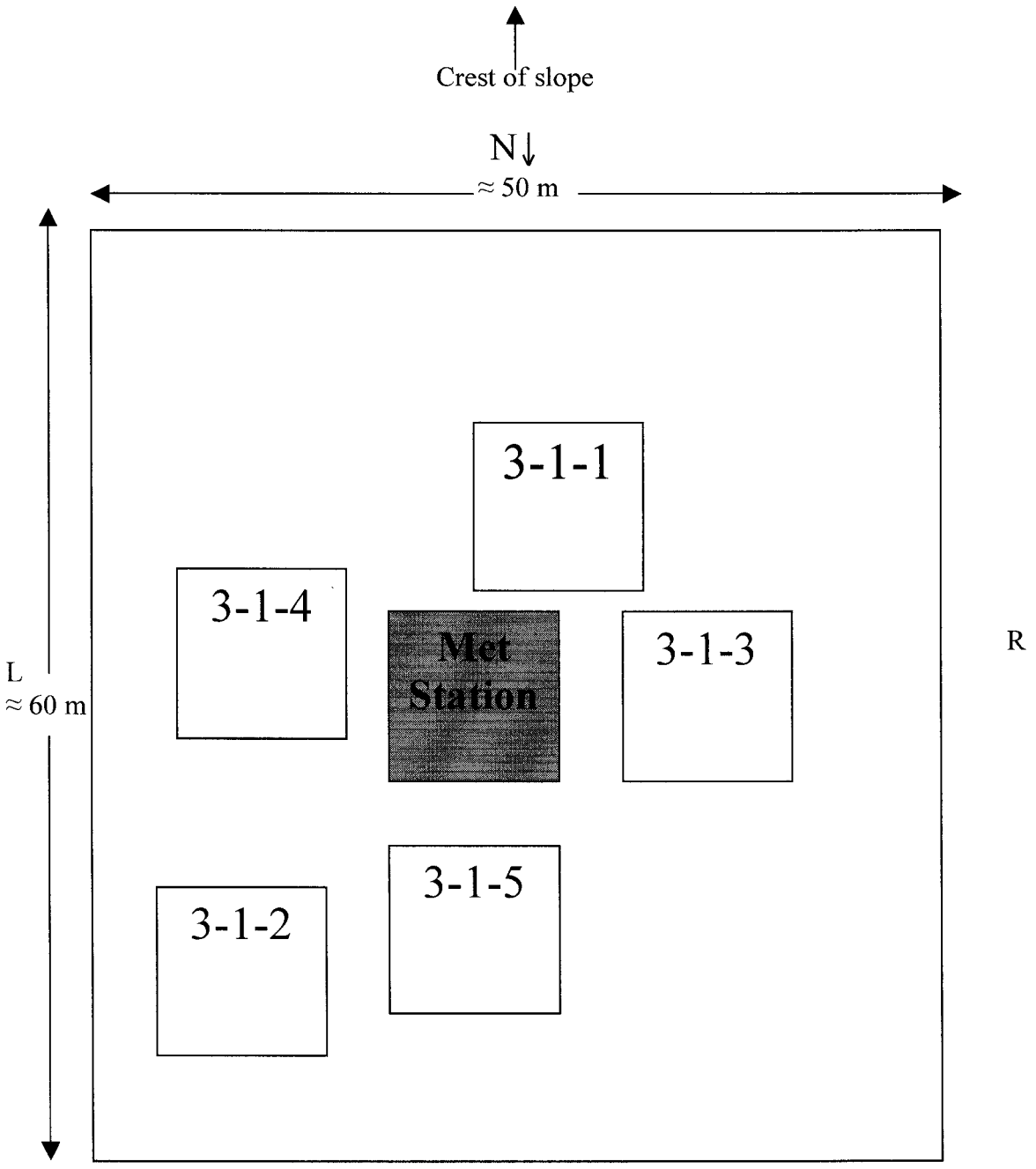

Towards crest of slope
←N
≈ 30 m
2-2-1
2-2-3
2-2-4
R
L
≈ 40 m
Road
2-2-2
Met Station
2-2-5

MESIC 3 SITE

The Mesic 3 site was located at Syncrude's 30 D, area D1. This was a relatively large slope, the second largest in the study. White spruce (*Picea glauca*) and aspen (*Populus tremuloides*) trees had been planted on the site and were seedlings at the time of the study. The replicates were chosen in the mid section of the slope near the meteorological station. The plot size for each of the replicates is 10 m x 10 m.

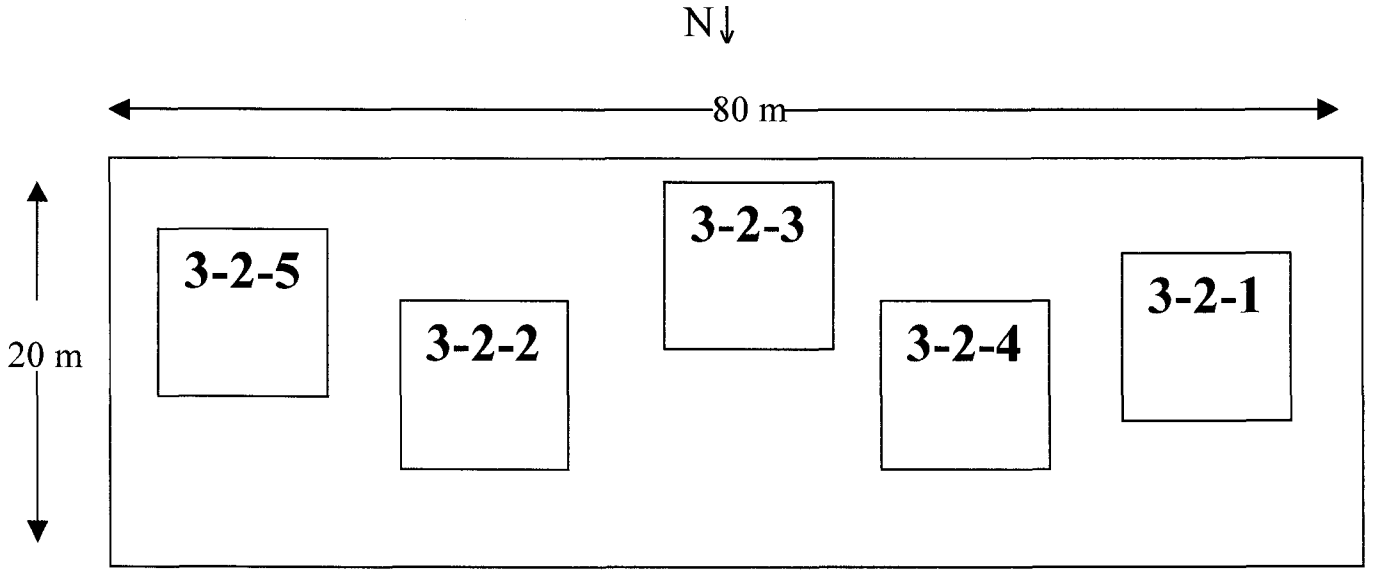
This north facing site had a 20 cm peat mix cap containing mesic peat overlying secondary material placed over overburden. Field observations at this site noted there was spatial variability within the material, for example some replicates contained much mineral soil and other replicates contained soil with mostly peat.

MESIC 3 SITE



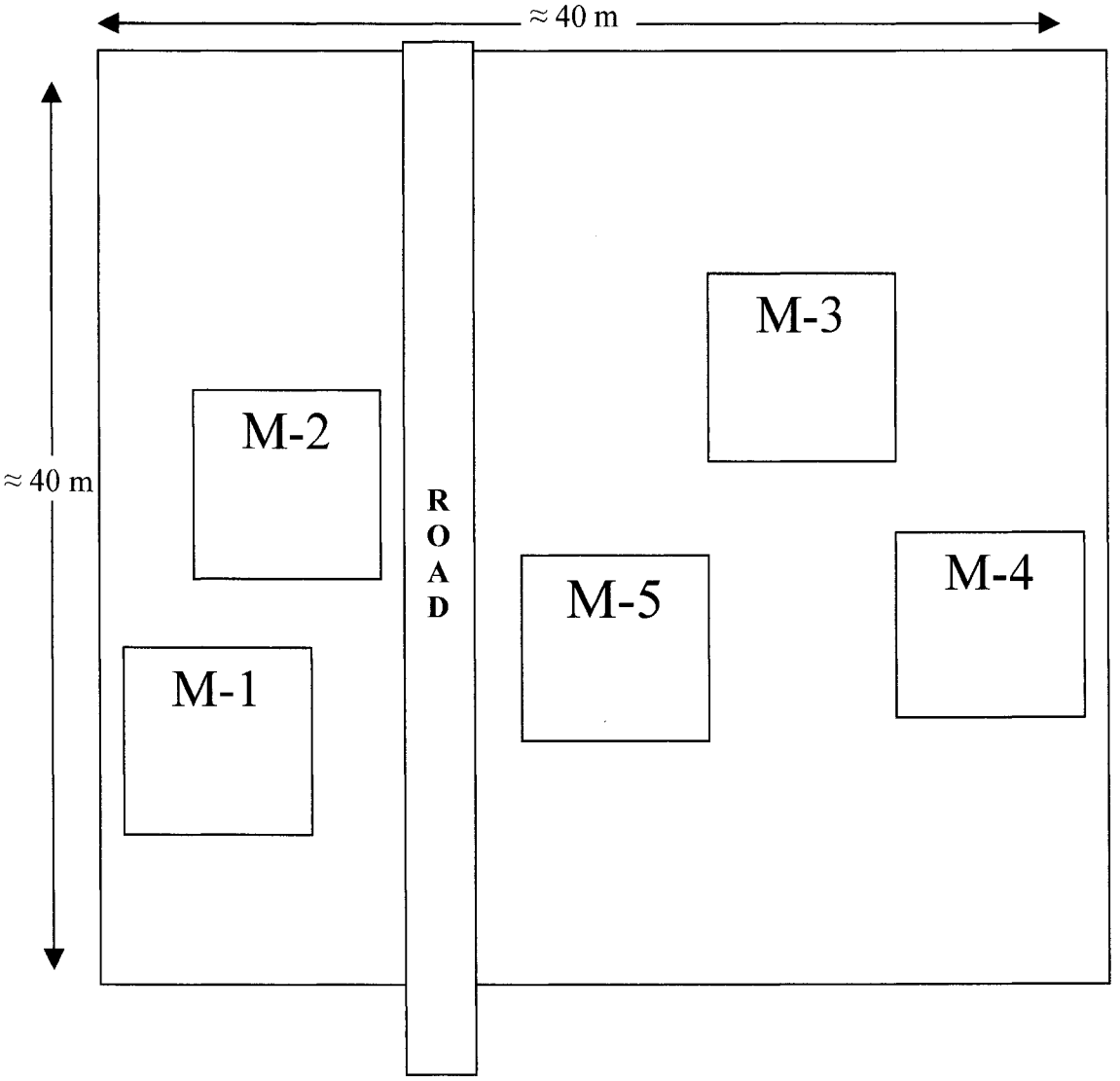
FIBRIC SITE

The north-facing Fibric site (field code 3-2) was located at Syncrude and was 80 m long by 20 m wide. The site contained a 20 cm peat mix containing fibric peat on top of secondary material over (saline sodic) overburden. The plot size for each of the replicates is 10 m x 10 m.



NATURAL SITE (SITE M): SEDGE FEN

The natural peat site contained sedge peat described as mesic peat by Dr. Quideau. This site was located at Albian Sands in a sedge fen in a depressional area with a level surface and had areas with open standing water in the spring of 2004. The plot size for each of the replicates is 10 m x 10 m.



APPENDIX B: ADDITIONAL SUMMARIZED DATA

B.1 ADDITIONAL DATA

Table B.1: Coring schedule for the 2004 field season.

Season	Days Incubated	Baseline	Resin-cores and Buried-bags
Summer	99	-Taken in May/June 2004	-Taken in May/June 2004 -Harvested late August 2004
Fall	75	-Taken in late August 2004	-Taken in late August 2004 -Harvested late November 2004
Winter	157	n/a	-Taken in late August 2004 -Harvested late April 2005
Spring	63	n/a	-Taken in late August 2004 -Harvest late June 2005

Table B.2: Raw data for the 2004 summer baseline.

Site	Moisture Content (% mass)	pH	NO3-N (ppm) in solution	NH4-N (ppm) in solution	Net N (ppm) in solution	DON (ppm) in solution	DOC (ppm) in solution
Humic/ mesic	18.6	7.27	0.34	0.25	0.59	1.11	19.74
	47.7	7.26	0.32	0.22	0.54	1.10	20.27
	18.1	7.45	0.36	0.24	0.60	0.95	25.78
	17.9	7.15	0.26	0.36	0.62	1.54	23.34
	52.5	7.30	0.24	0.21	0.45	0.79	20.00
Mesic 1	16.9	7.43	0.26	0.17	0.43	1.14	26.60
	16.2	7.51	0.25	0.18	0.43	0.93	26.65
	16.1	7.50	0.23	0.19	0.42	0.91	26.15
	15.6	7.47	0.24	0.16	0.40	0.93	28.13
	59.1	7.45	0.28	0.15	0.43	1.04	28.74
Mesic 2	14.3	7.27	0.30	0.18	0.48	0.68	15.62
	15.7	7.18	0.30	0.15	0.45	0.94	15.43
	15.1	7.30	0.35	0.16	0.51	0.65	15.39
	15.6	7.30	0.32	0.15	0.47	0.58	15.25
	16.1	7.03	0.13	0.17	0.30	1.02	17.46
Mesic 3	41.6	5.78	0.54	0.59	1.13	0.83	13.48
	56.6	7.23	0.50	0.24	0.74	0.85	15.35
	30.3	7.33	0.78	0.25	1.03	1.37	13.85
	48.5	7.35	0.82	0.26	1.08	0.74	12.13
	29.7	7.28	1.74	0.41	2.15	1.90	17.84
Fibric	127.8	3.66	0.29	0.52	0.81	0.84	24.18
	125.5	4.16	0.16	.	.	.	.
	127.3	4.28	0.25	0.24	0.49	0.78	19.29
	136.2	3.94	0.26	0.37	0.63	0.99	22.78
	148.5	4.28	0.22	0.36	0.58	0.71	19.33
Natural	720.8	7.07	0.07	0.77	0.83	2.29	72.21
	384.7	7.24	0.26	0.18	0.44	1.56	44.57
	538.8	7.17	0.26	0.55	0.81	1.97	46.09
	441.5	7.30	0.23	0.19	0.42	0.97	20.14
	421.8	7.19	0.23	0.91	1.14	1.60	59.03

Table B.3: Raw data for the 2004 fall baseline.

Site	Moisture Content (% mass)	Bulk Density (Mg m^{-3})	pH	NO3-N (ppm) in solution	NH4-N (ppm) in solution	Net N (ppm) in solution	DON (ppm) in solution	DOC (ppm) in solution
Humic/ mesic	6.1	0.92	7.02	1.44	0.23	1.67	1.85	27.57
	5.7	0.91	7.07	1.31	0.25	1.56	1.52	48.30
	4.5	0.99	7.03	0.86	0.14	1.00	1.61	25.79
	6.3	0.81	6.86	1.00	0.44	1.44	2.16	37.06
	5.3	0.94	6.71	0.80	0.15	0.95	1.73	35.35
Mesic 1	23.8	0.87	6.63	0.53	0.18	0.71	1.39	37.79
	28.7	0.68	6.87	0.52	0.21	0.73	1.57	38.52
	24.7	0.77	6.74	0.34	0.13	0.47	1.43	34.77
	21.4	0.72	6.70	0.44	0.23	0.67	2.20	48.74
	22.0	0.87	7.00	0.41	0.15	0.56	1.81	31.91
Mesic 2	28.9	0.73	6.46	1.74	0.16	1.90	1.93	29.33
	23.4	0.65	6.44	1.14	0.14	1.28	1.96	32.59
	26.2	0.81	6.58	0.39	0.07	0.46	1.37	20.68
	17.2	0.80	6.55	0.47	0.14	0.61	1.36	21.56
	22.8	0.72	6.64	0.58	0.13	0.71	1.57	34.18
Mesic 3	56.9	0.35	5.19	1.60	0.42	2.02	2.91	39.43
	20.3	0.68	6.48	1.00	0.10	1.10	1.42	22.61
	16.0	0.90	6.46	0.91	0.12	1.03	1.20	16.86
	16.0	0.85	6.62	1.45	0.20	1.65	1.72	23.64
	18.0	0.76	6.48	2.13	0.17	2.30	1.99	27.59
Fibric	69.1	0.27	3.64	0.61	0.66	1.27	2.18	49.63
	81.3	0.39	4.21	0.50	0.20	0.70	2.19	41.31
	102.1	0.31	4.23	0.36	0.11	0.47	2.05	39.84
	133.9	0.28	3.52	0.35	0.28	0.63	2.05	47.35
	92.3	0.28	4.10	0.63	0.32	0.95	2.08	39.51
Natural	478.8	0.13	6.83	0.34	1.53	1.87	2.90	75.25
	383.4	0.20	6.93	0.34	0.23	0.57	1.73	49.32
	536.0	0.13	7.14	0.60	0.84	1.44	2.23	67.23
	347.1	0.17	6.85	0.29	0.36	0.65	2.06	63.90
	346.4	0.18	6.87	0.75	0.43	1.18	1.83	56.35

Table B.4: Average monthly climate data for Fort McMurray and the experimental sites (Note: Fibric site has no met. station).

Date	Mesic 1		Humic/Mesic		Mesic 2		Mesic 3	
	Monthly Average Air Temperature (°C)	Monthly Total Precipitation (mm)	Monthly Average Air Temperature (°C)	Monthly Total Precipitation (mm)	Monthly Average Air Temperature (°C)	Monthly Total Precipitation (mm)	Monthly Average Air Temperature (°C)	Monthly Total Precipitation (mm)
Nov-03	-7.93	1.4	N/A	N/A	-7.7	12.7	-8.3	0.3
Dec-03	-10.72	0.25	N/A	N/A	-11	0	-10.9	0.0
Jan-04	-21.3	2.3	N/A	N/A	-20.7	0.8	-21.5	0.0
Feb-04	-10.7	0	N/A	N/A	-10.3	7.9	-10.5	7.4
Mar-04	-5.8	0	N/A	N/A	-5	13.2	-5.5	7.4
Apr-04	3.4	4.6	N/A	N/A	4.1	0	3.3	11.9
May-04	5.9	17.3	N/A	N/A	6.3	37.3	6.0	49.8
Jun-04	14.6	0.3	N/A	N/A	14.8	8.6	14.7	11.8
Jul-04	19.4	0.8	21.1	49	19.9	50.5	14.3	0.0
Aug-04	14	0.3	14.6	24.6	14.3	17	13.8	39.2
Sep-04	8.8	1	9.3	77.5	9.3	60.5	8.7	69.4
Oct-04	1.6	16	2.1	13.5	2.2	9.9	1.7	6.4
Nov-04	-4.6	46.2	-4	2.5	-4.1	2.3	-5.2	14.5
Dec-04	-17.7	17.8	-17.1	27.2	-17.9	9.7	-18.1	18.0
Jan-05	-18.8	5	-18.9	23.9	-19.2	13.7	-18.8	25.4
Feb-05	-11.1	4	-10	17.8	-10.3	11.4	-11.0	6.1
Mar-05	-4.4	5	-3.7	16.3	-3.8	11.7	-4.8	8.1
Apr-05	5.8	19	6.8	43.4	6.4	21.8	5.7	29.0
May-05	10.7	61	11.4	31.5	10.3	24.6	10.6	30.7
Jun-05	14.8	65	15.4	65.5	15.2	64.3	14.6	66.3
Overwinter (Nov 03-Apr 04)	-8.8	8.6	N/A	N/A	-8.4	34.6	-8.9	27.0
Growing Season (May 04-Oct 04)	10.7	35.7	N/A	N/A	11.1	183.8	9.9	176.6
Overwinter (Nov 04-Apr 05)	-8.5	97.0	-7.8	131.1	-8.2	70.6	-8.7	101.1
Growing Season (May 05-June 05)	12.8	126.0	11.4	97.0	12.8	88.9	12.6	97.0

Note: N/A indicates missing values due to a lack of meteorological stations

Table B.5: Additional soil characteristic data collected from seasonal harvests.

Site	Parameter	August 2004	November 2004	April 2005	June 2005
Humic/mesic	pH	6.92	6.79	7.29	6.81
	Moisture (%)	10.2	13.3	25.7	4.78
	Total C (%)	2.77	3.11	3.45	3.93
	Total N (%)	0.13	0.16	0.16	0.22
	C/N Ratio	22.1	19.1	22.0	18.1
Mesic 1	pH	7.15	6.92	7.30	7.11
	Moisture (%)	11.5	23.9	38.7	12.6
	Total C (%)	6.69	7.06	6.43	6.51
	Total N (%)	0.25	0.27	0.29	0.29
	C/N Ratio	26.7	26.3	22.3	24.7
Mesic 2	pH	7.07	6.87	7.21	6.56
	Moisture (%)	44.4	27.9	64.3	17.3
	Total C (%)	5.85	6.64	6.99	6.90
	Total N (%)	0.30	0.28	0.28	0.30
	C/N Ratio	19.6	23.5	25.0	23.2
Mesic 3	pH	6.47	6.59	7.16	6.58
	Moisture (%)	77.7	53.2	45.4	14.9
	Total C (%)	5.79	4.63	5.98	5.16
	Total N (%)	0.26	0.19	0.24	0.22
	C/N Ratio	20.9	24.0	25.0	21.8
Fibric	pH	4.36	4.09	4.15	4.30
	Moisture (%)	43.2	102	183	58.0
	Total C (%)	15.8	16.4	20.8	19.1
	Total N (%)	0.55	0.58	0.64	0.58
	C/N Ratio	28.6	28.2	32.9	33.0
Natural	pH	7.07	6.95	7.32	7.08
	Moisture (%)	134	172	231	69.6
	Total C (%)	21.9	24.4	25.4	30.7
	Total N (%)	1.12	1.38	1.44	1.53
	C/N Ratio	20.1	17.9	17.9	20.0

Table B.6: Multiple response permutations procedure (MRPP) results for species prominence vegetation data. A is the agreement statistic, T is the test statistic and P is the probability.

Comparison	A	T	P
All sites	0.706	-8.230	0.000
Mesic 1 + Mesic 3	0.732	-2.990	0.022
Mesic 1 + Humic/mesic	0.776	-2.996	0.022
Mesic 1 + Mesic 2	0.856	-2.998	0.021
Mesic 1 + Fibric	0.793	-2.967	0.022
Mesic 3 + Humic/mesic	0.404	-2.948	0.022
Mesic 3 + Mesic 2	0.468	-2.961	0.022
Mesic 3 + Fibric	0.593	-2.944	0.022
Humic/mesic + Mesic 2	0.546	-2.998	0.022
Humic/mesic + Fibric	0.583	-2.957	0.022
Mesic 2 + Fibric	0.490	-2.901	0.022

Table B.7: Multiple response permutations procedure (MRPP) results for vegetation data. A is the agreement statistic, T is the test statistic and P is the probability.

Sampling Date	Comparison	A	T	P
June 2005	All sites	0.61	-8.77	0.0000
	Mesic 1 + Mesic 3	0.75	-5.85	0.0017
	Mesic 1 + Humic/mesic	0.75	-5.83	0.0017
	Mesic 1 + Mesic 2	0.79	-5.74	0.0018
	Mesic 1 + Fibric	0.62	-5.61	0.0018
	Mesic 3 + Humic/mesic	0.05	-1.01	0.1466
	Mesic 3 + Mesic 2	0.30	-5.13	0.0019
	Mesic 3 + Fibric	0.46	-5.17	0.0025
	Humic/mesic + Mesic 2	0.18	-3.30	0.0094
	Humic/mesic + Fibric	0.40	-4.57	0.0039
	Mesic 2 + Fibric	0.25	-2.75	0.0239
July 2005	All sites	0.67	-9.49	0.0000
	Mesic 1 + Mesic 3	0.75	-5.85	0.0017
	Mesic 1 + Humic/mesic	0.79	-5.89	0.0016
	Mesic 1 + Mesic 2	0.80	-5.85	0.0017
	Mesic 1 + Fibric	0.76	-5.65	0.0018
	Mesic 3 + Humic/mesic	0.05	-0.87	0.1669
	Mesic 3 + Mesic 2	0.28	-4.74	0.0023
	Mesic 3 + Fibric	0.57	-5.70	0.0017
	Humic/mesic + Mesic 2	0.26	-3.94	0.0061
	Humic/mesic + Fibric	0.60	-5.78	0.0016
	Mesic 2 + Fibric	0.51	-5.50	0.0018
August 2005	All sites	0.64	10.27	0.0000
	Mesic 1 + Mesic 3	0.72	-5.82	0.0017
	Mesic 1 + Humic/mesic	0.73	-5.83	0.0017
	Mesic 1 + Mesic 2	0.80	-5.88	0.0017
	Mesic 1 + Fibric	0.74	-5.75	0.0017
	Mesic 3 + Humic/mesic	0.19	-2.89	0.0178
	Mesic 3 + Mesic 2	0.41	-4.86	0.0031
	Mesic 3 + Fibric	0.54	-5.54	0.0019
	Humic/mesic + Mesic 2	0.15	-2.93	0.0131
	Humic/mesic + Fibric	0.47	-5.31	0.0021
	Mesic 2 + Fibric	0.45	-5.10	0.0025

Table B.8: Multiple response permutations procedure (MRPP) results for the microbial community in 2005. A is the agreement statistic, T is the test statistic and P is the probability.

Dates	Site Comparisons	A	T	P
June/July				
	All	0.393	-9.141	0.000
	Mesic 2 & Humic/mesic	0.352	-4.917	0.002
	Mesic 2 & Mesic 3	0.271	-4.149	0.004
	Mesic 2 & Mesic 1	0.088	-1.994	0.038
	Mesic 2 & Fibric	0.392	-5.026	0.002
	Humic/mesic & Mesic 3	0.065	-1.446	0.083
	Humic/mesic & Mesic 1	0.435	-5.425	0.001
	Humic/mesic & Fibric	0.238	-3.955	0.004
	Mesic 3 & Mesic 1	0.340	-4.748	0.003
	Mesic 3 & Fibric	0.196	-3.408	0.009
	Mesic 1 & Fibric	0.456	-5.222	0.002
August				
	All	0.413	-10.175	0.000
	Mesic 2 & Humic/mesic	0.185	-3.741	0.005
	Mesic 2 & Mesic 3	0.158	-3.403	0.007
	Mesic 2 & Mesic 1	0.254	-4.749	0.002
	Mesic 2 & Fibric	0.482	-5.477	0.002
	Humic/mesic & Mesic 3	0.235	-4.844	0.002
	Humic/mesic & Mesic 1	0.116	-2.923	0.009
	Humic/mesic & Fibric	0.362	-4.935	0.003
	Mesic 3 & Mesic 1	0.248	-5.101	0.002
	Mesic 3 & Fibric	0.458	-5.581	0.002
	Mesic 1 & Fibric	0.432	-5.191	0.002

Table B.9: Mesic 1 vegetation survey June 2005.

Constituent	Coverage Mean %	Coverage Standard Deviation	Percent Frequency	Prominence
Coke/Coal	0.03	0.1	6.67	0.42
Rock	1.83	1.2	86.67	12.58
Soil	96.98	2.1	100.00	98.48
Wood	1.16	1.4	86.67	10.02

Table B.10: Humic/mesic vegetation survey June 2005.

Scientific Name	Common Name	Coverage Mean (%)	Coverage Standard Deviation	Percent Frequency	Prominence
Achillea millifolium L.	Common yarrow	0.06	0.1	26.67	1.22
Anemone canadensis L.	Canada anemone	0.05	0.1	6.67	0.60
Carex siccata Dewey	Hay sedge	1.15	1.7	26.67	5.54
Epilobium angustifolium L.	Fireweed	0.25	0.2	20.00	2.25
Festuca ovina L.	Rocky mountain fescue	7.29	8.3	46.67	18.44
Fragaria virginiana Duchesne	Wild strawberry	2.35	2.9	66.67	12.52
Galium boreale L.	Northern bedstraw	3.30	5.5	26.67	9.38
Hieracium umbellatum L.	Narrow-leaved hawkweed	1.23	1.3	53.33	8.09
Mertensia paniculata (Ait.) G. Don.	Tall lungwort	0.03	0.1	6.67	0.42
Petasites sagittatus (Pursh) A. Gray	Arrow-leaved coltsfoot	0.02	0.0	13.33	0.53
Pinus L.	Pine	0.43	0.7	6.67	1.69
Poa pratensis L.	Kentucky bluegrass	0.18	0.1	20.00	1.89
Rosa acicularis Lindl.	Prickly rose	4.19	4.7	33.33	11.82
Sonchus uliginosus Bieb.	Smooth perennial sow thistle	0.65	0.8	20.00	3.61
Taraxacum officinale Weber	Common dandelion	5.39	5.4	80.00	20.76
Urtica dioica L.	Stinging nettle	0.70	1.0	33.33	4.83
Vicia americana Muhl.	American milk vetch	0.08	0.1	13.33	1.05
	Cotyledon	0.20	0.3	26.67	2.29
	Dead plant matter	1.72	3.1	40.00	8.29
	Grass	5.30	4.2	46.67	15.72
	Litter	31.10	16.1	100.00	55.77
	Moss	16.87	11.2	93.33	39.69
	Rock	1.25	1.4	66.67	9.14
	Soil	14.57	12.3	93.33	36.87
	Unknown species 1 (forb)	0.11	0.2	20.00	1.46

	Unknown species 2 (forb)	0.07	0.1	6.67	0.68
	Unknown species 3 (forb)	0.23	0.4	6.67	1.23
	Wood	0.21	0.2	20.00	2.07

Note: Highlighted cells indicate characteristic species with a prominence over 20.

Table B.11: Mesic 2 vegetation survey June 2005.

Scientific Name	Common Name	Coverage Mean %	Coverage Standard Deviation	Percent Frequency	Prominence
Carex L.	Sedge	0.17	0.2	20.00	1.86
Epilobium angustifolium L.	Fireweed	2.01	3.1	33.33	8.19
Equisetum L.	Horsetail	8.17	5.7	80.00	25.57
Hieracium umbellatum L.	Narrow-leaved hawkweed	15.77	7.4	100.00	39.72
Poa pratensis L.	Kentucky bluegrass	0.07	0.1	6.67	0.67
Populus tremuloides Michx.	Trembling aspen	0.25	0.3	26.67	2.60
Rubus ideaus L.	Wild raspberry	2.36	3.9	40.00	9.72
Salix L.	Willow	1.32	2.2	13.33	4.20
Sonchus uliginosus Bieb.	Smooth perennial sow thistle	0.51	0.7	33.33	4.11
Taraxacum officinale Weber	Common dandelion	0.19	0.3	6.67	1.12
Urtica dioica L.	Stinging nettle	4.29	3.5	60.00	16.04
	Grass	0.75	1.3	20.00	3.86
	Litter	23.48	7.3	100.00	48.46
	Moss	0.35	0.3	13.33	2.15
	Rock	0.36	0.5	33.33	3.46
	Soil	38.69	17.4	100.00	62.20
	Unknown species (forb)	0.09	0.2	6.67	0.79
	Wood	1.08	1.2	46.67	7.10

Note: Highlighted cells indicate characteristic species with a prominence over 20.

Table B.12: Mesic 3 vegetation survey June 2005.

Scientific Name	Common Name	Coverage Mean %	Coverage Standard Deviation	Percent Frequency	Prominence
Achillea millefolium L.	Common yarrow	0.91	1.4	20.00	4.26
Aster L.	Aster	1.00	1.6	26.67	5.16
Bromus inermis Leyss.	Smooth brome	0.35	0.3	20.00	2.63
Calamagrostis canadensis (Michx.) Beauv.	Blue-joint	11.97	11.0	73.33	29.63
Cirsium arvense (L.) Scop.	Canada thistle	0.07	0.1	6.67	0.67
Epilobium angustifolium L.	Fireweed	9.27	4.6	86.67	28.35
Equisetum L.	Horsetail	1.46	1.9	66.67	9.88
Fragaria virginiana Duchesne	Wild strawberry	0.07	0.3	13.33	0.94
Geum aleppicum Jacq.	Yellow avens	0.95	1.6	13.33	3.55
Koeleria macrantha (Ledeb.) J.A. Schultes f.	June grass	8.52	10.2	60.00	22.61
Melilotus alba Desr.	White sweet clover	0.09	0.2	6.67	0.79
Populus tremuloides Michx.	Trembling aspen	0.27	0.5	13.33	1.89
Sonchus uliginosus Bieb.	Smooth perennial sow thistle	9.13	9.1	86.67	28.13
Taraxacum officinale Weber	Common dandelion	4.40	3.5	66.67	17.13
Trifolium hybridum L.	Alsike clover	11.00	11.2	66.67	27.08
Vicia americana Muhl.	American milk vetch	1.35	1.9	13.33	4.25
	Litter	30.83	15.0	93.33	53.64
	Moss	3.28	4.7	33.33	10.46
	Rock	0.07	0.1	6.67	0.67
	Soil	4.79	4.0	46.67	14.95

Note: Highlighted cells indicate characteristic species with a prominence over 20.

Table B.13: Fibric vegetation survey June 2005.

Scientific Name	Common Name	Coverage Mean %	Coverage Standard Deviation	Percent Frequency	Prominence
Carex L.	Sedge	1.95	2.8	33.33	8.06
Epilobium angustifolium L.	Fireweed	24.15	10.7	100.00	49.14
Equisetum L.	Horsetail	0.00	0.0	6.67	0.13
Festuca ovina L.	Rocky mountain fescue	0.41	0.5	26.67	3.32
Potentilla norvegica L.	Rough cinquefoil	0.43	0.6	33.33	3.77
Salix L.	Willow	0.03	0.1	6.67	0.42
Sonchus uliginosus Bieb.	Smooth perennial sow thistle	0.08	0.1	6.67	0.73
	Dead plant	0.03	0.1	6.67	0.42
	Litter	8.91	11.2	60.00	23.12
	Soil	63.56	19.8	86.67	74.22
	Wood	0.47	0.5	33.33	3.94

Note: Highlighted cells indicate characteristic species with a prominence over 20.

Table B.14: Mesic 1 vegetation survey July 2005.

Constituent	Coverage Mean %	Coverage Standard Deviation	Percent Frequency	Prominence
Coke/Coal	0.03	0.1	6.67	0.42
Rock	1.83	1.2	86.67	12.58
Soil	96.98	2.1	100.00	98.48
Wood	1.16	1.4	86.67	10.02

Table B.15: Humic/mesic vegetation survey July 2005.

Scientific Name	Common Name	Coverage Mean (%)	Coverage Standard Deviation	Percent Frequency	Prominence
Achillea millifolium L.	Common yarrow	0.03	0.1	6.67	0.45
*Agropyron sp.*Gaertn.	Wheat grass	11.48	9.8	33.33	19.56
Anemone canadensis L.	Canada anemone	0.19	0.3	13.33	1.59
Aster L.	Aster	0.21	0.4	6.67	1.18
Carex siccata Dewey	Hay sedge	0.48	0.8	6.67	1.79
Dracocephalum parviflorum Nutt.	American dragonhead	2.04	2.8	40.00	9.03
Epilobium angustifolium L.	Fireweed	0.17	0.3	20.00	1.84
Festuca ovina L.	Rocky mountain fescue	2.92	3.2	40.00	10.81
Fragaria virginiana Duchesne	Wild strawberry	5.65	6.8	53.33	17.36
Galium boreale L.	Northern bedstraw	2.37	3.8	40.00	9.74
Hieracium umbellatum L.	Narrow-leaved hawkweed	2.98	2.9	80.00	15.44
Mertensia paniculata (Ait.) G. Don.	Tall lungwort	0.07	0.1	13.33	0.97
Pinus L.	Pine	1.19	2.1	13.33	3.98
Poa pratensis L.	Kentucky bluegrass	0.35	0.4	13.33	2.16
Rosa acicularis Lindl.	Prickly rose	2.56	3.4	26.67	8.26
Sonchus uliginosus Bieb.	Smooth perennial sow thistle	1.91	1.9	20.00	6.18
Taraxacum officinale Weber	Common dandelion	6.17	7.6	73.33	21.27
Unknown species		0.07	0.1	13.33	0.97
Urtica dioica L.	Stinging nettle	0.11	0.1	13.33	1.21
Vicia americana Muhl.	American milk vetch	0.04	0.1	6.67	0.52
	Dead plant	7.49	11.5	26.67	14.13
	Litter	20.81	19.4	86.67	42.47
	Moss	21.57	12.8	93.33	44.87
	Mushroom	0.08	0.1	6.67	0.73
	Needles	0.21	0.4	6.67	1.18
	Rock	1.04	1.2	60.00	7.90
	Soil	7.71	5.6	73.33	23.78
	Wood	0.11	0.2	6.67	0.86

Note: Highlighted cells indicate characteristic species with a prominence over 20.

Table B.16: Mesic 2 vegetation survey July 2005.

Scientific Name	Common Name	Coverage Mean %	Coverage Standard Deviation	Percent Frequency	Prominence
Carex L.	Sedge	0.65	0.1	26.67	4.18
Epilobium angustifolium L.	Fireweed	1.07	1.4	40.00	6.54
Equisetum arvense L.	Horsetail	15.48	9.0	93.33	38.01
Hieracium umbellatum L.	Narrow-leaved hawkweed	9.75	5.4	93.33	30.16
Poa pratensis L.	Kentucky bluegrass	0.31	0.5	13.33	2.02
Populus tremuloides Michx.	Trembling aspen	0.56	0.8	13.33	2.73
Rubus idaeus L.	Wild raspberry	3.53	4.8	33.33	10.85
Salix L.	Willow	0.65	1.0	26.67	4.18
Sonchus uliginosus Bieb.	Smooth perennial sow thistle	0.31	0.4	20.00	2.47
Urtica dioica L.	Stinging nettle	1.80	2.5	60.00	10.39
	Dead plant matter	0.56	1.0	6.67	1.93
	Litter	29.57	10.6	100.00	54.38
	Moss	1.15	1.9	33.33	6.18
	Rock	0.24	0.3	33.33	2.83
	Soil	33.87	18.6	100.00	58.20
	Wood	0.48	0.8	26.67	3.58

Note: Highlighted cells indicate characteristic species with a prominence over 20.

Table B.17: Mesic 3 vegetation survey July 2005.

Scientific Name	Common Name	Coverage Mean %	Coverage Standard Deviation	Percent Frequency	Prominence
Achillea millefolium L.	Common yarrow	1.19	1.8	13.33	3.98
Calamagrostis canadensis (Michx.) Beauv.	Blue-joint	18.15	18.9	86.67	39.66
Epilobium angustifolium L.	Fireweed	9.29	6.5	80.00	27.26
Equisetum arvense L.	Common horsetail	1.04	0.8	33.33	5.89
Equisetum pratense Ehrh.	Horsetail	0.19	0.2	13.33	1.59
Festuca ovina L.	Rocky mountain fescue	0.07	0.1	6.67	0.68
Geum aleppicum Jacq.	Yellow avens	1.33	2.3	13.33	4.21
Hieracium umbellatum L.	Narrow-leaved hawkweed	0.13	0.2	13.33	1.32
Hordeum jubatum L.	Foxtail barley	1.17	2.0	20.00	4.84
Koeleria macrantha (Ledeb.) J.A. Schultes f.	June grass	7.11	6.4	53.33	19.47
Melilotus alba Desr.	White sweet clover	0.36	0.6	6.67	1.55
Populus tremuloides Michx.	Trembling aspen	0.52	0.9	13.33	2.63
Rubus idaeus L.	Wild raspberry	0.01	0.0	6.67	0.26
Sonchus uliginosus Bieb.	Smooth perenial sow thistle	10.69	7.8	86.67	30.44
Taraxacum officinale Weber	Common dandelion	3.56	1.4	46.67	12.89
Trifolium hybridum L.	Alsike clover	16.55	15.5	66.67	33.22
Trifolium repens L.	White clover	1.31	2.5	13.33	4.18
Trifolium pratense L.	Red clover	0.84	1.0	20.00	4.10
Unknown species	Unknown species	0.05	0.1	6.67	0.58
	Dead plant matter	0.83	1.4	13.33	3.33
	Gopher hole	0.28	0.3	13.33	1.93
	Litter	23.28	7.4	73.33	41.32
	Moss	1.76	1.9	20.00	5.93
	Soil	0.29	0.5	13.33	1.97

Note: Highlighted cells indicate characteristic species with a prominence over 20.

Table B.18: Fibric vegetation survey July 2005.

Scientific Name	Common Name	Coverage Mean %	Coverage Standard Deviation	Percent Frequency	Prominence
Carex L.	Sedge	1.95	2.8	33.33	8.06
Epilobium angustifolium L.	Fireweed	23.97	11.0	100.00	48.96
Equisetum L.	Horsetail	0.03	0.1	6.67	0.42
Festuca ovina L.	Rocky mountain fescue	1.29	0.6	26.67	5.87
Potentilla norvegica L.	Rough cinquefoil	0.24	0.8	33.33	2.84
Salix L.	Willow	0.32	0.1	6.67	1.46
Sonchus uliginosus Bieb.	Smooth perennial sow thistle	0.00	0.1	13.33	0.00
	Dead plant matter	0.03	0.1	6.67	0.42
	Litter	14.68	5.2	53.33	27.98
	Soil	56.57	12.3	100.00	75.21
	Wood	0.47	4.0	33.33	3.94

Note: Highlighted cells indicate characteristic species with a prominence over 20.

Table B.19: Mesic 1 vegetation survey August 2005.

Constituent	Coverage Mean %	Coverage Standard Deviation	Percent Frequency	Prominence
Coke/Coal	0.03	0.1	6.67	0.42
Rock	1.83	1.2	86.67	12.58
Soil	96.98	2.1	100.00	98.48
Wood	1.16	1.4	86.67	10.02

Table B.20: Humic/mesic vegetation survey August 2005.

Scientific Name	Common Name	Coverage Mean %	Coverage Standard Deviation	Percent Frequency	Prominence
Achillea millifolium L.	Common yarrow	0.28	0.5	13.33	1.93
Agropyron sp. Gaertn.	Wheat grass	6.19	10.7	6.67	6.42
Anemone canadensis L.	Canada anemone	0.04	0.0	6.67	0.52
Bromus inermis Leyss.	Smooth brome	0.03	0.1	6.67	0.42
Calamagrostis canadensis (Michx.) Beauv.	Blue-joint	1.25	1.1	13.33	4.09
Carex siccata Dewey	Hay sedge	4.96	6.0	20.00	9.96
Carex L.	Sedge	1.23	1.7	33.33	6.39
Dracocephalum parviflorum Nutt.	American dragonhead	0.88	0.4	26.67	4.84
Epilobium angustifolium L.	Fireweed	0.39	0.6	26.67	3.21
Erigeron L.	Fleabane	0.19	0.5	6.67	1.12
Festuca ovina L.	Rocky mountain fescue	0.99	1.2	20.00	4.44
Fragaria virginiana Duchesne	Wild strawberry	7.12	8.4	66.67	21.79
Galium boreale L.	Northern bedstraw	1.96	3.4	20.00	6.26
Hieracium umbellatum L.	Narrow-leaved hawkweed	1.63	1.2	40.00	8.07
Hordeum jubatum L.	Foxtail barley	0.21	0.4	6.67	1.19
Mertensia paniculata (Ait.) G. Don.	Tall lungwort	0.19	0.2	13.33	1.58
Moss	Moss	13.75	11.6	100.00	37.08
Petasites palmatus (Ait.) A. Gray	Palmate-leaved coltsfoot	0.01	0.0	6.67	0.30
Rosa acicularis Lindl.	Prickly rose	8.75	16.2	33.33	17.07
Sonchus uliginosus Bieb.	Smooth perennial sow thistle	3.05	5.2	33.33	10.09
Taraxacum officinale Weber	Common dandelion	5.97	9.2	73.33	20.93
Trifolium hybridum L.	Alsike clover	0.21	0.6	13.33	1.69
	Unknown species 1	0.51	0.8	33.33	4.11
	Unknown species 2	0.03	0.1	6.67	0.42
	Litter	18.89	9.9	80.00	38.88
	Rock	4.97	3.3	60.00	17.27
	Soil	16.33	10.1	86.67	37.62
	Wood	0.11	0.2	13.33	1.19

Note: Highlighted cells indicate characteristic species with a prominence over 20.

Table B.21: Mesic 2 vegetation survey August 2005.

Scientific Name	Common Name	Coverage Mean %	Coverage Standard Deviation	Percent Frequency	Prominence
Bromus inermis Leyss.	Smooth Brome	3.15	1.8	6.67	4.58
Carex L.	Sedge	0.59	0.3	20.00	3.43
Epilobium angustifolium L.	Fireweed	1.23	2.0	33.33	6.39
Equisetum arvense L.	Horsetail	12.32	6.4	93.33	33.91
Hieracium umbellatum L.	Narrow-leaved hawkweed	9.15	3.8	93.33	29.22
Populus tremuloides Michx.	Trembling aspen	0.51	0.7	26.67	3.68
Potentilla norvosa L.	Rough cinquefoil	0.07	0.1	6.67	0.67
Rubus idaeus L.	Wild raspberry	3.27	4.5	40.00	11.43
Salix L.	Willow	0.05	0.1	6.67	0.60
Sonchus uliginosus Bieb.	Smooth perennial sow thistle	1.56	1.2	20.00	5.59
Taraxacum officinale Weber	Common dandelion	0.31	0.4	20.00	2.48
Urtica dioica L.	Stinging nettle	0.68	1.1	46.67	5.63
	Dead plant matter	0.08	0.1	6.67	0.73
	Litter	27.91	12.0	100.00	52.83
	Moss	0.83	1.2	26.67	4.70
	Rock	0.37	0.4	46.67	4.17
	Soil	38.24	18.1	100.00	61.84
	Wood	0.27	0.4	26.67	2.67

Note: Highlighted cells indicate characteristic species with a prominence over 20.

Table B.22: Mesic 3 vegetation survey August 2005.

Scientific Name	Common Name	Coverage Mean %	Coverage Standard Deviation	Percent Frequency	Prominence
Achillea millefolium L.	Common yarrow	0.75	1.3	6.67	2.23
Bromus inermis Leyss.	Smooth brome	1.44	2.5	6.67	3.10
Calamagrostis canadensis (Michx.) Beauv.	Blue-joint	8.99	10.6	26.67	15.48
Epilobium angustifolium L.	Fireweed	17.57	12.7	73.33	35.90
Equisetum arvense L.	Horsetail	1.64	1.6	26.67	6.61
Festuca ovina L.	Rocky mountain fescue	0.47	0.8	6.67	1.76
Galium boreale L.	Northern bedstraw	0.08	0.1	6.67	0.73
Grass	Grass	0.15	0.2	6.67	0.99
Hieracium umbellatum L.	Narrow-leaved hawkweed	0.03	0.1	13.33	0.67
Hordeum jubatum L.	Foxtail barley	13.53	14.2	60.00	28.50
Koeleria macrantha (Ledeb.) J.A. Schultes f.	June grass	1.09	1.5	20.00	4.68
Poa pratensis L.	Kentucky bluegrass	4.99	1.6	13.33	8.15
Poa L.	Grass	1.41	2.4	6.67	3.07
Populus tremuloides Michx.	Trembling aspen	0.60	1.0	13.33	2.83
Potentilla norvegica L.	Rough cinquefoil	0.57	1.0	6.67	1.96
Rubus idaeus L.	Wild raspberry	0.25	0.4	13.33	1.84
Sonchus uliginosus Bieb.	Smooth perennial sow thistle	23.97	15.0	86.67	45.58
Taraxacum officinale Weber	Common dandelion	0.40	0.7	6.67	1.63
Trifolium hybridum L.	Alsike clover	5.23	6.6	46.67	15.62
Trifolium L.	Clover	0.15	0.3	6.67	0.99
Elk Scat	Scat	0.13	0.2	6.67	0.94
	Litter	8.61	12.2	66.67	23.96
	Moss	5.60	1.8	33.33	13.66
	Soil	2.35	3.0	20.00	6.85

Note: Highlighted cells indicate characteristic species with a prominence over 20.

Table B.23: Fibric vegetation survey August 2005.

Scientific Name	Common Name	Coverage Mean %	Coverage Standard Deviation	Percent Frequency	Prominence
Agropyron Gaertn.	Wheat grass	0.29	0.4	20.00	2.42
Calamagrostis canadensis (Michx.) Beauv.	Blue-joint	0.60	1.0	6.67	2.00
Carex L.	Sedge	0.51	0.5	13.33	2.60
Epilobium angustifolium L.	Fireweed	24.28	11.7	100.00	49.27
Equisetum L.	Horsetail	0.12	0.2	6.67	0.89
Festuca ovina L.	Rocky mountain fescue	0.08	0.1	6.67	0.73
Poa L.	Grass	0.36	0.6	6.67	1.55
Populus balsamifera L.	Balsam poplar	0.11	0.1	13.33	1.19
Populus tremuloides Michx.	Aspen poplar	0.12	0.2	13.33	1.26
Potentilla norvegica L.	Rough cinquefoil	0.35	0.5	26.67	3.04
Rubus idaeus L.	Wild raspberry	0.13	0.2	6.67	0.94
Taraxacum officinale Weber	Common dandelion	0.03	0.1	6.67	0.42
	Litter	4.65	3.8	86.67	20.08
	Rock	0.59	1.0	13.33	2.80
	Soil	67.51	14.7	100.00	82.16
	Wood	0.47	0.3	33.33	3.94

Note: Highlighted cells indicate characteristic species with a prominence over 20.

Table B.24: Pair wise comparisons of microbial community structure between sites for 2005, derived from PLFA analysis.

Sampling Date	Comparison	Total Biomass	Gram (-)	Gram (+)	Gram (-)/ Gram (+)	Diver-sity	Richness	Even-ness	Fungi	Actino - mycetes	Fungi/ Bacteria	AM
June/ July	Mesic 2 & Humic/mesic	0.963	0.015	0.751	0.563	0.995	0.000	0.017	0.572	0.240	0.196	0.051
	Mesic 2 & Mesic 3	0.059	0.473	0.035	0.957	0.999	0.016	0.224	0.662	0.001	0.011	0.973
	Mesic 2 & Mesic 1	1.000	0.909	0.210	0.964	0.962	0.945	0.996	0.183	0.038	0.038	0.862
	Mesic 2 & Fibric	0.882	0.000	0.780	0.096	0.161	0.013	0.958	0.497	0.076	0.492	0.001
	Humic/mesic & Mesic 3	0.307	0.459	0.495	0.218	1.000	0.260	0.763	1.000	0.404	0.840	0.198
	Humic/mesic & Mesic 1	0.920	0.001	0.017	0.902	0.843	0.000	0.041	0.973	0.000	0.980	0.004
	Humic/mesic & Fibric	0.993	0.282	0.999	0.650	0.306	0.980	0.030	0.989	0.833	1.000	0.212
	Mesic 3 & Mesic 1	0.039	0.106	0.000	0.664	0.885	0.002	0.403	0.916	0.000	0.987	0.519
	Mesic 3 & Fibric	0.837	0.014	0.866	0.030	0.241	0.869	0.195	0.969	0.999	0.928	0.003
	Mesic 1 & Fibric	0.827	0.000	0.070	0.247	0.056	0.003	0.876	1.000	0.000	0.991	0.000
August	Mesic 2 & Humic/mesic	0.999	0.545	0.317	0.218	0.973	0.707	0.973	0.706	0.175	0.999	0.029
	Mesic 2 & Mesic 3	0.935	0.961	1.000	0.883	0.993	0.314	0.993	0.000	1.000	0.030	0.222
	Mesic 2 & Mesic 1	0.000	0.996	0.360	0.503	0.954	0.028	0.954	0.002	0.673	0.021	0.195
	Mesic 2 & Fibric	0.000	0.010	1.000	0.249	0.246	1.000	0.246	0.003	0.999	0.937	0.000
	Humic/mesic & Mesic 3	0.875	0.231	0.349	0.037	1.000	0.981	1.000	0.035	0.151	0.030	0.866
	Humic/mesic & Mesic 1	0.000	0.750	0.999	0.962	0.717	0.551	0.717	0.146	0.855	0.022	0.848
	Humic/mesic &	0.000	0.209	0.743	0.994	0.542	0.907	0.542	0.082	0.353	0.887	0.240

	Fibric										
	Mesic 3 & Mesic 1	0.004	0.844	0.398	0.111	0.801	0.853	0.801	0.945	0.615	1.000	1.000
	Mesic 3 & Fibric	0.001	0.003	1.000	0.067	0.425	0.681	0.425	1.000	1.000	0.450	0.031
	Mesic 1 & Fibric	0.644	0.020	0.810	0.882	0.089	0.243	0.089	0.950	0.772	0.417	0.025

Note: The values in the table are p values; shaded cells indicate significant differences. AM stands for arbuscular mycorrhizal fungi,

Table B.25: PLFA indices from June/July and August 2005 sampling events.

Collection	Site		% AM	Total Biomass	% Gram (-)	% Gram (+)	% Fungi Degrood	% Fungi Hannam	% Actinomycetes	Fungal/ Bacterial Biomass	Gram (-) /Gram (+)
June/July	Humic/mesic	average	2.21	72.96	24.64	17.79	0.44	5.37	4.54	0.01	1.39
		sd	0.54	25.27	4.09	2.28	0.34	0.97	1.22	0.01	0.25
	Mesic 1	average	1.62	51.50	20.13	15.41	0.31	4.43	2.34	0.01	1.32
		sd	0.09	5.08	0.94	1.36	0.48	0.50	1.04	0.01	0.16
	Mesic 2	average	1.77	55.70	21.02	16.91	0.76	4.93	3.59	0.02	1.27
		sd	0.15	4.91	1.38	1.81	0.86	1.07	1.38	0.03	0.22
	Mesic 3	average	1.86	124.00	22.77	18.99	0.48	4.61	5.35	0.00	1.21
		sd	0.44	135.89	3.44	2.22	0.44	0.72	0.96	0.01	0.20
	Fibric	average	2.68	87.97	27.66	18.03	0.30	7.27	5.19	0.01	1.55
		sd	0.90	20.67	4.38	1.44	0.19	1.38	1.27	0.01	0.34
August	Humic/mesic	average	2.70	86.94	27.65	18.60	0.67	6.65	5.90	0.01	1.49
		sd	0.67	42.47	3.46	1.41	0.20	0.97	0.85	0.01	0.19
	Mesic 1	average	3.04	196.07	29.30	18.36	1.10	6.94	5.30	0.03	1.60
		sd	1.21	99.54	3.02	1.25	0.51	1.37	0.91	0.01	0.21
	Mesic 2	average	3.71	92.59	29.76	16.60	0.44	7.65	4.46	0.01	1.85
		sd	0.39	25.49	3.47	2.88	0.23	2.20	2.38	0.01	0.45
	Mesic 3	average	3.04	110.39	30.65	16.61	1.23	7.26	4.44	0.03	2.00
		sd	0.57	43.34	4.35	4.11	0.44	2.77	2.26	0.01	0.70
	Fibric	average	1.19	238.88	23.60	16.89	1.60	7.46	4.09	0.02	1.40
		sd	0.27	41.92	1.41	1.30	0.80	0.85	1.07	0.02	0.03

The following are the descriptions of the prominent species surveyed at each site: AM stands for arbuscular mycorrhizal fungi.

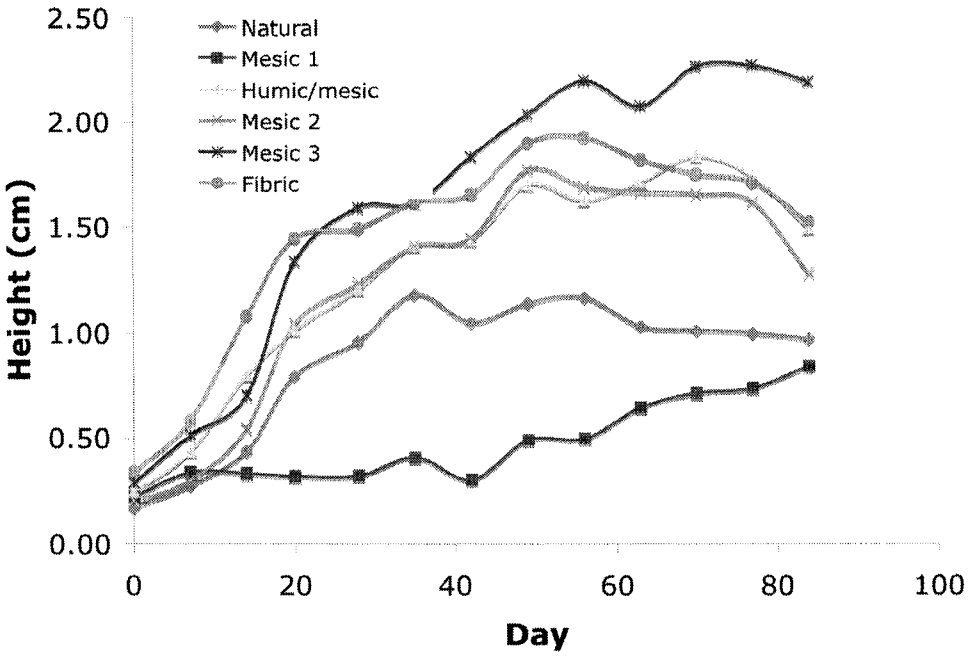


Figure B.1: Average height of *Calamagrostis canadensis* during a 92-day greenhouse bioassay.

B.2 DESCRIPTIONS OF PROMINENT SPECIES

Humic/mesic common dandelion/wild strawberry

Common dandelion is a naturalized exotic, common in disturbed areas (Johnson et al., 1995). In August wild strawberry becomes a characteristic species, this is a native species common in the Boreal forest. Rocky mountain fescue does not meet the criteria for a characteristic species, but with a prominence of 18%, it is the second most common plant on site. Rocky mountain fescue is a drought resistant species, widespread across the Boreal forest. The Humic/mesic site was the oldest site and common non-native species and a native grass characterized the understory. With a south aspect the drought resistant fescue is likely to remain established on this slope.

Mesic 2 narrow-leaved hawkweed/horsetail

Narrow-leaved hawkweed is a native species and can cover large areas quickly by cloning itself. This species is commonly found on disturbed ground. Horsetail is also common on this site and is commonly found in moist ecosystems and disturbed ground (Johnson et al., 1995). As the peat amendment comes from a saturated system it is likely that horsetail was in the seedbank. Stinging nettle is also widespread in disturbed sites and was common on site (16% prominence). The Mesic 2 site was characterized by primary successor species that occupy recently disturbed lands.

Mesic 3 bluejoint/fireweed/smooth perennial sow thistle/alsike clover/june grass

Bluejoint is a native species that is widespread throughout the Boreal forest. Another native grass on site was June grass, which is widespread across the Southern Boreal forest (Johnson et al., 1995).

Smooth perennial sow thistle is an introduced, noxious weed, common on disturbed sites. Noxious weeds have the ability to spread rapidly, and may cause severe crop losses and economic hardship. It should be controlled to prevent further spread. Alsike clover is also an introduced species that is common on disturbed areas. Unlike sow thistle, clover is known to improve nitrogen poor soils (Johnson et al., 1995).

The Mesic 3 site was characterized by natural grasses and introduced species. Fireweed, sow thistle and clover are all primary successors. The sow thistle poses a threat to this vegetation community with its status as a noxious weed.

Fibric fireweed

Fireweed is a natural primary successor, widespread in burned and disturbed areas. It is very important in controlling erosion of disturbed areas (Johnson et al., 1995). The Fibric site did not have many non-native species in comparison to the other sites. The acidic nature of the Fibric peat may help to reduce weed growth (Lucas et al., 1965).

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APPENDIX C: A COMPARISON OF THE RESIN-CORE AND BURIED-BAG INCUBATION METHODS

Numerous methods have been proposed to quantify net N mineralization rates under field conditions (Hart et al., 1994). The "buried-bag" technique, where soils are incubated in buried polyethylene bags, has been the most commonly used experimental approach in boreal forest floors (Table 2.1). Net rates of N mineralization are estimated from the difference in inorganic N content between post- and pre-incubated soils. The bags are semi-permeable to gases but impermeable to liquids. Hence, this method represents soil water content at the beginning of the incubation period (Gordon et al., 1987; Hart et al., 1994) but does not reflect *in situ* soil moisture fluctuations that may occur during incubation. Another potential disadvantage is that the soil does not remain intact in this method, causing disturbance that may increase mineralization (Raison et al., 1987).

Another method uses ion exchange resins (IER) in porous bags to trap soluble ions. Binkley and colleagues have spent the better part of a decade researching IER bag incubations (Binkley and Matson, 1983; Binkley, 1984; Binkley et al., 1986; Binkley et al., 1992). Measurements with IERs are sensitive to fluctuations in soil water status, but do not prevent competition from root uptake when buried unconfined in soils (Binkley, 1984). Distefano and Gholz (1986) combined the IER technique with a soil containment system by incubating soil cores in PVC tubes that are sandwiched between two IER bags; the top bag deionizes incoming water, while the bottom bag traps ions leaching from the core. While not as cost-effective and more labor-intensive than the simpler buried-bag approach, this so-called "resin-core" method allows water fluctuations during incubation, and is potentially superior because it may provide a more realistic value for net N mineralization and nitrification than other in-field incubation methods (Hart et al., 1994). Further, it may provide more realistic measurements of *in situ* rates by allowing continuous removal of mineralization products during incubation (Hart et al., 1994).

This experiment paired two in-field incubation techniques to measure soil N mineralization rates, the resin-core and the buried bag incubations. The objective of this study was to determine if the two techniques provide similar results.

Samples in buried-bags were buried to 7 cm depth to be consistent with the depth the resin-cores were buried. The net ammonification rate was calculated as NH_4-N after incubation minus NH_4-N at the beginning of the field incubation, the net nitrification rate as NO_3-N at the end minus NO_3-N at the beginning of the incubation, and the net mineralization rate as inorganic N (NH_4-N + NO_3-N) at the end minus inorganic N at the beginning (Jerabkova et al., 2006). A paired t-test was used to compare results from the two incubation methods with an alpha value of 0.05 to detect significant levels.

Comparison of the resin-core and buried-bag incubation techniques showed significant differences in net N mineralization rates between the two methods, although these were not consistent among sites or seasons (Figure C.1 a). The resin-core method yielded a significantly higher net mineralization rate than the buried-bag method for the Mesic 1 peat material in winter ($p = 0.020$), and for the Humic/mesic material in the fall ($p = 0.030$). On the other hand, in summer, estimated net N mineralization rates for site Humic/mesic were significantly higher from the buried-bag incubation ($p = 0.002$). Results for net nitrification rates (Figure C.1 b) were similar to the net mineralization results in that the resin-core incubations showed higher rates for the Mesic 1 material in winter ($p = 0.021$) and the Humic/mesic material in the fall ($p = 0.029$), but a lower rate for Humic/mesic in the summer ($p= 0.002$). When significant differences occurred for net ammonification rates (Figure C.1 c), the resin-core method always yielded a higher rate than the buried-bag method (Mesic 1 summer $p = 0.025$, Natural summer $p = 0.009$, winter $p = 0.013$). Differences in net ammonification rates between methods, however, were typically much smaller than what was observed for differences in net nitrification rates.

Post-incubation DON concentrations showed consistent differences between the two incubation methods (Figure C.2 a). When significant differences occurred, concentrations were always higher in the resin cores than in the buried bags (Mesic 1 summer $p = 0.001$; Humic/mesic summer $p = 0.002$, Fall $p = 0.044$, spring $p = 0.016$; Fibric fall $p = 0.047$, spring $p = 0.004$; Natural spring $p = 0.003$). Post-incubation moisture measurements showed consistently higher moisture content in the soil from the buried-bag incubations than in the soil from the resin-core incubations (Mesic 1 summer $p = 0.0001$, fall $p = 0.031$, spring $p = <0.0001$; Humic/mesic winter $p = 0.017$, spring $p =$

0.006; Fibric summer p = 0.0004; Natural summer p = 0.004, fall p = 0.003, winter p = 0.037, spring p = 0.001) (Figure C.2 b).

Our study did not find that the resin-core and buried-bag techniques correlated well. The differences between the buried-bag and resin-core incubation methods were inconsistent. There are few comparable studies that contrast incubation techniques. Binkley et al. (1986) stated that IER bag and buried-bag data correlated well for N dynamics. Considering that Binkley et al. (1986) had a three-year difference between the buried-bag and the IER bags experiments, the results of this study were surprising and are unique from any previous research conducted.

One drawback to the resin-core design is that water may not infiltrate the soil within the core in a natural manner. There may be preferential flow, or the tendency of the water to flow more around the edges of the core. Although this may not be representative of natural infiltration, it is an improvement to not having water infiltration at all, as in the buried-bag design.

In conclusion, the results of this experiment showed that the two incubation techniques do not provide similar results for a variety of parameters, including soil moisture, DON, and net N mineralization and nitrification rates. However, this study could not pinpoint why the differences in net mineralization and nitrification rates occurred. Based on the reduced amount of disturbance to the soil and the seasonal influxes of moisture content that occurred in the resin-cores, the resin-core methods was chosen as the preferable method for analyzing seasonal N dynamics and was used for all analyses in chapter 2.

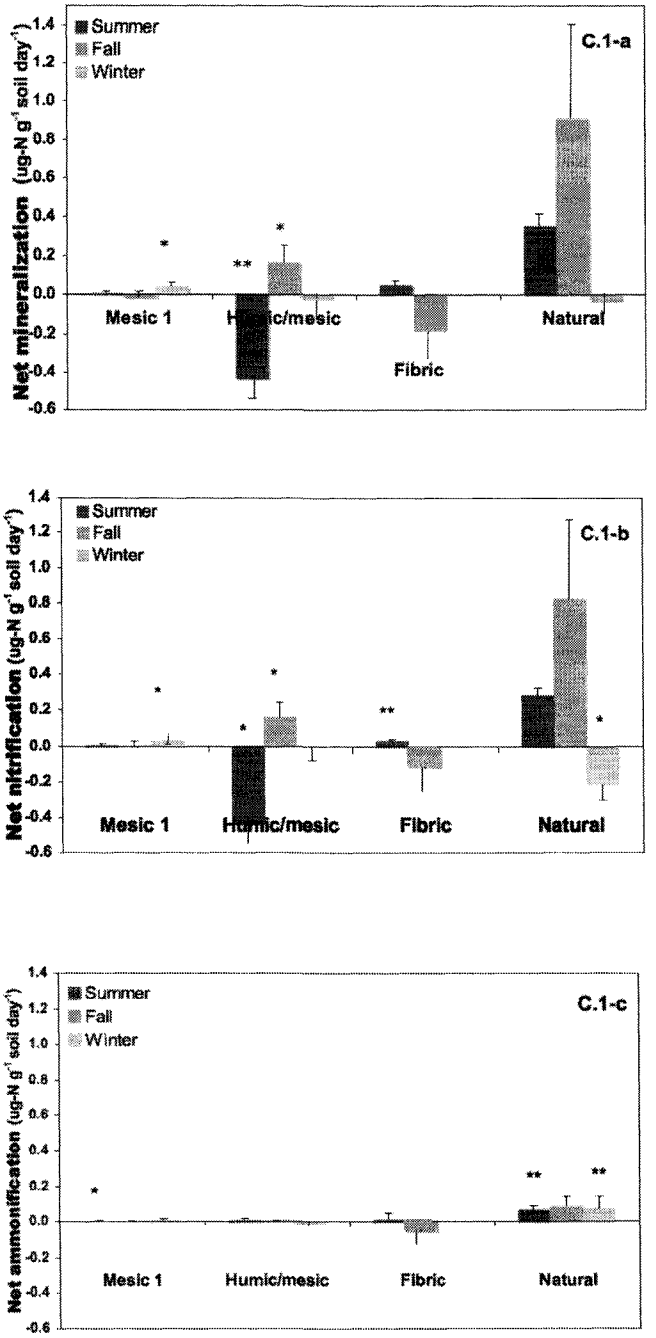

Figure C.1: Differences in a) net mineralization, b) nitrification, and c) ammonification rates (μg-N g^{-1}soil day^{-1}) between the resin-core and buried-bag method for peat materials from the Mesic 1, Humic/mesic, Fibric and Natural sites. Positive values indicate larger rates for the resin-core than the buried-bag method. For each incubation period and peat material, * indicates a significant difference between the two methods at α =0.05, and ** at α =0.01. Error bars represent 1 standard deviation from the mean (n=5).

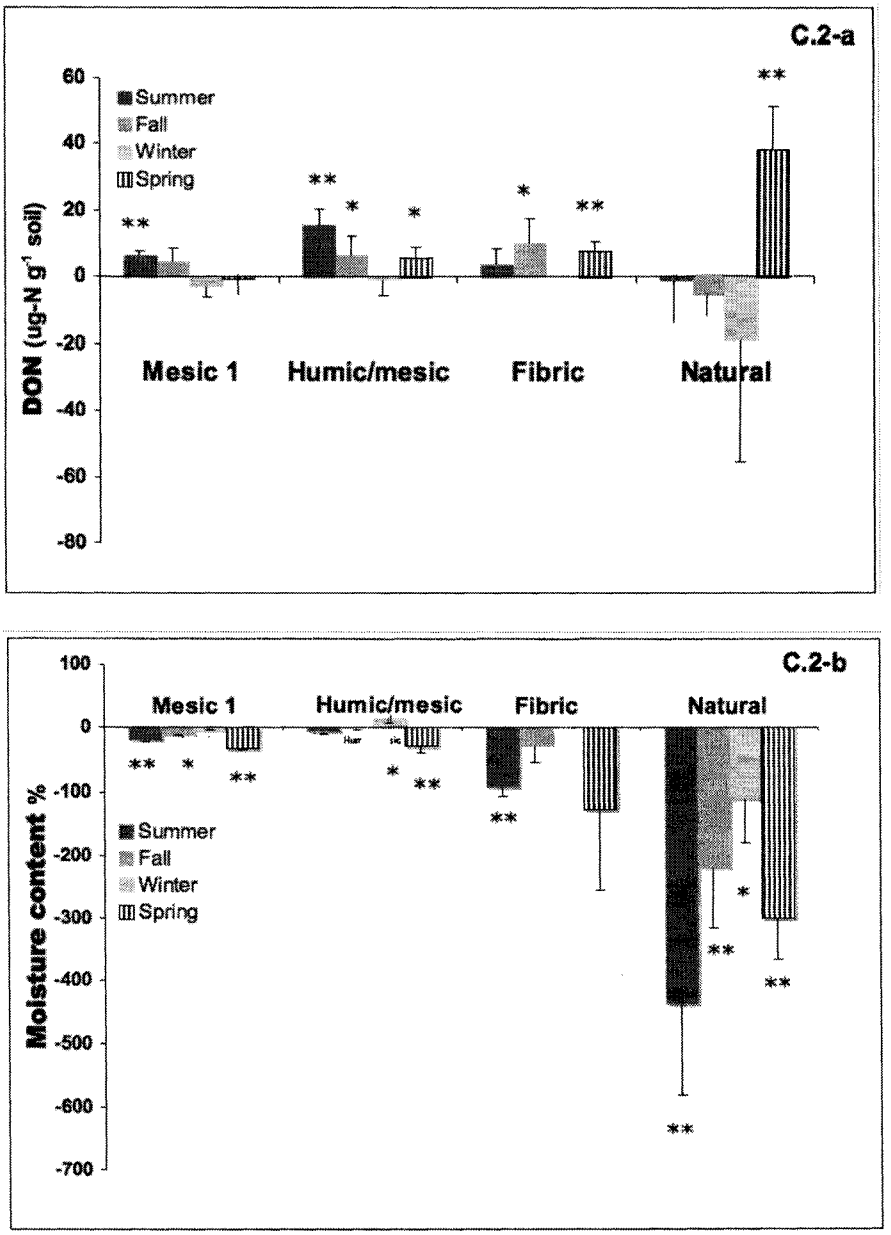

Figure C.2: Differences in post-incubation a) dissolved organic nitrogen (DON) concentrations (μg-N g^{-1} soil), and b) moisture content (%) between the resin-core and buried-bag method for peat materials from the Mesic 1, Humic/mesic, Fibric and Natural sites. Positive values indicate larger rates for the resin-core than the buried-bag method. For each incubation period and peat material, * indicates a significant difference between the two methods at α =0.05, and ** at α =0.01. Error bars represent 1 standard deviation from the mean (n=5).

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